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Agenda item 4 : **Progress report on activities carried out by SPA/RAC since the twelfth meeting of Focal Points for SPAs**

Guidelines for the long term Monitoring programmes for marine turtles nesting beaches and standardized monitoring methods for nesting beaches, feeding and wintering areas

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Content :

1. OVERVIEW

1.1. Prerequisites to realise the monitoring of the sea turtles in the Mediterranean

- The main research and sea turtles monitoring bodies in the Mediterranean are willing to cooperate and follow collaborative agreement of the parties.

- A central unit of researchers collects the data annually from all collaborators and opportunistic sources, to collate and analyse them, along with the results of intensive uniform surveys every 5-years.

- A central dynamic (and interactive) database that will include all existing, ongoing and future data (including existing and potential nesting, foraging and wintering areas, nesting data, data from foraging areas, tagging data, genetic data, etc.) is developed.

- This database would be used to identify gaps in the distribution of monitoring and monitoring techniques applied, and to suggest solutions for the collaborating parties.

- Ensure advantages for researchers that share and contribute with their data in the database.

- Elaboration of data quality checks to ensure that the data collected are accurate and representative.

1.2. Summary

This Guideline describes and suggests improvement on the methodology for the long-term standardized collection and assimilation of data on adult and juvenile loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles at nesting, foraging and wintering areas throughout the Mediterranean. In particular, it suggests (i) standardised monitoring techniques for establishing the current distribution of nesting, wintering and feeding areas in parallel to detecting shifts in distribution over time and (ii) standardised monitoring techniques for establishing the population size of selected nesting, wintering and feeding areas, along with proposed selection criteria to assimilate a representative cross-section of sites nationally based on the provisions of the UNEP(DEPI)/MED IG.22/Inf.7, the IMAP and the Common Indicators factsheets. The combined use of a variety of assessment techniques is suggested to facilitate demographic analyses, which should be covered in the '*Standardization of methodologies to estimate demographic parameters for population dynamics analysis, such as population modelling*'.

Due to the different financial, personnel, equipment and National Security status of the countries bordering the Mediterranean, the document has been structured to suggest (1) essential baseline information for collection throughout all countries and (2) additional information for collection at a network of sites with different characteristics to enhance demographic models and the assessment of key pressures to sea turtles. It is essential to obtain a broad understanding of the current distribution and numbers of sea turtles across all sites to record future shifts in response to changes in anthropogenic pressure, including climate change.

In order to ensure that data are standardised with the aim of facilitating the sharing and analysis of information at the Mediterranean scale, the agreement of the collaborative parties is required along with the willingness to participate from the main contributors (research organisations, universities, fishers, individuals etc.). A central body should be assigned to liaise with participants/contributors, ensure that the information are inserted in the appropriate database with relevant linkages among all databases (ultimately, genetics data should link all other data inputs). Depending on the type and detail of information, layers could be generated on a GIS database, building on Mediterranean wide surveys conducted every 5-years (supporting the suggestion of the Demography Working Group 2015). This approach would allow gaps in monitoring (location and level of information provided) to be identified at the Mediterranean scale, and addressed at regular intervals. Furthermore, by assimilating all data in a central database, the access and use can be standardised, allowing nesting areas to be connected with foraging and wintering areas, which generally occur in different countries.

Ultimately, as bias will always exist, this suggested approach will facilitate variation in effort across all involved nations, maximising our understanding and ability to improve the protection of sea turtles at the Mediterranean scale.

1.3. Key Suggestions

The below recommendations support and expand on those suggested by the Demography Working Group (2015):

1. Standardised 5-yearly aerial surveys (plane, unmanned aerial vehicles [UAV]) throughout the Mediterranean for the delineation of all sandy beaches and those used for nesting, with calibration by ground surveys.

2. Maintenance of ongoing beach monitoring projects and expansion to other areas based on the above-mentioned recommendation.

3. Improved estimates of female numbers, to also include male numbers, for operational and adult sex ratio predictions and demographic assessments, including reproductive longevity, remigration intervals and clutch frequency information.

4. Improved monitoring of existing sites to link females to their nests and offspring output within and across years, including fitness and health predictions.

5. Standardised 5-yearly aerial surveys (plane, UAV or satellite imagery) throughout the Mediterranean across all marine and coastal areas (in combination with cetacean surveys) to delineate key foraging and wintering areas of adults and juveniles.

6. Year-round aerial and boat surveys of focal foraging habitats throughout the year to delineate population structure and demography.

7. Satellite tracking (combined with genetics and stable isotope sampling for validation of non-tracked individuals) of at least 20 adult males and females from each breeding area (4 individuals per sex per year per site to gain information on breeding periodicity, internesting intervals and clutch frequency) and of 60 adult males and females and juveniles from foraging grounds to delineate connectivity between breeding-foraging and foraging-wintering and foraging-wintering grounds across the region.

8. Standardised bycatch projects to update bycatch figures and assess post-release mortality.

9. Mediterranean-wide genetics analysis, blood and stable isotopes at breeding (adult males and females, hatchings) and foraging and wintering sites, as well as of stranded turtles. The genetic component is essential as it will help overcome the challenges associated with the complex population structure of sea turtles and will facilitate the consolidation of all other data collection types.

10. Stranding networks in every Mediterranean country to collect data and samples, including skeletochronology of all dead stranded individuals.

1.4. Standardised Monitoring Techniques for Implementation

Nesting areas

5 year intervals:	Essential:	Potential nesting beach identification Standardised aerial surveys (plane/UAV) of all beaches Mid-season confirmation of turtle activity (aerial or foot patrols) detected by previous and proximate 5-year surveys
Annual:	Essential:	1-3day track/nest counts all season (ongoing and newly identified key areas from 5-year surveys) at key areas and additional selected areas representing different characteristics Nesting area strandings records and associated sampling
	Preferred:	Capture-mark-recapture of females on beaches (2-3 parallel techniques; flipper/PIT/photo-id/genetic fingerprinting); Capture-mark-recapture of males in the sea Satellite transmitter attachment to males and females prior to the onset of nesting (clutch frequency/remigration) Blood/tissue sampling for genetics (nucleotide and mitochondrial), health and stable isotope analyses of all recorded individuals; and laparoscopy

Foraging and wintering areas

5 year intervals:	Essential:	Standardised aerial surveys (plane, UAV) across all marine areas at least once (September), preferably 4 times, to account for seasonal variation in habitat use Use of these data to select 10 key sites (which can be revised every 5 years based

		on analysis of information/gaps) per country for annual monitoring
Annual	Essential:	Four aerial surveys (plane, UAV) per year to document seasonal use of selected representative foraging wintering sites (aerial surveys)
		Nationwide strandings records and associated sampling
	Preferred:	Boat-based capture-mark-recapture surveys (2-3 parallel techniques; flipper/PIT/photo-id/genetic fingerprinting) of all sea turtles (adult male, adult female, juvenile) Satellite transmitter attachment to representative individuals from each size class to determine connectivity with other foraging habitats, wintering habitats and nesting sites Blood/tissue sampling for genetics (nucleotide and mitochondrial), health and stable isotope analyses of all recorded individuals; and laparoscopy Sampling of benthos for stable isotope correlation and marine health assessments
Opportunistic records	information:	GPS location, descriptive and photographic
		Blood/tissue sampling for genetics (nucleotide and mitochondrial), health and stable isotope analyses of all recorded individuals; and laparoscopy Database to detail sightings by all organisations and contribution by laypersons/fishers etc.
Bibliographic review:		Continuous consolidation of all published information (past, present, reports, papers, newspapers webpage information – including videos) at regular intervals making use of all potential sources

Notes:
1. For the best results, the suggested techniques should be integrated and combined with regular validation assessments.
2. Demographic information should be integrated with abundance information to enhance population trends models.

2. General Introduction

Demographic (vital-rate) parameters are needed in combination with abundance estimates to understand and predict trends in sea turtle populations. As noted by the National Research Council (NRC) of the USA (2010), the techniques used to measure the abundance and other demographic parameters of sea turtles on nesting beaches and in the water, vary widely with respect to the type of sampling, what is counted, how counts are made, and how the data are used for estimates. For instance, most projects only focus on nesting habitat, where females (and males) aggregate to breed, with this behaviour providing an easy opportunity to count females or their nests on beaches (not usually the males, though). However, these females represent only a small component of the total population. Monitoring only female nesting activity provides insufficient information for population assessment because adult females usually skip one or more breeding seasons, and nest counts provide no information on demographic structure because immature, adult male, and non-breeding female components are not sampled (NRC, 2010). Furthermore, the long generation time and oceanic habitat of juveniles presents major obstacles to studying immature stages.

Survey techniques vary with respect to species, life stage, sea conditions (depth and clarity, currents and sea state), accessibility, personnel and equipment availability and funding. In particular, in the Mediterranean, the funding allocated to the conservation of wildlife differs noticeably among countries, along with the national security risks of each country (Mazaris *et al.* 2016). Consequently, the lack of coordination of sea-turtle databases and lack of information on Mediterranean wide distribution impede the management and conservation of sea turtles, due to projects being biased to certain areas or countries or life-history phases (e.g. nesting).

A population is usually defined as a group of organisms whose members interbreed and are subjected to processes that result in a common birth, mortality and growth rate. All members of a species can potentially interbreed, and some migration occurs among populations. However, a single nesting site might represent a subpopulation, rather than a distinct population in the Mediterranean, with the gene flow of males being assumed to represent the upper geographical scale of a nesting population (Bowen and Karl 2007; Lee 2008; Wallace *et al.* 2009). Several studies have confirmed that males, and some females, frequent more than one nesting area in the Mediterranean (Schofield *et al.* 2013ab; Casale *et al.* 2013). Thus, Wallace *et al.* (2010, 2011) grouped all species of sea turtles globally into regional management units (RMUs), which are geographically distinct population segments, to determine the population status and threat level. RMUs may be restricted to a single isolated nesting population or may encompass several nesting populations and are analogous to evolutionarily significant units as defined by Moritz *et al.* (1995). These regional population units are used to assimilate biogeographical information (i.e. genetics, distribution, movement, demography) of sea turtle nesting sites, providing a spatial basis for assessing management challenges. The Mediterranean has been divided into two RMUs for loggerheads and 1 RMU for green turtles (Wallace *et al.* 2010, 2011), which are assumed to reflect genetically-based population units based on nDNA (inherited nuclear DNA of males).

Ultimately, due to the complex structure of sea turtle populations it is not possible for a single organisation to collect information on all life stages of animals originating from a single nesting area, as such individuals may be dispersed across multiple countries. Yet, knowledge about the age structure, spatial distribution and genetic diversity of a population facilitates the development of sophisticated models (with lower uncertainty) to assess current and future population trends for the implementation of appropriate conservation actions that are viable for future management, which are addressed in the '*Standardization of methodologies to estimate demographic parameters for population analysis, such as population modelling*'.

The collection of data in a consistent manner by coordinated programs and opportunistic sampling (e.g. strandings) would facilitate the combination of information at a Mediterranean, particularly if genetic samples were consistently collected across all surveys involving the capture of individuals (beach and sea). This would require the establishment of accessible archives, potentially including access to means of storing and analysing collected tissue samples beyond a national level to connect turtles from the same nesting area that frequent different foraging and wintering areas.

This guideline seeks to address these issues by providing baseline suggestions on how to standardise data collected throughout the Mediterranean region for the improved protection of loggerhead and green sea turtle habitat.

3. Monitoring of Nesting Areas

The Mediterranean hosts two sea turtle species, the loggerhead turtle (*Caretta caretta*) and the green turtle (*Chelonia mydas*), which breed in this region. Despite significant advances in recent years, there are still significant knowledge gaps that preclude effective evidence-based conservation at a regional scale.

In brief, over 100 loggerhead nesting sites around the Mediterranean have scattered (i.e. intermittent) to stable (i.e. every year) nesting (Halpin *et al.* 2009; Kot *et al.* 2013; SWOT 2006a, 2006b, 2008, 2009, 2010, 2011, 2012), of which just 13 sites support more than 100 nests each (Casale and Margaritoulis 2010). Greece and Turkey alone represent more than 75% of the nesting effort in the Mediterranean; for details on nest numbers at the different sites in the Mediterranean see Casale and Margaritoulis (2010). Most sites are located in the eastern and central basins of the Mediterranean. Sporadic to regular nesting has been recorded in Cyprus, Egypt, Greece, Israel, Italy, Lebanon, Libya, Malta, Syria, Tunisia and Turkey (Margaritoulis *et al.* 2003; Casale and Margaritoulis 2010). Surveys have been conducted for tracks in Algeria (last surveyed 1980s), Croatia (last surveyed 1990s), France (last surveyed 1990s), Morocco (last surveyed 1980s) and Spain (last surveyed 1990s) (Margaritoulis *et al.* 2003; Casale and Margaritoulis 2010). Information on nesting has not been gathered for Albania, Montenegro, Monaco, Slovenia or Bosnia (Margaritoulis *et al.* 2003; Casale and Margaritoulis 2010). A recent IUCN analysis suggests that, when all loggerhead nesting sites in the Mediterranean are considered together, the geographic distribution of loggerheads in the Mediterranean is broad, and the species is considered of Least Concern (LC) under current IUCN Red List criteria, though conservation dependent (Casale 2015). An average of 7200 nests are made per year across all sites (Casale and Margaritoulis 2010), which are estimated to be made by 2,280–2,787 females assuming 2 or 3 clutches per female (Broderick *et al.* 2002).

Most green turtle nests (99%) are laid in Turkey, Cyprus and Syria, with the remainder being found in Lebanon, Israel and Egypt (Kasparek *et al.* 2001; Casale and Margaritoulis 2010). Out of 30 documented sites, just six host more than 100 nests per season (Stokes *et al.* 2014), with a maximum of just over 200 nests at two sites (both in Turkey). The five key nesting beaches include: Akyatan, Samadağ, Kazanli (Turkey), Latakia (Syria) and Alagadi (northern Cyprus), with Ronnas Bay also being a priority area (Stokes *et al.* 2015). For details on nest numbers at the different sites in the Mediterranean see Stokes *et al.* (2015). An average of 1500 nests are documented each year (range 350 to 1750 nests), from which an annual nesting population of around 339–360 females has been estimated assuming two to three clutches (Broderick *et al.* 2002). Unlike loggerheads, green turtles globally strong exhibit interannual fluctuations in the number of nests, which has been associated with annual changes in forage resource availability (Broderick *et al.* 2001). Consequently, our knowledge about the population dynamics of green turtles in the Mediterranean remains insufficient.

The nest numbers and/or counts of female sea turtles are often used to infer population trends and associated extinction risk, because counts of individuals in the sea or when nesting on (often) remote beaches is tricky. Estimates of sea turtle abundance are obtained from foot patrols on nesting beaches counting either the number of females (usually during the peak 2-3 weeks of nesting) and/or their nests (Limpus 2005; Katselidis *et al.* 2013; Whiting *et al.* 2013, 2014; Pfaller *et al.* 2013; Hays *et al.* 2014). However, females may not be detected by foot patrols because they do not all initiate and end nesting at the same time and might not nest on the same beach or section of beach within or across seasons; consequently, monitoring effort could fail to detect turtles or miss them altogether on unpatrolled beaches. Consequently, it is assumed that females lay two (Broderick *et al.* 2001), three (Zbinden *et al.* 2007a; Schofield *et al.* 2013a) or at least four clutches (Zbinden *et al.* 2007a; in this case transmitters were attached on the nesting beaches at least 2 weeks after the onset of nesting), depending on the beach being assessed in the Mediterranean. High environmental variability leads to overestimates of female population size in warmer years and under-estimates in cooler years (Hays *et al.* 2002). This is because sea turtles are ectotherms, with environmental conditions, such as sea temperature and forage resource availability, influencing the seasonality and timing of reproduction (Hays *et al.* 2002; Broderick *et al.* 2001, 2003; Fuentes *et al.* 2011; Schofield *et al.* 2009a; Hamann *et al.* 2010; Limpus 2005). As a result, concerns have been raised about the reliability of using nest counts of females alone to infer sea turtle population trends (Pfaller *et al.* 2013; Whiting *et al.* 2013, 2014). Furthermore, in light of global climate change, shifts in nesting area use and the location of key sites need to be monitored (Almpanidou *et al.* 2016). Thus, these issues should be addressed in the '*Standardization of methodologies to estimate demographic parameters for population analysis, such as population modelling*'.

Another well-established parameter is the emergence success rate of hatchlings from the nests, along with the offspring sex ratios at hatching. Globally, highly female-biased offspring sex ratios have been predicted (Witt *et al.* 2010; Hays *et al.* 2014). This high female bias is of concern because sea turtles exhibit temperature dependent sex determination, with the warming climate ultimately leading to even more biased female production (Poloczanska *et al.* 2009; Saba *et al.* 2012; Katselidis *et al.* 2012). Thus, it is essential to determine how the offspring sex ratio transforms into the adult sex ratio, to determine the minimum number of males needed to keep a population viable and genetically healthy, which are not necessarily the same. Because males tend to breed more frequently than females (i.e. every 1-2 years versus 2 or more years by females; Casale *et al.* 2013; Hays *et al.* 2014), fewer males might be needed in the population to mate with all females. However, biased sex ratios can induce deleterious genetic effects within populations with a decline in the effective population size and increase the odds of inbreeding and random genetic drift (Bowen and Karl 2007; Girondot *et al.* 2004; Mitchell *et al.* 2010). However, most sea turtle populations exhibit high multiple paternity (i.e. the eggs of individual females are fathered by multiple males; for review see Lee et al. in submission). This behaviour is considered to be a strategy to enhance genetic diversity; thus, if male numbers further declined, this could have deleterious effects on the population (Girondot *et al.* 2004). Furthermore, differences in survival between the sexes might occur in different age classes (Sprogis *et al.* 2016); thus, it is essential to quantify sex ratios and sex-specific mortality across the different size/age classes.

3.1. Identification and Evaluation of Nesting Areas

Every 5 years (following the suggestion of the Demography Working Group, 2015), standardized surveys of all sandy beaches should be conducted uniformly throughout the Mediterranean region to delineate all areas with nesting activity and record shifts in area use over time due to different pressures.

3.1.1. Essential: Existing and Potential Nesting Beach Characterization

A database of all existing and potential nesting beaches present in each country should be assimilated during May-June (following winter storms) of the survey year. Beach-based surveys with hand-held GPS units combined with aerial surveys using Unmanned Aerial Vehicles (UAVs) or small aeroplanes should be used to map the beach structure, as well as regular (permanent / semi-permanent) environmental and anthropogenic features on the sea turtle nesting beaches. With UAVs or small aeroplanes, overlapping photographs are taken, 3d models and orthorectified images can be generated, from which beach characteristics can be quantified, allowing changes in structure at 5-year intervals to be delineated (Allen *et al.* 2015). On the beaches, transects can be delineated at regular intervals (100-500 m) from the vegetation to the sea. At 5 m intervals (depending on beach width), sand compaction and moisture can be measured with the appropriate instruments (meters). If it is not possible to obtain 3D maps using UAVs/plane surveys, then theodolite measurements should be taken at 5 m intervals down each transect. In particular, the presence of steep/scarp slopes/changes in slope and the presence of running or seasonal (i.e. dry out in summer) riverbeds should be documented (as these deter nesting). Sand samples should be collected from 50 cm depth at 1 m elevation (representing average nest depth and nest location on the beach), and analysed for: particle composition (i.e. sand, clay and silt) calcium carbonate content, organic content and pH levels. This information will provide a baseline to identify changes in composition and their source (anthropogenic or environmentally oriented).

The distribution of roots from vegetation adjacent or on the beach can be assessed (and correlated with encroachment in hatched nests too) by digging to 50 cm depth in the sand at 5 m intervals from vegetation (i.e. at 5, 10, 15, 20 m etc., as often fine roots are the most dangerous to nests and occur at the furthest points from trees/shrubs). The presence of predators (dogs, cats, rats, seabirds, foxes etc.) on the beach can be recorded by direct observation and the documentation of tracks during 5-yearly surveys. The presence of development, including roads, tracks and walls, lamp posts, along the back of beaches should be documented (GPS) annually, along with any changes. Light pollution sources should be documented, including recording the GPS range of direct lighting backing beaches and that of sky glow, during cloudless and moonless nights. Light meters could be operated on cloudless and moonless nights to gauge the level of light pollution at regular intervals (e.g. 100 m) along the beach.

For every single measurement location or sampling collection point, the GPS position should be recorded, so as to allow repeatability across surveys and feed all information on a GIS database. This information can then be collated with that of sea turtle emergence and nesting activity of the survey year to determine any trends. In addition, this information can be compared with the one collected 5-years previously to document changes to the status of the beach, and whether changes to the status of nesting have occurred in parallel.

3.1.2. Essential: Aerial Surveys

Aerial surveys should be used to record the presence/absence of turtle tracks, and if possible nests, at 1 km intervals along surveyed beaches. The aerial counts should be calibrated to ground checks at certain intervals (or where accessible), which could also be used to clarify species (green turtle versus loggerhead turtle) based on track and nest morphology. Plane surveys should be conducted by collaborating organisations, which will need to be organised to achieve the uniform coverage of key sections of coastline (delineated by **Section 3.1.1**) in each country. This can be revised/added depending on opportunistic sightings or potential gaps that become visible in repeated analyses over time. UAV surveys could be used in lieu of or in parallel to plane surveys, but may be more time consuming. Surveys should be conducted between 20 June and 20 July (period of peak nesting in the Mediterranean); twice if possible.

Aerial surveys could be done using planes or UAVs. During surveys, counts of all tracks should be made by two people independently (regardless of tide line, as these surveys are not regular surveys aimed at obtaining total track/nest counts). Video footage or overlapping photographic images allow the validation, and where necessary, recount of tracks, particularly in areas with high levels of activity. This post-hoc method also minimises observer error that might otherwise result from surveyor fatigue. The aerial counts are typically calibrated to ground counts at certain intervals (or where accessible), which is important to validate activity and species (green turtle versus loggerhead turtle) based on track and nest morphology.

Aerial surveys could be conducted using light aircraft (Cessna 182 and 202) travelling at speeds of 180–190 km/h at an altitude of 15–100 m; the lower the altitude the better the chances for detection and identification of nests; (Witt *et al.* 2009; Davis and Whiting 1977), but if photographic/video images are being collected, a height that encompasses the entire beach width is advised. During each survey the position of the aircraft should be recorded using two global positioning system (GPS) hand-held receivers (Witt *et al.* 2009). Ideally, surveys should be conducted after sunrise and before 10:00 (local time), so that a low sun angle would aid track detection for observing turtle tracks (crawls) over beaches (Davis and Whiting 1977; Schroeder and Murphy, 1999).

Ideally, UAV surveys should be conducted following a similar altitude delineation to that used by planes (i.e. 15 to 100 m) to allow comparability. Note that nests/aborted attempts can be detected at 30 m, while tracks alone can be detected above this. Thus, the height flown on each beach will be ultimately decided based on the width of the beach, with multiple sweeps at different heights being potentially required for wide beaches to record full tracks (higher altitude) versus validation of nests (lower altitude). As with plane surveys, UAV surveys should be conducted after sunrise and before 10:00 (local time), so that a low sun angle aids track detection for observing turtle tracks (crawls) over beaches. Note also that, commercially available UAVs are affordable (e.g. equivalent cost of a single satellite transmitter, and becoming cheaper).

Plane surveys can cover more area than UAV surveys at a single time, whereas UAVs can fly sufficiently low enough to allow good track identification (including the capacity to hover over tracks of interest). For some countries, sufficient funding might not be available for aerial surveys, or national security risks may exist. If aerial surveys are not possible, then coordinated foot patrols, with checks at intermittent 500 m intervals and the use of binoculars/telescopes or raised land to survey wider areas (this suggestion is not supported, and could be revised based on the analysis of aerial survey data) along beaches.

All plane/UAV survey transect lines should be recorded on GPS and fed into a GIS system. All tracks and nests should be identified, with corresponding GPS data points. All locations that are checked on foot to validate aerials surveys must have the GPS location recorded, along with validation of whether the detected nesting species was a loggerhead or green turtle. Depending on the capacity of different countries, if only 500 m checks of presence/absence are possible on foot in some regions; then the aerial data must be adjusted to be comparable for general overviews. However, the detailed information can be retained for nationwide specific analyses or future analyses potentially requiring this level of detail. See **Section 3.1.4** for more details.

3.1.3. Preferred: Trial of Satellite Images

High resolution remote sensing imagery could be used to survey all beaches throughout the Mediterranean. A single central body could then confirm that collaborating organisations are focusing their resources on the correct sites, or redirect them to other sites. In 2017, the European Space Agency (ESA) is launching the Sentinel-2B satellite (https://sentinel.esa.int/web/sentinel/home), which is targeted towards facilitating environmental surveys. It is advised that ESA is contacted to collaborate/coordinate the use of satellite imagery for sea turtle nesting surveys. This approach will facilitate an unbiased way of monitoring all beaches uniformly and in a standardised way, providing a base map on which to add more detailed layers compiled by collaborating organisations. This approach will overcome differences in resource availability of different countries, in parallel to allowing different levels of information to be collected from different sites objectively. This approach could be used to redefine nesting area conservation priorities for the sea turtle population in the Mediterranean Sea.

3.1.4. Mediterranean-wide Nesting Area Evaluation Procedure

These 5-year surveys represent a type of sampling termed “one-time sampling,” describing presence/absence records made during a short visit to a nesting area. Such sampling is used to determine presence and absence and approximate abundance. These surveys would help buffer temporal sampling biases of index beaches.

Information should be fed into a GIS platform including: (1) date range of surveys, (2) coastline covered by surveys (foot patrols, plane surveys, UAV surveys, calibration with ground surveys at designated locations, etc.) as GPS data points/ranges, (3) distribution of tracks (GPS points per track), plus the number of tracks recorded for every 500 m section of beach on the single survey day, (4) the minimum number of nests detected, if nests can be identified; (5) locations where calibration was conducted and where nests were confirmed to be by green or loggerhead turtles. (3) and (4) will reflect the likely distribution of tracks and nests in surveyed areas, and could be extrapolated to predict actual seasonal nest counts of surveyed areas, with careful calibration at sites with known ground data.

The GIS database will provide an overview of the entire Mediterranean coast showing the beaches used by and suitable for sea turtle nesting. It will be developed following the provisions of the UNEP(DEPI)/MED IG.22/Inf.7, the Integrated Monitoring and Assessment Programme (IMAP) and the Common Indicators factsheets. Updating this database every 5 years, will allow the detection of spatial and temporal shifts in nesting habitat use, which, when combined with information on beach characteristics, will help improve our understanding on the relationship between human activities (which may be causing adverse pressures on the environment) and the environment, including biodiversity. Overlapping maps in a GIS will help give a holistic visualization of the assessment area, the anthropogenic pressures acting upon it and locations of current monitoring programs. This will enable informed decision making on how to prioritise the areas considered for monitoring. Finally, this database will highlight areas where the 5-year monitoring has been missed but where nesting might be occurred, requiring action by the collaborating organisations. It will also highlight areas where more regular, annual, monitoring may need to be initiated. The open free data policy of IMAP will facilitate easy access, allowing experts from different fields of research to access the database and evaluate anthropogenic impacts.

3.2. Standardisation of Monitoring on Nesting Beaches

Standardised beach monitoring should be continued at already monitored areas, and adjusted to ensure the minimum (**Section 3.2.1** and **3.2.2**) consistent across all areas. In addition, key areas identified from **Section 3.1** should be included. Furthermore, the selected sites for standardised monitoring should be assessed to ensure they cover all potential characteristics (i.e. different ranges of nest numbers) that are required for demographic models (see *'Standardization of methodologies to estimate demographic parameters for population dynamics analysis, such as population modelling'*) based only on track/nest parameters, with the inclusion of additional sites to meet these requirements. All sites should achieve "**Section 3.2.1** and **3.2.2**" as a baseline monitoring requirement, with the additional levels implemented being dependent on financial, equipment, personnel, and national security limitations.

3.2.1. Essential: Monitoring nesting and hatching activity

In the Mediterranean, most sea turtles nest between late May and early August, with occasional nests in April and September at some sites. The hatching period generally extends from 42 to around 70 days after this (depending on sand composition, sand temperature and season). Thus, it is recommended that once weekly surveys are initiated from 15 April (and shifting 5-days earlier the subsequent year when/if activity occurs before this date), with daily surveys being conducted from 15 May until 15 August (allowing adjustment for beach specific variation), with once weekly checks after this, in case of late season nesting activity (and again revised based on the site and activity levels). Hatching activity should be daily checked for from 1 July until 1 October (allowing adjustment for beach specific variation), and once weekly until 1 November (allowing adjustment for beach specific variation).

To ensure the accurate prediction of nests (visually, without requiring to dig nests and change nest topography), it is advised that a core team of trained and validated personnel (at 15-day intervals throughout each season per person) are involved in the monitoring activities. Validation should take the form of confirming nests that were not tampered with, through night observation, quantifying the level of accuracy of each person. These trained and regularly re-validated persons can be supported by volunteers. Assessment of female turtle abundance on nesting beaches may be based on counts of eggs, tracks, nests, and/or nesting females. Counts of all tracks and potential nests should be made. Surveys can be done on foot or by using aerial techniques (UAVs/planes), combined with calibration on the ground at regular 15 day intervals, when there is extensive population range, discontinuous beaches, and few personnel.

Aerial surveys

See **Section 3.1** for details on the suggested technology, methodology and flight altitude. Where multiple surveys are conducted (i.e. daily), only tracks visible below the most recent high tide line each day should be recorded to count only recent activity (i.e., around <12 h old; Witt *et al.* 2009). For key sites where it is not possible to monitor beaches that have been detected by **Section 3.1** by any possible means on the ground or through aerial surveys, periodic (i.e. once weekly) satellite images (or more frequent, if possible) could be collected to provide comparable information on track (and possibly nest) counts in these areas. This will facilitate objective demographic counts of adult females at the Mediterranean scale.

Foot patrols

For foot patrols, the GPS locations of track apex (highest point of a turtle track), failed nesting attempts and nests should be collected, which can then be transferred to GIS layers. This information shows the area use of beach and shift in use over time. By combining GIS layers on environmental and anthropogenic parameters at the 5-year level or annually (as suggested below), correlations in how nesting characteristics change in response to these parameters can be evaluated, following the provisions of the UNEP(DEPI)/MED IG.22/Inf.7, the IMAP and the Common Indicators factsheets.

Surveys should be initiated from the same location. The official weather status should be obtained from the nearest weather station. The fresh tracks (emergences) of adult female turtles from the previous night (or previous 2 nights, depending on regularity of surveys) should be recorded. All adult female emergences should be counted, recorded and classified by the morphology of the track as ‘nesting’ or ‘non-nesting’. Non-nesting tracks should be classified as (1) ‘false crawls’ – where no nesting attempt was made, (2) ‘failed nesting attempts’, where the turtle began clearing sand in a “swim” or “body pit”, or proceeded to the digging an “egg chamber” but did not complete nesting (i.e. the turtle crawl was interrupted). The reasons for failure, such as the presence of obstructions like stones, roots or dry sand causing the hole to collapse, should be recorded where possible. The presence of scarp slopes or other obstacles preventing movement up the beach should also be recorded. For published guides see: Eckert *et al.* (1999); SWOT (2011).

In brief, the data collected provided information on:

a. The ‘nesting success’ i.e. how many emergences resulted in egg-laying.
b. Nest location with respect to distance from the sea.
c. The spatial distribution of nesting on each beach and across the rookery
d. Nesting densities per beach and across the rookery
e. Reasons for failed nesting attempts.

On observing a turtle track, the skilled personnel should:

a. identify the up-track
b. follow the track from start to finish
c. record the track events:
    - false crawl (i.e. no evidence of failed nesting attempts or nesting)
    - failed nesting attempts (FNA) and specify, swim, body pit, egg chamber
    - nest – specifying whether it is a confirmed nest (i.e. egg chamber location found for caging or relocation, or marked during night survey) or an estimated/possible nest (i.e. egg chamber not found)
d. record evidence of nest predation activity and/or damage by human activity

Wherever possible the reasons for false crawls (i.e. hit wall, hit dune, unknown) or failed nesting attempts (i.e. hit stones, hit roots, unknown) were recorded. A GPS should be used to record:

a. the apex (highest point of each track)
b. the central point of every swim, body pit or egg chamber
c. the estimated or accurate nest egg chamber location (for relocated nests the original egg chamber and new egg chamber GPS location were recorded).

Additional measurements recorded for nest locations include:

a. The distance of the nest to permanent poles placed at the back of the beach
b. The distance of the nest to the sea
c. The elevation of nest above sea level and in relation to fixed points at the back of the beach (using a theodolite or, if available, a highly accurate hand-held GPS system, or by deriving it from photogrammetry from UAV or small plane surveys; Katselidis *et al.* 2013; Ierodiaconou 2016).

If nests are relocated by a program, the original and new nest GPS coordinates need to be made freely available. At the end of each morning survey the following parameters were recorded:

a. The total number of estimated new nests (confirmed and estimated combined) laid that morning
b. The number of total estimated nests (confirmed and estimated combined) laid on each beach
c. The last laid nest code recorded for each beach
d. Evidence of predation activity
e. Evidence of inappropriate human beach use

Table 1: Methods and data for monitoring nesting and hatching activity

Data collected / recorded
identify & record: 1. false crawls 2. failed nesting attempts 3. nests (confirmed or estimated) 4. evidence of nest predation activity 5. the reasons for false crawls (i.e. hit wall, hit dune, unknown) or failed nesting attempts 6. the apex (highest point of each track) 7. the central point of all failed nesting attempts (e.g. swim, body pit or egg chamber) 8. the reasons for failed nesting attempts (dry sand, roots, stones etc) 9. estimated or accurate nest egg chamber location 10. distance of the nest to permanent markers placed at the back of the beach (if present) 11. distance of the nest to the sea 12. distance of the nest to vegetation 13. elevation of nest above sea level
Methods
Foot patrols Aerial surveys (for 3,4, 9, 10, 11*)

3.2.2. Essential: Hatched nest excavations

Surveys of beach hatching activity should be conducted every morning from mid-July onwards and initiated from the same point. The official weather status should be obtained from a weather station based in the field.

At least 30% of all nests should ideally be excavated, with a random cross section across locations and time. Excavations should only be conducted by trained personnel, whose ability to assess the nest contents is regularly re-validated (every 2 weeks), to ensure that data collection is consistent over time and across personnel. Gloves must be worn to avoid the risk of disease. Nest excavations should be conducted at most 10 days after first hatching to ensure the contents are recognisable. Developing from the guidelines in Eckert *et al.* (1999), it is recommended that all eggs are removed from the nest and all empty egg shells (i.e. hatched) are separated from unopened eggs. The technique used to designate empty egg shells representing ‘one’ egg by counting a group of pieces that together visually appeared to comprise 100% of the total egg shell, should be used. With respect to all unopened eggs, the personnel should count eggs before opening. On opening each egg, the presence of an embryo should be recorded and whether it is early (<30% of content), mid (30-60% of content) or late (>60% of content) stage, along with whether it is alive or dead. All live and dead hatchlings found in the nest or emerging from the nest should be recorded. If dead hatchlings are no longer intact, the heads should be counted to obtain an estimate of numbers. All eggs and dead hatchlings should be returned to the nest following the procedure. Live hatchling should be released on the beach to crawl to the sea after dark (or based on local environmental conditions).

The number of hatched eggs, dead and live hatchings in the nest, unhatched eggs and status of the eggs should be documented. This provides information on hatching and emergence success; if the nest has an identifier, it can be linked back to the date of nesting and the specific female that laid the nest (if observed for tagging); this information could be used to assess the fitness of individual females in relation to nest output (and could be combined with the number of emergences required before successful nesting by the same individual). Fecundity is calculated in age-specific birth/hatch rates, which may be expressed as the number of births per unit of time, the number of births/hatchlings per female per unit of time, or the number of births/hatchlings per individuals per unit of time.

In brief, the data collected should provide information on:

a. The number of laid nests that hatched
b. The incubation period for caged/relocated nests (i.e. time lapsed from egg laying to emergence of the first hatchling)
c. The hatching success of the nest (i.e. the number of eggs that hatched in the nest) derived from hatched nests only at excavation
d. The hatchling emergence success rates (i.e. the number of hatchlings that made it out of the nest onto the beach) derived from hatched nests only at excavation
e. Parameters that may inhibit egg development or inhibit hatchling emergence from the nest observed during excavations.

On observing a hatching track, personnel should:

a. Identify the nest emergence site by following the tracks to their source
b. Record the GPS location of the nest emergence site
c. Record the distance of the nest to the permanent marker poles and sea
d. Record the approximate number of hatchling tracks (i.e. 1,2,3…8,9,10, 10-20, 20+/mass)
e. Record the orientation of hatchlings from the nest to the sea using a compass including widest track angle, modal track angle, shortest angle to the sea (see **Section 3.5.4** below) – and the widest track angle points will be recorded on GPS units
f. In cases of mis/disorientation the suspected light source should be recorded (and/or the beach was returned to at night to confirm source) – the GPS location of these tracks should be recorded
g. Record the number of live hatchlings observed on the beach (naturally – i.e. not aided out of the nest prematurely) during (and when possible after) morning survey
h. Record evidence of nest and/or hatchling predation activity on the beach and within 300 m of shore; this included (a) nest digging – successful/unsuccessful; (b) circumstantial evidence of hatchling track termination with predator tracks in the soft dry sand (tracks were too faint in salt encrusted and wet sand); (c) observed actual predation attempts on the beach and/or sea with recorded successful/possible/failed outcomes; (d) evidence from tracks of escaped hatchlings – i.e. track leading to sea but not initiating at a nest; (e) washed up hatchlings without innards – possible predation from polychaetes.
i. Record the number of desiccated hatchlings found on the beach.

Hatchling orientation from the nest to the sea is measured for a sample of nests on all beaches. A minimum of 20 tracks had to be present, and the following parameters are staked out using sticks at a distance of 7 m from the nest, to record using a compass:

a. main-track angle; angular range in which most of the tracks are included
b. modal-direction; the direction to the sea followed by the majority of hatchlings
c. ocean-direction; the most direct angle to the sea
d. the number of loopings/outliers.

Witherington and Martin's (2000) method to confirm mis/disorientation when (a) angular range is >90º and (b) angular range between modal and ocean direction >30º should be used. This information should provide a basis from which the current situation of hatchling sea finding process can be evaluated. This analysis should be assessed in combination with light-pollution survey information to determine if there is a correlation between light pollution associated with coastal development and the disruption of hatchling sea-finding ability. Whether disorientation patterns overlap with natural/geographical characteristics should also be considered. Hatchlings may also be disorientated by the sun or moon. Weather information was taken into consideration as low air temperatures may result in high levels of looping, disorientation and the presence of weak hatchlings on the beach following sunrise. On mornings where similar lethargic and mass looping/disorientated track patterns are recorded on more than 1 beach – the data will not be included in this analysis.

Table 2: Methods and data collected for hatched nest excavations

Data collected / recorded
identify & record: 1. number of laid nests that hatched 2. incubation period for caged/relocated nests 3. hatching success of the nest 4. hatchling emergence success rates 5. parameters that may inhibit egg development or inhibit hatchling emergence 6. location of the nest emergence site 7. distance of the nest to the permanent markers, sea and vegetation 8. approximate number of hatchling tracks 9. orientation of hatchlings 10. suspected light source 11. number of live hatchlings 12. evidence of nest and/or hatchling predation activity 13. desiccated hatchlings
Methods
Foot patrols Aerial surveys (1,6, 7, 10, 11, 12)

During periods of heavy rain during hatching, surveys should be adjusted to detect hatching activity based on the presence of holes in the beach (tracks are not visible). In order to increase the chances of locating such holes and hence to not miss recording hatched nests, known nests should be mapped out and marked at this time pending hatching with bamboo sticks. Personnel should also check the entire beach systematically for holes of unmapped nests which were missed during the egg laying period.

It is important to validate that a nest documented at egg laying is the same as that documented at hatching and excavation to investigate certain demographic parameters, linking an identified female through to the emergence success of its nests. For all nests for which the GPS location is recorded at laying (or estimated the morning after), the nest can be matched by the GPS location at hatching. Some nests may be caged, while this can be validated for other nests in which identifiers are placed during egg laying.

3.2.3. Preferred: Identification of individual nesting females

For organisations that have personnel available to implement a comprehensive tagging/identification program, all tagging programs should use a combination of at least two techniques, three where possible. For instance, internal passive integrated transponder (PIT, i.e. an internal microchip) tags should be prioritized and combined with external flipper tags, but also complemented with photo-identification or genetic fingerprinting to ensure long-term records of unique individuals are built, which is essential for accurate demographic models.

Night surveys should be conducted during the prime nesting period and should be stopped as soon as hatching activity begins (due to risk of trampling nests that are about to hatch, leading to crushed hatchings detected during excavations). Surveys can be conducted from sunset to sunrise (or for shorter periods). The beach is patrolled along the water's edge searching for turtles. On observing a turtle, the researcher crawls behind the turtle to determine the stage of emergence activity. On completion of nesting the researcher:

a. Places a marker in the nest for matching at hatching and records GPS location of nest
b. Checks for an existing PIT tag
c. insert a PIT into the flesh.
d. records existing external tag information
e. attaches an external tag
f. photographs the top of the head and sides of the head
g. morphometric measurements are taken
h. body mass is obtained, where possible

Morphometric measurements (i.e. body measurements of curved and straight carapace lengths and widths) should be taken, if possible (Eckert *et al.* 1999; Georges *et al.* 2006). Ideally, one standard ID external tag type and model should be used for all turtles released into the Mediterranean, along with a standard type and model for PIT tags, including the location of PIT tag application, to ensure that all PIT tags are checked for in the correct body location and that all recorders are able to read all PIT tags. The country and rescue centre details, progressive ID number assigned to each animal, address and phone number of the central organization (e.g. RAC/SPA) should be indicated on the external and PIT tags. A central database should be established on which all applied external and PIT tags are recorded. This approach will allow clear and fast identification and classification of Mediterranean marine turtles. A similar approach should be used for potential future DNA fingerprinting and photo-id records.

If the DNA fingerprinting of individual females becomes possible (Peare and Parker 1996; Jeffreys 2005, Bowen and Karl 2007; NRC 2010), this could be used as an alternative tagging technique, improving estimates of remigration interval and clutch frequency. A tissue voucher should be collected from each female, male and juvenile, as well as from nests and hatchings for future collective analysis. See **Section 3.2.4** for more details.

Photo-identification has also been proven effective as a complementary tool (Dutton *et al.* 2005; Stokes *et al.* 2015), with the nesting areas in the Mediterranean supporting sufficiently small numbers of turtles (i.e. within 1000 max at a given site) to promote this technique.

It is recommended that photographs are taken of the top of the head, and when possible the left and right sides. A red light could be used without flash (no light is necessary on the full moon) – many cameras have this function. A good modern camera with low noise in high ISOs is also an asset (full frame DSLRs are perfect, though a bit expensive), accompanied with bright lenses (f1.4 or f2.0) and manual or autofocus focus. Note that sand may need to be gently removed from the facial area. This technique would also facilitate the collection of records of the majority emerging turtles as they return to the sea (regardless of nesting activity), which would facilitate record saturation in the central nesting period. Photo-id is an extremely useful tool facilitating the acquisition of long-term records on longevity of nesting, remigration intervals and recruitment into the population. A combination of manual and automated identification is now possible, with several automated programs existing (PITMAR http://www.pitmar.net/index.php/en/, I3S http://www.reijns.com/i3s/, among others).

While some tagging programs may have personnel to operate on all nights throughout the nesting period, all programs should focus on saturation tagging during the central nesting period (e.g. around 25 June to 10 July) when all individuals of the population may be present (complemented with photographing every emerging turtle if possible) (e.g. Dutton *et al.* 2005; Pfaller *et al.* 2013; Whiting *et al.* 2013, 2014). On these dates, multiple teams should focus on targeting areas supporting >50% of activity, and on tagging nesting individuals throughout the night. Through this strategy, saturation of all nesting individuals is likely to occur (with some exceptions due to the use of more than one beach/area by some individuals), at which point, focus on less intensive areas could become more spread. As around 80% fidelity to specific nesting sites exists, this approach will facilitate the development of long-term records based on tagging, although assumptions of recruitment of previously untagged turtles into the population should be treated with caution, as they could be from other beaches.

The PIT tag is a microchip that is usually placed in the flesh, i.e. internally (e.g. Wyneken *et al.* 2010; Eckert *et al.* 1999). It can be placed in the shoulder, forelimbs or hind limbs, after initially checking with a reader that a PIT has not been previously placed; if it has, the existing PIT number should be recorded. Following a study on the potential migration of PIT tags placed in different locations of the body (Wyneken *et al.* 2010), it is recommended that the PIT tag is placed just above the second-third digit of the front flippers (one PIT should be placed in each flipper to reduce the risk of loss due to migration through the body or the tag falling out after being poorly inserted), following cleaning with iodine solution. This location is ideal as the turtle does not use the front flippers in the initial stage of camouflaging (so the fore-flippers are not moving), the turtle is minimally disturbed, and there is little or no blood flow. Following application, the flipper is again cleaned with iodine solution, and the tag is checked using the PIT tag reader.

3.2.4. Preferred: Blood/tissue sampling

For organisations that have the capacity (i.e. funds, equipment, skilled and available personnel), collect tissue and blood samples from random nesting females for genetics, health (contaminant or microbe), and stable isotope analyses. Sterile surgical gloves should always be worn.

Blood is usually collected from the cervical sinus of adults (Owens 1999; Jacobson 1999), after cleaning the site with ethanol prior to sampling. The site should be cleaned with 70% ethanol first. The sinus is on either side of the midline of the neck about 1/3 to 1/2 way toward the back of the head from the anterior edge of the carapace. Depending on the size of the turtle, the sinus is from 0.5-3 cm lateral to the midline. With practice the sample can be taken within 30 seconds. Lithium or sodium heparin is best for an anticoagulant. EDTA (also an anticoagulant) should be avoided since it causes haemolysis in sea turtle blood. It is important to position the turtle so that the sinus fills with blood. For this reason, consistent results have been obtained when the turtle’s head is lower than the body. An angled restraining rack, a slanted table or bench, or an inclined nesting beach (with assistants doing the restraining) all work well. Always carefully clean the neck with alcohol (containing at least 70% concentration of ethanol), or other antiseptic prior to sampling.

The most common solid tissue biopsied is the skin (Jacobson 1999). The biopsy site is treated with a local anaesthetic and then a surgical scrub (including ethanol and iodine). The sample can be obtained using a scalpel blade (#10 or #15) or a disposable biopsy punch. Subsequently, the site should be cleaned and then sutured or left to heal (Jacobson 1999). For histologic evaluation, a portion of each sample should be fixed in neutral buffered 10% formalin (NBF), with a tissue to fixative volume ratio of 1:10 within 24 hours and with ethanol beyond 48 hours. For microbial isolation, they should be cleaned with sterile saline and then placed in the specified transport media. Samples should never be frozen.

Ideally, this information should be assimilated by a central body, to which all countries should send the samples for analysis (requiring CITES permit). This will overcome issues of cost and expertise in some countries, and ensure all collected samples are analysed (rather than stored for use at some unspecified future date). In addition, tissue and/or blood samples can be collected from live and dead hatchlings. Skin is probably the best tissue type for stable isotope analysis (Seminoff *et al.* 2006; Reich *et al.* 2007; Vander Zanden *et al.* 2012), and can be sampled easily from both dead and live individuals and integrates diet over several months. In nesting areas, hatchlings and eggs could be sampled as proxies of samples from females.

This information can be used to confirm sex and infer multiple paternities of the nests (Zbinden *et al.* 2007b). To date, most genetics data are based on the use of mitochondrial haplotypes and nuclear microsatellites, which allow the individual assignment of loggerhead and green turtles to major nesting areas within the Atlantic (Carreras *et al.* 2011; Carreras *et al.* 2014). However, it has not been possible to the assignation to distinct populations within the Mediterranean. The use of SNPs is recommended to improve individual assignation (NRC 2010). See **Section 3.2.3** for sampling from adult females for genetic fingerprinting.

The stable isotope technique could be used to complement satellite tracking studies, to increase the sample size of monitored individuals and establish the proportions of turtles frequenting different general broad areas. Stable isotope analysis is an inexpensive method for mass monitoring in conjunction with telemetry (Zbinden *et al.* 2011; Cardona *et al.* 2014) and genetic information. Thus, the regular collection of tissue samples from adult females, adult males, and juveniles will allow the identification of the foraging grounds used by individuals from different nesting areas, as well as possible connectivity between foraging grounds. Stable isotope samples can be calibrated when used in combination with satellite telemetry. The stable isotopes of potential prey at different foraging grounds should be collected – this will help improve the level of accuracy of stable isotope information.

3.2.5. Preferred: Other beach-related parameters

For groups that have the resources, more detailed information in beach characteristics, including predation and human impact should be collected every year.

Sand temperature

It is recommended that temperature loggers are placed at 50 cm depth and 1 m elevation in all beaches used for nesting or potential use for nesting (e.g. Katselidis *et al.* 2012; Laloe *et al.* 2014), where possible 1 unit per 1 km section (more if possible), year-round. This will provide important baseline data on climatic changes to sand temperature and the capacity to support nesting activity. Temperature loggers are very low cost and highly accurate (Katselidis *et al.* 2012; Laloe *et al.* 2014). It is possible to infer offspring sex ratio from sand temperatures and incubation duration (e.g. Godley *et al.* 2001; Katselidis *et al.* 2012), which is relatively straight forward.

Beach structure

For details on various beach parameters that should be collected see **Section 3.1.1**. For organisations that have the resources, these measurements should be implemented annually, to monitor how various nesting characteristics vary with the environment or anthropogenic pressure (e.g. Katselidis *et al.* 2012, 2013). This would help identify potential issues with 5-year sampling that could be overcome via models.

3.3. Marine Habitat Monitoring Around Nesting Areas

The marine habitat at the key nesting areas delineated in **Section 3.2** should be monitored in parallel to obtain important information about the numbers of reproductively active adult males and the relative numbers of reproductively active adult males and females, which will also improve demographic surveys.

It is essential to determine the number of males that are present in a breeding population, as sea turtle offspring sex ratios are highly female skewed (Hays *et al.* 2014), and we do not know the minimum number of males required to maintain the genetic health of a breeding population. Already, the modelling of in-water photo–id surveys combined with information on the relative return rates of males (generally annually) and females (generally biannually) at the Zakynthos loggerhead population (one of the largest known populations in the Mediterranean), suggests that the area supports a minimum of 100 unique males (i.e. almost all individuals returning to breed annually) versus around 800 unique females (i.e. around 400 females breeding in a given year, and returning every other year in general) (Hays *et al.* 2010; Schofield *et al.* 2009b, 2010, 2013a). Thus, for nesting areas supporting far fewer breeding females, it is essential to determine the numbers of males, which could be just ones of individuals, potentially indicating limited genetic diversity at these sites.

A combination of aerial (planes or UAVs) and in-water (snorkel/dive) surveys are recommended to obtain information on the relative abundance of male and female turtles, mating activity and the numbers of unique individuals to improve the accuracy of population modelling.

3.3.1. Preferred: Aerial surveys

For organisations with sufficient resources, monitoring the marine area during the breeding period with UAVs is recommended over plane surveys to distinguish males from females (minimum 30 m altitude needed) and mating activity, as they can be conducted close to the sea surface (<10 m altitude) without disturbing turtles (Bevan *et al.* 2016), whereas plane surveys do disturb mating activity (Frick *et al.* 2000). UAV surveys should be run parallel to shore to reflect the general swimming direction of males searching for females (Schofield *et al.* 2006). However, plane surveys may be beneficial when large tracts of coast need to be checked initially (see **Section 3.1** on plane survey techniques), then shifting to the use of UAVs to distinguish males, females and mating activity once core aggregation areas have been delineated. It is possible to fly UAVs at a maximum of 2 km distance from the operator (with intermediate observers along the flight path to ensure continual line of sight). Hovering should not be implemented and UAVs should run at the same height and speed throughout the survey. Video or overlapping stills images should be collected during flight. The latter are preferred as they allow sufficient resolution for zooming on images to distinguish sex during post-survey analysis.

Within each country, aerial surveys of the breeding area should be conducted at a selection of sites with different levels of nesting activity (i.e. <10 nests, 10-50 nests; 50-100 nests; 100-500 nests; 500-1000 nests; 1000+ nests) to define the male versus female sex ratios within these different populations. At a minimum a single site should be selected as an example for each level and two if possible. At best, all sites should be monitored.

Mating activity occurs as early as March, peaking in late April/early May (Schofield *et al.* 2013a). Thus, surveys of males and mating activity should be run every 15 days in March and every 3-4 days from 15 April onwards through to 30 June. Most males depart breeding areas in late May, but some remain and/or are resident; thus, mating activity likely continues until late June, when the last females arrive at the breeding areas.

In general, mating activity primarily occurs in nearshore waters fronting the nesting beaches; if no mating activity is detected in front of nesting beaches, then exploratory surveys at 60 m altitude could be done with UAVs or planes along the coast and offshore at a given area. UAVs should be flown at 30 m altitude to detect surface and underwater mating activity, and tails of patrolling males (Bevan *et al.* 2016). When flying at 30 m altitude, transect lines should be separated by 50-100 m to obtain maximum coverage; at 60 m altitude, 100-200 m separations could be used to cover a wider area. The distance offshore selected should be at the point where turtles are no longer detected along transects. Thus, surveys may need to be repeated at greater distances initially before establishing the selected range for analysis. This distance will vary from site to site and may require the UAV to be operated from a boat at some locations. At regular intervals i.e. once every 1-2 weeks, the outer limits should be re-tested to ensure the turtle distribution remains within the selected limits. If, not, then these limits need to be revised again. During surveys at different altitudes, gaps or overlaps in transect lines must be taken into account, depending on the spacing selected. This spacing information should be incorporated into subsequent models (e.g. distance sampling).

In all surveys, weather conditions and sea conditions should be recorded. Weather conditions include cloud coverage, wind speed and wind direction (wind information is recorded on the UAV, but should be validated with a local weather station). The UAV should not be operated in strong winds or during rain/thunder. Sea conditions can be determined visually and from the UAV footage of known items on the seabed (e.g. rocks) over which the UAV consistently passes on pre-programmed transects. This can be used to determine the depth to which turtles could potentially be seen. Sea state, glare and glitter can be recorded on the post-flight analysis of the images. In waters where the sea is turbid (no underwater visibility), only surface activity will be detected; thus, these surveys can only be used as an indicator of surface individuals.

The analyses of the images will provide a minimum estimate of the number of males (and females if surveys are continued until 30 June) frequenting each nesting area, including seasonal changes in mating activity.

If satellite imagery (see **Section 4.1**) proves effective at identifying the presence/absence of turtles in the marine environment, it would be advisable to use this technique to make counts of mating activity at 1 week intervals from 15 April to 15 June at a selection of sites of low to high nesting activity to determine minimum numbers of males in each nesting area.

3.3.2. Preferred: Boat surveys

In areas with good sea conditions, in-water boat surveys, including snorkelling and diving surveys, should be conducted to obtain photographic records of males and females for unique identification. Counts of unique individual males can only be obtained from in-water surveys. Boat surveys should initially follow set transect lines, parallel or perpendicular to shore; once, key areas have been established, then in-water surveys in areas with expected high capture-recapture rates can be conducted.

By marking captured individuals, the capture-mark-recapture (CMR) method can be completed (Ehrhart and Ogren 1999; Limpus 1993). Models using CMR allows demographic structure and survivorship rates to be analysed (Ehrhart and Ogren 1999). CMR can be used to estimate regional population abundance more powerfully if it uses multiple capture sites (Ehrhart and Ogren 1999; NRC 2010). For instance, CMR data collection coordinated within a networked array of sites, including nesting beaches, would provide one of the most detailed and powerful datasets possible for assessments of sea-turtle abundance and for measurement of many important demographic rates (Ehrhart and Ogren 1999; Chaloupka and Limpus 2001; Bjorndal *et al.* 2005). Wider networking of capture sites allows a wider inclusion of turtle status, such as sex, genetic identity, size, physiological condition, breeding status, and geographic location.

Existing capture methods include the use of nets (for different times in different ways and of different sizes/types) (Ehrhart and Ogren 1999), as well as hand capture (rodeo) (Limpus 1993; Bjorndal *et al.* 2005), dip nets (Witherington, 2002), hoop nets (James and Mrosovsky 2004), and strike nets (Ehrhart and Ogren 1999).

At a minimum, transect surveys could simply be used to record the sightings of turtles, sex and size/age class where possible, recording the co-ordinates of all sighted turtles (Schofield *et al.* 2010). Photo-identification does not require the capture of individuals and can be achieved by researchers entering the water to take photos (Schofield *et al.* 2010), or from cameras deployed from the boat (including ROV, although cameras attached to the bottom of the boat can be sufficient in shallow areas) (Smolowitz *et al.* 2015).

If personnel are trained and have the appropriate equipment, turtles can be captured and tagged with PIT and external tags in addition to photo-id and the collection of blood/tissue samples for DNA fingerprinting, stable isotope and health information (see **Section 3.2.3-4**). Laparoscopic examination should also be made to distinguish adult males from adult females. Note that rodeo capture techniques or netting can be used. This approach can prove effective for saturation tagging the females in the population prior to the onset of nesting too.

3.3.3. Preferred: Attachment of satellite transmitters

Satellite tracking provides detailed information about the movements of individuals within a population, including breeding area use before and during mating/nesting, clutch frequency of individuals (i.e. number of nests laid by specific individuals), internesting period (duration between each nesting event), date of departure from breeding grounds, migration distance and time, identification of foraging and wintering sites, wintering/foraging site fidelity and/or the use of multiple sites, remigration intervals to breed (1-2 years in males and 1-3+ years for females, depending on foraging site and animal condition) and residency at breeding sites, prospecting of alternative (possibly future under climate change) nesting sites (for overview see Luschi and Casale 2014; Godley *et al.* 2008). This information would provide information of variability in internesting interval and clutch frequency between individuals within the same year and across years with different environmental conditions (Tucker 2010), particularly as the Mediterranean is a temperate breeding area for both loggerheads and greens and internesting interval and clutch frequency vary with local weather conditions. Existing research recommends that a minimum of 20–30 individuals (for populations of >100 individuals) are required for population-level inferences, while 50–100 individuals are required to address more complex issues of animal survival or home-range studies (Borger *et al.* 2006; Murray 2006; Lindberg & Walker 2007). This number is also likely to vary depending on environmental variables, range of resource requirements and sociality (i.e. solitary versus group living) of target species. However, it is difficult to attain these numbers, due to this technique being very expensive.

It is recommended that satellite transmitters (preferably GPS for high resolution movement information) are attached to 4 selected females each year over each 5-year period prior to the onset of nesting (i.e. using in-water capture techniques during the pre-nesting period in May) to record interannual variation in clutch frequency and improve estimating female numbers from nest counts. By attaching transmitters in May in the sea, it is guaranteed that the females have not yet nested and random females are selected. In contrast, by selecting females that emerge in the first week of nesting, the sample could be biased towards individuals that nest more frequently; although, this latter approach may be required in some areas where the sea conditions are not viable to boat-based work.

See **Section 3.3.2** for in-water capture techniques; however, the feasibility of in-water capture must be assessed at each site independently, as different issues exist (sea state, seabed depth, reefs, turbidity, exposure, availability of skilled personnel, other safety issues, etc.).

See **Section 4.2.4** for information on the parallel attachment of satellite transmitters in foraging areas.

Table 3: Methods and data collected for monitoring the marine habitat around nesting areas

<table>
<tr><td colspan="3">Aerial surveys</td></tr>
<tr><td>Tasks</td><td colspan="2">Data collected / recorded</td></tr>
<tr><td>Distinguish males from females
Mating activity</td><td colspan="2">Sighting
Sex
Size class
GPS location of all individuals</td></tr>
<tr><td colspan="3">Boat surveys</td></tr>
<tr><td>Methods</td><td>Tasks</td><td>Data collected / recorded</td></tr>
<tr><td>Onboard census
Diving surveys
Snorkelling surveys</td><td>Photo-identification
GPS location of all sighted individuals</td><td>Sighting
Sex
Size class</td></tr>
<tr><td>Capture-mark-recapture (CMR) method</td><td>PIT and external flipper tagging
Photo identification
Blood/tissue sampling
Laparoscopic examination
Census counts</td><td>Sighting
Sex
Size class
DNA fingerprinting
Stable isotope analysis
Health analysis</td></tr>
<tr><td colspan="3">Satellite tracking device attachment</td></tr>
<tr><td colspan="3">Data collected / recorded</td></tr>
<tr><td colspan="3">1. Distribution of individuals (overlay with bathymetry and habitat information)
2. Clutch frequency of females
3. Internesting period of females
4. Date of departure from breeding grounds
5. Migration timing, distance and duration
6. Identification of foraging and wintering sites
7. Wintering/foraging site fidelity and/or the use of multiple sites
8. Remigration intervals
9. Residency at breeding sites
10. Prospecting of alternative or future nesting sites.</td></tr>
</table>

3.4. Data requirements for demographic analyses

The methods used to calculate different aspects of population abundance and demography will provide a confidence interval, dependent on the level of confidence of the original census data. To reduce uncertainty in the original census/sampling data, it is important that the personnel obtaining the data have received proper training and are maintained over extensive periods. The field data collected on the nesting beaches and, where possible, in the marine environment around the breeding areas, will provide the baseline data required to calculate the index of population abundance, along with various other demographic parameters.

Track/nest/female counts

At present, estimates of population size (note that this is actually just female population size) at breeding areas are based on the number of females, number of nests and/or number of tracks (total nests vs total tracks = nest success rate), with appropriate modelling to extrapolate population numbers depending on the method used:

1. for total number of nesting sites, number of sites (n)
2. for average nesting site size, size of the nesting area versus number of females, number of nests or number of tracks, with appropriate modelling to extrapolate population numbers depending on the method used (i.e. to obtain density/km) (n)

Track counts have the greatest data requirements for estimating mature-female abundance, whereas counts of unique females during nesting (relying on external flipper tags, internal PIT tags, photo-id or genetic fingerprinting) have the fewest data requirements (NRC 2010). In each type of annual count, abundance estimates must account for nesting females that skip breeding seasons, which is a common trait in sea turtles. Horwitz–Thompson estimators can allow for the effect of skipped breeding on detection (Dutton *et al.* 2005) and have provided abundance estimates based on nesting-female counts over multiple nesting seasons. Modelling abundance, using uniquely identified nesting females, requires minimal additional data on reproductive rates, because these rates are measured as part of the method. Identification of nesting females over multiple nesting seasons allows mark–recapture rates to be modelled. How counts of females reflect abundance varies with detectability and availability of the item being counted, and with systematic error, such as misidentification due to lost tags (see NRC 2010; Dutton *et al.* 2005).

In brief, at discrete sampling locations and times, estimates of nesting-female abundance are often modelled by using an observation probability function, such as the Horvitz-Thompson estimator, a general estimator for a population total, which can be used for any probability sampling plan with or without replacement (Balazs and Chaloupka 2006), or other estimators of population totals used for varied sampling plans and encounter probabilities. The models include covariates (i.e. two or more random variables that exhibit correlated variation) that describe how available a nesting turtle is for being counted, given a specified measure of effort. Effort often varies across a time series due to changes in personnel or other factors. When counts are collected as an index (standardized locations and season) and a fine spatiotemporal scale is used, missing data can be filled in by using Poisson and negative binomial models (Witherington *et al.* 2009). Tag-loss models describe the probability of misidentifying previously counted turtles as new ones (Rivalan *et al.* 2006). Although that identification error can be factored into models by using repeat observation rates of nesting females, the use of PIT or genetic fingerprinting would reduce this error to insignificant rates. Open robust-design modelling using mark–recapture data can be used to estimate nesting female abundance probabilities, along with recruitment, survival, and breeding estimates (Dutton *et al.* 2005; Troëng and Chaloupka 2007; Troëng and Rankin 2005).

Overall, the suggested essential (identified by * below) and preferred monitoring approaches in Section 3 would facilitate:

1. *Estimates of nest success (i.e. all emergences divided by estimated nests).

2. *Estimates of females based on track counts, assuming constant nesting success (nests and crawls) and constant clutch frequency (the number of clutches deposited by an individual turtle in a nesting season; see NRC 2010 for overview).

3. *Estimates of females based on nest counts, assuming constant clutch frequency (see NRC 2010 for overview).

4. Quantification of actual female numbers based on the identification of unique individuals, rather than estimates from nests or tracks (through knowing actual female numbers plus actual nest numbers, it is possible to quantify clutch frequency with more accuracy too).

5. Satellite tracking can be used to reveal variation in clutch frequency between individuals and years.

6. If 4/5 is achieved, then recruitment of individuals to the nesting area can be estimated through various models, along with information on breeding frequency and longevity of breeding by individual females, survival rates etc.

7. Information on female fitness (nest and hatch success rates and nest frequency), where data on the same individual is available across years.

8. Remigration intervals of females based on tracking datasets and saturation tagging – allowing these parameters to be linked with foraging ground status.

9. Information on the genetic diversity of the population and the health of individuals, using genetic, stable isotope and blood characteristics (contamination etc.) data. This could be connected to foraging areas, through the use of satellite telemetry.

* = the most basic information to be collected across all sites.

The remaining items will likely only ever be collected from key ongoing monitored areas, but existing programs should be adjusted to ensure these latter objectives are incorporated.

Genetic data

Population genetic assessments (both mitochondrial and nucleotide) use F statistics (FST; Wright 1943), which measure departures from random mating within and among populations on the basis of genotype frequencies (NRC 2010). Values of F statistics generally range from zero (no population differentiation) to one (complete population differentiation). An analog that takes DNA sequence divergence into account is ΦST (Excoffier *et al.* 1992), usually performed in the program ARLEQUIN (Excoffier *et al.* 2005) or SAMOVA (Spatial Analysis of Molecular Variance; Dupanloup *et al.* 2002). Additional analogs are available to address the maternal inheritance of mtDNA (GST; Takahata and Palumbi 1985); potential biases in highly polymorphic datasets (when genes exist in several allele forms), such as microsatellites (Jost, 2008); and the mutational model for microsatellites (RST; Slatkin 1995). Many of those estimators are available from the Web service SMOGD (Software for the Measurement of Genetic Diversity; Crawford, 2009). All the genetic-distance estimators can be used to rank barriers to gene flow, as implemented in BARRIER (Manni *et al.* 2004). It is also possible to make bidirectional estimates of gene flow with the software programs MIGRATE (Beerli and Felsenstein 2001), IMA (Hey and Neilsen 2007), and BayesAss+ (Wilson and Rannala 2003), which allows at least for some resolution of historical sources of migrants and founders.

Relative in-water abundance of males and females

In-water data can be used to measure the relative abundance or density by using point-count methods, strip-transect methods (Marsh and Saalfeld 1989) or line-transect methods (Epperly *et al.* 1995), each with assumptions regarding detectability and availability (Buckland *et al.* 1993). Point-count methods are generally thought of as methods to approximate indexes of relative abundance and are not commonly used to estimate abundance or density. Although they have an assumption of constant proportionality between observation periods (a constant probability of detection), the methods do not allow the assumption to be tested. Transect observations are most frequently used to model relative abundance using distance-sampling methods (Buckland *et al.* 1993; Eguchi and Gerrodette 2009), in which observers measure the distance to each observed animal. These methods are used to model the detectability of subjects and their density by using observed distances and counts; researchers subsequently then model the reduction in detection probability with distance from a transect, assuming perfect detectability along the transect line itself, or they can specify an effective strip width that includes a high proportion of observed animals (NRC 2010). Assumptions of line-transect versus strip-transect theory dictate survey protocols and sampling design, and reviews have concluded that line transects are preferred because they require fewer assumptions about detectability and they use all the sightings in the analysis (Burnham *et al.* 1985; Marsh and Sinclair, 1989).

4. Monitoring of Feeding and Wintering Areas

The two nesting sea turtle species (loggerhead and green turtles) are considered as a reliable indicator on the status of biodiversity across the Mediterranean (Coll *et al.* 2011). Green turtles are primarily herbivores, whereas loggerheads are primarily omnivores, resulting in their occupying important components of the food chain; thus, changes to the status in sea turtles, will be reflected at all levels of the food chain.

Sea turtle populations are dispersed throughout the entire region because, while individuals from the same population frequent the same breeding site, they disperse to multiple foraging, wintering and developmental habitats (Casale and Margaritoulis 2010). However, the population structure is complex because these species are highly migratory (Luschi & Casale 2013; Casale & Mariani 2014). Thus, it is necessary to survey every life stage to determine the extent of connectivity among populations.

Studies indicate that loggerheads probably forage throughout all oceanic and neritic marine areas of the west and east basins of the Mediterranean (Hays *et al.* 2014; Casale and Marianni 2014). Most satellite tracking studies have been conducted in Spain (of juvenile turtles), Italy (a mix of juvenile and adult turtles), Greece (adult males and females) and Cyprus (adult females) (UNEP(DEPI)/MED 2011; Luschi and Casale 2014). Due to these biases, the results of tracking studies alone should be treated with caution. Green turtles have been primarily documented foraging and wintering along the Levantine basin (Turkey, Syria, Cyprus, Lebanon, Israel, Egypt) (Broderick *et al.* 2007; Stokes *et al.* 2015). However, foraging areas have also been documented in Greece (particularly, Lakonikos Bay and Amvrakikos Bay; Rees *et al.* 2013; Margaritoulis and Teneketzis 2003) and along the north coast of Africa, primarily Libya and some sites in Tunisia. Some green turtles have been documented in the Adriatic Sea (Lazar *et al.* 2004) and around Italian waters (Bentivegna *et al.* 2011), with some records occurring in the western basin. In addition, Broderick et al. (2007) detected wintering behaviour for greens off of Libya, with high fidelity to the same sites across years; however, further documentation has not been recorded for the other populations or other areas of the Mediterranean.

Juvenile and immature turtles represent the greatest component of sea turtle populations; thus information on the size structure and abundance at foraging grounds is essential to understand changes in nest counts, based on changes in mortality and recruitment into adult breeding populations (Demography Working Group 2015). However, because the juveniles of each nesting population may be dispersed across multiple habitats, and appear to use different sites across seasons, obtaining such counts is difficult requiring the complementary use of genetic sampling (Casale and Margaritoulis 2010). While information on the number of juveniles alone at given habitats does not reflect on any given nesting population, the relative numbers of immature to mature animals will provide baseline information about key juvenile developmental habitats and actual numbers relative to those obtained to adults.

The extent of knowledge on the occurrence, distribution, abundance and conservation status of Mediterranean marine species is uneven. It is therefore necessary to establish minimum information standards to reflect the known distribution of the two selected species. Species distribution ranges can be gauged at local (i.e. within a small area like a national park) or regional (i.e. across the entire Mediterranean basin) scales using a variety of approaches. Given the breadth of the Mediterranean, it is not feasible to obtain adequate information about the entire region (since the marine environment is 3 dimensional, with sea turtles only surfacing briefly to breathe), so it is necessary to choose sampling methods that allow adequate knowledge of the distribution range of each species. Such sampling involves high effort for areas that have not been fully surveyed to date. Monitoring effort should be long term and should cover all seasons to ensure that the information obtained is as complete as possible.

4.1. Identification and Evaluation of Feeding and Wintering Areas

Every 5 years (following the suggestion of the Demography Working Group, 2015), standardized aerial surveys of the entire marine area of the Mediterranean should be completed uniformly to delineate all potential feeding (or foraging) and wintering areas and record shifts in the use of previously delineated areas over time. These shifts can then be correlated to different environmental and anthropogenic pressures documented in the region from other sources (e.g. Coll *et al.* 2011; Halpern *et al.* 2008).

4.1.1. Essential: Aerial surveys

Plane surveys should be conducted covering all marine areas of each country. The central organisation should delineate the transect areas (and effort should be coordinated with cetacean surveys). If only a single survey period is possible, this should be 15 September to 15 October, when all turtles are foraging (with none wintering or breeding at this time). If multiple surveys are possible, these should be conducted on 4 occasions through the year to record foraging, wintering, summer foraging and breeding (e.g. December, March, June, September).

Survey methods should follow that of Lauriano *et al.* (2011) and Cardona *et al.* (2005) and Fortuna *et al.* (2015), with the survey design being in accordance with line-transect distance sampling methodology (Buckland *et al.* 1993). Ideally, a two engine high-wing aircraft (Partenavia P-68) equipped with bubble windows (to allow direct observation of the trackline below the plane) should be used. Alternatively, a high-wing aircraft (Cessna 172) that allows side viewing; however, a glass floor is recommended to obtain a full range of view. The plane should be flown at a constant altitude of 500 feet (152 m; Cardona *et al.* 2005) with a ground speed of approximately 100 knots (185 km/h). This altitude was designed to be optimal for whales and dolphins (Panigada *et al.* 2011). Ideally, two to three experienced researchers should be present onboard: two seated in the rear seats searching for animals through the bubble windows and the third observer was in the co-pilot seat, recording the data (on paper, on laptop or verbally into a recorder). In addition, it is advised that photographic/video equipment with ultra-high definition is attached to the plane to obtain supplementary records that could be reviewed subsequently.

The transects should be spaced at 10-15 km (software Distance can be used to design the transects: www.ruwpa.st-and.ac.uk/distance/; Thomas *et al.* 2009, 2010) to provide equal coverage probability. For instance, 53 transects of 15 km spacing were sufficient to monitor the entire Adriatic Sea (Fortuna *et al.* 2015). The Mediterranean should be divided into manageable survey regions and designated to different groups (i.e. Adriatic forms 1 survey region). Surveys should not exceed 6 hours at a time, and should be conducted between 09:00 and 17:00, during optimal daylight hours. Stills photographs or video footage allows the entire transect width to be analysed (i.e. beneath the plane, which is not possible by observers seated in the plane); furthermore, such data provide a way of validating counts of turtles post-survey, particularly in areas with high numbers of individuals. This post-hoc method also minimises observer error that might otherwise result from surveyor fatigue.

At each sighting, specific data should be collected including: GPS position (latitude, longitude), group size, declination angle when the sighting was estimated to be abeam and observer. Primary search effort data (distance flown in acceptable conditions) and altitude should be recorded directly from the GPS. All surveys should be conducted under specific flying conditions, namely Beaufort state 3 or less. Additional relevant information should be collected at the start of each transect line: sea state, glare, cloud cover and subjective sighting condition (the observers' view as to their ability to see an animal at the surface if present on a 3-point scale) and/or whenever changes occurred. Declination angle to the sighting should be measured, which, together with the plane altitude, allows the perpendicular distance from the track line to the sighting to be measured, according to the formula $X = h \times \tan(90 - \alpha)$, where h is the plane altitude and α is the declination angle. Distance analyses of the sightings data should be performed using the dedicated software Distance; multiple covariate distance sampling (MCDS) methods (Buckland *et al.* 1993, Thomas *et al.* 2009, 2010).

Turtles smaller than 60 cm in carapace length are difficult to detect from fixed-wing aircraft flying at any altitude or speed. Thus, estimation of density or absolute abundance presents a number of sensitivity issues (Burnham *et al.* 1985; Gerrodette 2000). However, such surveys can be used to identify key areas for focal surveys using other techniques, like UAVs. Some surveys incorporate measurements of the surfacing time of turtles, so that an availability function can be used to estimate absolute density and abundance or a correction factor can be used for unobserved animals; however, dive patterns vary with size, species, ambient temperature, and activity (Lutcavage and Lutz 1997).

Note: Transects must be corrected for the likelihood of observing surfacing animals, according to species. For instance, sea turtles are much smaller (particularly juveniles) and spend less time at the surface than sea birds or mammals. Furthermore, turtles are more likely to be sighted in shallow waters (<10 m depth) versus deeper waters. These issues need to be incorporated into the survey techniques and subsequent extrapolation/analyses.

Focal coastal aerial surveys

The plane-based aerial surveys across the marine areas should be complemented by extensive coastal surveys of foraging and wintering habitat using UAVs or supplementary dedicated nearshore aerial surveys, as animals in shallow waters might be more likely to be missed due to turbidity.

Plane surveys could follow a systematic saw-tooth pattern in this instance (e.g. Cardona *et al.*

2005), with a maximal perpendicular distance to the continental shelf (depth <200 m). Alternatively, 5 km intervals could be used for intensive survey areas (Frick *et al.* 2000). All other parameters should be consistent with the description above.

UAV technology is continuously improving. This technology could complement plane surveys, as it is easier to use on a more regular basis. Thus, once key areas are delimited, the UAV could be operated at selected locations from shore or a boat. See **Section 3.3.1** for details. UAVs can fly sufficiently low enough to differentiate species, size class and sex.

4.1.2. Preferred: LIDAR Trial

Ships with known route trajectories over open ocean basins could support, vessel-mounted multibeam sonar to estimate the numbers and sizes of individual animals in decimetres at a distance of 90 m from the vessel (see NRC 2010). Laser-based ranging systems (using light detection and ranging [LIDAR]) and radar-based ranging systems could also be used (NRC 2010). These technologies could be integrated into Mediterranean wide surveys to complement key survey types and validate the presence/absence of turtles in different areas.

4.1.3. Preferred: Satellite Images Trial

High resolution remote sensing imagery is proposed to record the presence/absence of sea turtles of potential marine and coastal areas in the Mediterranean. Imagery could be obtained for random areas, validating presence in known sites and determining presence/absence in sites where gaps exist. Also imagery could be preferentially used for focal foraging sites. In 2017, the ESA is launching the Sentinel-2B satellite (https://sentinel.esa.int/web/sentinel/home), which is targeted towards facilitating environmental surveys. It is advised that ESA is contacted to collaborate/coordinate the use of satellite imagery for sea turtle nesting surveys. This technology could be used to survey different regions of the Mediterranean in an objective and standardised way for the presence/absence of sea turtles, possibly with the capacity to detect species and size classes too. It would be superior to plane aerial surveys due to the resolution obtained and the ability to review still images and detect turtles that might be missed by visual sightings in plane based surveys. Because different countries have different resources; this approach would provide a way of monitoring the region objectively, as well as providing a baseline and centralised database on which to build and standardise all additional information collected opportunistically and at focal sites during annual surveys. The surveys, conducted every 5-years, should be implemented in September or October (on calm fine weather days) when all turtles are at foraging grounds and before the onset of wintering. Calibration would be necessary, using areas that are well monitored with a known mix of adults and juveniles, loggerheads and greens.

4.1.4. Area Evaluation Procedure

The 5-year surveys across all marine and coastal habitats will establish a minimum information standard on the presence/absence of turtles throughout the Mediterranean region. Where conducted, data from annual surveys in nesting areas will be overlaid on 5-year surveys to validate inferences from the 5-year datasets and to provide a means of additional analyses in relation to different pressures. The 5-year surveys will detect any variation over time (trends in the number of occupied grid cells) in the total surface area occupied by sea turtles for breeding, wintering and feeding.

The European (ETRS) 10 x 10 km² grid is used for mapping the distribution and range of the target species, accounting each known location along the Mediterranean coast. Three different maps (grids) are produced yearly for each species accounting for breeding sites, wintering sites and feeding/developmental sites of loggerheads (*Caretta caretta*) and greens (*Chelonia mydas*). For both species, information on spatial distribution within the assessment would be transferred in a 10 × 10 km grid (or finer for small countries, 1 x 1 km or 5 x 5 km). The distribution area is the sum of area of the cells where the species is "present"; filled cells will indicate the presence of the species.

To report the range of the target species and to describe and detect changes in the extent of the distribution, a tool to calculate the range size from the map of the actual breeding (or wintering or feeding) distribution is required (i.e. occurrences). The Home Range Tool extension for ArcGIS software (Rodgers *et al.* 2005) provides a standardised process that ensures the repeatability of the range calculation in different reporting rounds. After the automated calculation of the range, it is possible to correct any gaps to obtain a complete overview of the data following a standardised protocol. The resulting range map is a combination of the automated procedure completed by expert judgement.

The trends in the number of occupied cells or area occupied is a basic and immediate parameter for which the significance may be statistically assessed. This objective requires the use of different but widely available GIS geoprocessing techniques and geodatabases tools (ArcGis, QGIS, R platform, etc.) (see also Section 4.1.2 Mapping tools and methods, UNEP(DEPI)/MED IG.22/Inf.7).

The quality of the source data should be assigned scores (i.e. 3, Good; 2, Moderate; 1, Poor; 0, Uncertain). Following the "Common Indicator 3: Species distributional range" for seabirds: A helpful rule for assessing the quality of the range calculation could consist of a scaling system, combining the reliability of the distribution at the time it was mapped, how recently it was mapped, and the method used to map it.

4.2. Monitoring of Key Feeding and Wintering Areas

"Key" areas used by turtles in oceanic and coastal foraging and wintering habitats should be initially delineated for more detailed surveys (see **Section 4.1)**. Such areas could include smaller geographic areas where large numbers of animals aggregate (e.g. Amvrakikos Gulf, Greece or Drini Bay, Albania), broader ones containing a large number of individuals as a whole (e.g. stretches of coastline along which tracked turtles are distributed), or shallow (<150 m seabed depth) open water areas like the central Adriatic or the Gulf of Gabes in Tunisia.

It is suggested that, 5 key sites are initially selected in each country, which encompass different habitats and are widely spread across the country (see **Section 4.1.3 on** satellite imagery). As knowledge builds, monitoring foraging/wintering habitats skills are developed and gaps are identified during the 5-year surveys. Additional areas should be included to cover a broad-cross section of conditions. Where it is not possible to regularly monitor 5 key sites, a minimum of two key sites should be selected initially, adding a further single key area each subsequent year. The identification/selection of key sites may be complemented with existing data from aerial surveys, bycatch data, telemetry data and the study of the distribution of prey species. In addition, if only a part of a wider site where turtles are known to aggregate is monitored, this must be clarified and the representativeness of the monitored site to the entire area must be validated.

For foraging sites, the numbers of individuals need to be counted at different periods throughout the year (e.g. December, March, June, September). In some regions (e.g. north coast of Africa) foraging may occur year round for loggerheads, whereas green turtles exhibit wintering behaviour (Broderick *et al.* 2007; Hochscheid *et al.* 2007a, Hochscheid *et al.* 2007b; Patel *et al.* 2015); furthermore, while adult loggerheads appear to shift during the winter from the cooler Adriatic waters to winter or forage in the Ionian Sea, juvenile loggerheads have been recorded foraging in Adriatic waters during winter (Snape *et al.* in submission). To date, most wintering behaviour has been documented between November and February (Broderick *et al.* 2007; Patel et al. 2015).

Monitoring approach

Phase 1: Initially, potential foraging and wintering habitat that has been identified from previous satellite tracking studies combined with the 5-yearly aerial surveys (**Section 4.1**) should be selected to determine the behaviour of foraging versus wintering turtles.

Phase 2: Subsequently, key foraging habitat should be surveyed during the wintering period to determine whether turtles using these areas are foraging or wintering (i.e. some habitat could be used as foraging habitat year round, but by adults in summer and juveniles in winter, or it could be used as foraging habitat in summer and wintering habitat in winter). It is advised that focal aerial surveys (plane or UAV) are initially used to run transects of the selected habitat to determine the location/distribution/aggregation of turtles (see **Section 3.3.1** and **4.1** for details).

Phase 3: Finally, boat (underwater – dive/snorkel/videography) surveys and direct observations of targeted individuals should be implemented (see **Section 3.3.2** for details).

For sea turtles, direct counts at foraging areas may require the development of underwater monitoring techniques (where visibility is good), due to their low surfacing frequency, in parallel to emerging techniques (e.g. ROVs, LIDAR, UAVs; Patel *et al.* 2015; NRC 2010). This approach would be particularly important in major feeding areas that are not coastal, such as in the central Adriatic, the Gulf of Gabes in Tunisia, etc. In addition, juvenile foraging grounds for sea turtles are not necessarily in the same location as those of adults; therefore, dedicated surveys of areas used by juvenile life stages are also required; however, all aerial surveys are limited by the fact that water clarity restricts the depth of detection.

If all individuals surveyed within a habitat are deemed to be wintering, then UAV surveys could be used to detect the number and size class of individuals, with sex ratios being extrapolated from the sampled individuals. Alternatively, as wintering turtles surface less frequently than during breeding or foraging, underwater survey techniques may need to be developed (e.g. ROV; Smolowitz *et al.* 2015).

The proximity of foraging habitats to the nearest breeding areas should be recorded in all instances, as this parameter will influence the ratios of adult males to females in each habitat, as more males tend to use foraging/wintering habitats closer to breeding grounds compared to females. Also, it should be clarified whether intensive surveys are conducted across the entire habitat or in part of it; if the latter, the representativeness of this selected survey site to the whole area must be validated.

Note: the initial selected areas by a given country may be subject to re-evaluation following the collection of data on species, sex, size class and genetic origin.

4.2.1. Essential: Aerial Surveys

Coastal

Depending on resource availability, UAVs should be prioritised for use in survey coastal habitats. Two survey levels should be used: 60 m and 30 m above sea level to obtain maximum coverage and to identify the presence of males (Bevan *et al.* 2016). Adult males are distinguished by a tail that noticeably protrudes beyond the length of the carapace (Casale *et al.* 2005; Rees *et al.* 2013; Bevan *et al.* 2016) when swimming. The ratio of males detected in surveys across all the key foraging habitat can be validated by the ratio detected at focal sampling areas, especially when the entire foraging habitat cannot be surveyed by boat. Aerial surveys should be completed at a minimum of every 1-2 months, more frequently where possible. See **Section 3.1.2**, **3.3.1** and **4.1.1** for suggested UAV survey protocols.

Open sea

UAV surveys could be conducted while onboard fisheries vessels (see **Section 4.2.2** and **4.2.3**) to obtain an overview of the numbers of surfacing turtles within a 2 km radius of the vessel (distance sampling can be used to elucidate actual numbers; see **Section 3.4** and **4.3**). Surveys could be conducted on selected trips at regular intervals. At a minimum, UAV surveys should be conducted across the entire key habitat every 2-3 months every year to identify seasonal changes in foraging, along with wintering. This will provide a minimum estimate of the numbers of turtles using the area, and shift in use by species, size class and sex over the course of the year. See **Section 3.3.1** and **4.1** for suggested UAV survey protocols.

Alternatively, plane surveys could be conducted every 2-3 months (i.e. 4-6 times in total over the course of each year) due to greater expense. These surveys will only facilitate the identification of the presence/absence of turtles; thus UAV surveys should be prioritised. See **Section 4.1.1**.

4.2.2. Essential: Fishery Bycatch Surveys

Where fisheries strongly overlap with key foraging areas, bycatch surveys could be used as a complementary sampling technique. Fishery-bycatch data are an important independent source of turtle stock assessments. Catch per unit effort (CPUE) usually applies to fisheries data, and is the measure of relative abundance of turtles removed from the population. However, this approach is subject to sample bias with variable capture rates, making it difficult to validate its use as a quantitative index of abundance statistically. To improve its utility, the standardization of sampling season, capture gear, and other methods that affect capture efficiency is needed, with random sampling in space and time at a regional scale.

Comprehensive or standardized methods are needed that allow the incorporation of bycatch data into population assessments, with all sources being made available for examination at a Mediterranean scale. The monitoring and assessment of biodiversity related common indicators regarding sea turtles at breeding, foraging, wintering and developmental habitats and towards achieving the targets on Good Environmental Status (GES) in the Mediterranean could be facilitated by monitoring implemented by the General Fisheries Commission for the Mediterranean (GFCM) under the Ecological Objective 3 (EO3) (Harvest of commercially exploited fish and shellfish). Within this framework, live/dead sea turtle captures by incidental catch could be documented, including collecting morphometric and demographic data on sea turtles.

Researchers should be present onboard craft to document all captured individuals, and to record morphometrics (body mass where possible), sex, species, tag (PIT, external tag and photo-id), and collect tissue and blood samples for genetics, stable isotope and health assessments, along with the collection of bone samples from dead animals for skeletochronology (See **Sections 3.2.3 3.2.4 and 5.1)**. Laparoscopic examination should also be made to distinguish adult males from adult females (Eckert *et al.* 1999) (See **Sections 5.1**). The movement of vessels should be recorded by GPS to establish the area covered in relation to the key area identified by **Section 4.1**.

Ideally, post-release mortality should be measured; however, this is difficult as not all dead individuals are washed up on shore; yet, if all live captured turtles are tagged with both external and PIT tags (along with photo-identification and genetic samples for genetic fingerprinting, which could be used to identify individuals; see **Section 3.2.3**), information on the numbers of individuals stranded near shore could be obtained, and models of those that remain undetected at sea could be developed.

Through the activities mentioned above, it should be possible to establish models to account for differences in the coverage of fleets with different dynamics in different areas. GPS data will also help identify gaps in coverage for focused field surveys, potentially using different techniques.

4.2.3. Preferred: Boat Surveys

In key coastal areas, a combination of bycatch and dedicated capture-recapture boat-based surveys should be conducted. If the entire foraging/wintering site cannot be monitored (i.e. it is too large), a focal area should be selected, but must be validated as being representative of the entire foraging/wintering site. In areas with good sea conditions, in-water boat surveys should be complemented with snorkelling and diving surveys to obtain photographic records of males and females for unique identification and to record behaviour.

For all captured individuals, morphometrics (and body mass where possible), sex, species and tagging (PIT, external tag, photo-id, genetic fingerprinting) should be completed, along with laparoscopy and the collection of tissue and blood samples for genetics, stable isotope and health assessments. See **Section 3.2.3**, **3.2.4**, **3.3.3** and **5.1** for more details.

4.2.4. Preferred: Attachment of satellite transmitters

Within each 5-year period, a key foraging site for which previous satellite tracking information is limited (i.e. <5 turtles have been tracked entering and using the area or tracked from the area), should be selected in each country for one of the two species. Ideally, Fastloc GPS technology should be preferentially used (Schofield *et al.* 2010; Dujon *et al.* 2015), as fine-scale movement patterns (locations accurate within 20 m) are needed to facilitate the delineation of maritime zoning where required in breeding and foraging habitat.

This information will facilitate:

1. The delineation of potential protection zoning (and timing of zoning) at breeding and foraging grounds, taking into account the findings of previous tracking studies within the Mediterranean.

2. The delineation of migratory corridors between breeding and foraging habitats, will demonstrate sex-specific differences in migratory distance, which would be incorporated into models of sex-ratios at foraging grounds located at different distances from breeding areas.

This information will help determine key dispersal routes and connectivity with other foraging sites, wintering sites and the breeding grounds. Also, by tracking turtles from the foraging sites (rather than breeding sites), additional information on remigration rates can be built, in parallel with annual capture-mark-recapture study outputs from these key sites.

4.3. Data requirements for demographic analyses

It is not possible to count all individuals in a given habitat/population. Transects must be corrected for the likelihood of observing surfacing animals, according to species. For instance, sea turtles are much smaller (particularly juveniles) and spend less time at the surface than sea birds or mammals. Furthermore, animals are more likely to be sighted in shallow waters (<10 m depth) versus deeper waters. All of these issues need to be incorporated into the survey techniques and subsequent extrapolation/analyses.

The information on turtles collected through a combination of aerial, boat-based (monitoring and bycatch) surveys will provide the baseline data required to:

1. Calculate the abundance of non-breeding individuals (different species, adults and juveniles) and sex ratios (adults only) at wintering/foraging/developmental sites through the use of appropriate models (i.e. distance sampling Buckland *et al.* 1993); the possibility that individuals are not observed due to low surfacing frequency and poor underwater visibility in the marine environment should be taken into account.

2. Estimate seasonal variability in the abundance of different species, size classes and sexes at foraging and wintering habitat.

3. Obtain measures of trauma/mortality (all size/age classes), and hence survival rates, that are being injured/killed in foraging/wintering/developmental areas via the strandings network (**Section 5.1**) and bycatch data (**Section 4.2.2**) for incorporation into models on population trends.

4. Establish the distribution of turtles across marine and coastal habitats to delineate key areas for focal surveys.

5. Build models of differences in growth rates at different foraging grounds across the Mediterranean, by the recapture of the same juveniles and adults

6. Reveal shifts in foraging and wintering habitat use in relation to environmental or human factors, including climate change, through long-term surveys.

7. Uncover the genetic structure of individuals within foraging areas; genetic analyses of cohort origin will facilitate the identification of key sites for MPA establishment and other factors that might need considering when local numbers are low but amount to representative numbers at population level over a wide area.

8. Estimate the genetic structure of strandings at a Mediterranean wide scale to determine the extent and overlap of different populations.

9. Provide an understanding of age (based on size class) in relation to size of turtles from different nesting areas (including Mediterranean and Atlantic turtles, particularly in the west Mediterranean) will be obtained through skeletochronological analyses of dead stranded turtles collected from foraging/wintering/developmental areas.

10. Identify potential key areas through existing tracking information (although existing bias to a couple of sites exists and should be taken into account), including connectivity between breeding and foraging areas, different foraging areas, and foraging areas with wintering areas.

The data collected at the different sites across different countries can be overlaid with that of various pressures (fisheries, industry, shipping, tourism etc.; Halpern *et al.* 2008; Coll *et al.* 2011). The vulnerability/resilience of these sites in relation to physical pressures could then be quantified, including the 'Analysis of pressure/impact relationships for these sites and definition of qualitative GES' (See Common Indicator 1: Habitat distributional range for list of all possible outputs, and UNEP(DEPI)/MED IG.22/Inf.7).

Direct counts of sea turtle bycatch, or stranded individuals on beaches throughout the Mediterranean could be combined with appropriate modelling, to estimate the location where the animal was traumatized (i.e. how it was carried by sea currents) in cases of strandings. It is also important to model how these losses (i.e. rate of loss of adults vs juveniles, males vs females) potentially impact the resilience of the Mediterranean sea turtle population as a whole, as well as for individual population and sub-population units (Wallace *et al.* 2010).

Individuals could be sorted into age-specific categories called cohorts or age/stage classes (such as "juveniles" or "sub-adults"). Then, a profile of the abundance and different age classes can be created. The demographic structure may provide an estimate of the annual survival probability and/or reproductive potential of that population, which is critical information along with other parameters, from which current and future growth may be estimated.

Population analyses of wintering and foraging areas

Mixed-stock analyses should be used for foraging and wintering areas. Mixed-stock analyses are mathematical models that compare the genotypes (genetic profiles) of natal areas (nesting populations in the case of sea turtles) with the genotypes in feeding areas (pelagic [open sea] or benthic [seafloor] habitats; Bolker *et al.* 2003). These models use maximum-likelihood or Bayesian algorithms, with the ultimate goal of estimating the contribution of each natal area to the shared feeding habitat. Such methods have been applied for assessing mixed stocks of sea turtles (e.g. Laurent *et al.* 1998).

5. Other Essential Data Sources for the Analysis of Nesting and Feeding/Wintering Areas

5.1. Stranding Data – Stranding Network

Sea turtle strandings represent a useful index of population abundance and can be used if data are appropriately collected and standardized.

It is important to establish a functioning network of strandings and beached individual census throughout the Mediterranean, to obtain valuable and necessary information. Dedicated stranding networks already exist for sea turtles in several Mediterranean countries (Medasset 2016), with stranding information being confirmed to reflect distribution patterns based on satellite telemetry studies (Schofield *et al.* 2013b; Zbinden *et al.* 2008). All collaborating countries should establish strandings networks, all strandings should be reported to relevant authorities and details should be documented on an online database, with a central repository for genetic and blood sample analyses. For this purpose, specific tracts of coast can be selected as index zones, or coastlines may be opportunistically surveyed with the assistance of the general public.

Sea turtle strandings are a good indicator for the presence/absence of sea turtles in different geographical regions. Strandings account for an unknown proportion of total mortality that probably varies among regions. If carcass-recovery efforts are standardized and data are pooled over broad spatiotemporal scales, the patterns of strandings in time and space can provide information about seasonal distribution and interactions with fisheries. Changes in the number or size distributions of strandings may be a valuable indicator of shifts in age structure or distribution of juveniles (NRC 2010; Shoop et al. 1999). Strandings may also be a reasonable indicator of the nearshore turtle population, at least on broad spatial and temporal scale (Zbinden *et al.* 2008: Schofield *et al.* 2013b). This can be further validated by collecting genetic samples from stranded turtles and from those in focal study populations. Every stranded animal represents a valuable source of information for assessment if recovery efforts are standardized; i.e. proper measurements are taken, samples are collected, processed and archived according to established protocols. To improve the value of strandings data for assessment, each nation’s program needs to be reviewed and evaluated for consistency in recovery effort, volunteer training, and protocols. Areas that have low or inconsistent sampling effort could be identified to improve extrapolation methods. Tissue, blood and flipper (for bones) collection could become standard protocol, but a considerable investment in time and resources will be needed to process and evaluate those samples. In particular, officials should be trained to record all specified stranding details (for live and dead animals), and to take tissue samples of dead animals for various analyses analysis. In addition, all stranded and rehabilitation animals should be recorded on an online database with supporting genetics analyses (to link to nesting areas) for Mediterranean scale evaluation.

The following information should be documented for all live and dead stranded animals in each country:

1. Essential: Record all strandings: GPS location of stranding, photograph individuals and injuries; record species, sex; record morphometrics, take tissue samples and store appropriately for genetic and health (e.g. heavy metal contamination) analysis.

Where possible, also:
2. Preferred: Record biomass if turtles are alive or freshly dead; collect bone samples to conduct skeletochronology analyses of dead specimens.

3. Preferred: Trained officials (veterinarians) perform necropsies are also beneficial to determine internal blockages (rubbish, sand, fishing gear).

All live and dead stranded animals should be identified to the species level, estimated sex (based on tail morphometrics), checked for external tags and PIT tags, and recorded by date and location (including a GPS position). Carapace morphometrics and general condition should also be recorded, along with biomass where possible. Laparoscopic examination should also be made to distinguish adult males from adult females. Samples should be collected from live and dead turtles including tissue and blood for genetics, contaminant/health and stable isotope analyses. Such information would help determine the genetic diversity of different animal groups and their general health. Furthermore, the animals could be used as indicators of ocean health due to the effects of toxins building in the bodies of animals from higher trophic classes.

Recovered dead turtles should be necropsied by trained staff (in conjunction with a veterinarian) to identify sex and state of maturation, to record ingestion of plastic, sand or other debris, and to conduct a general evaluation of the potential cause of death (although this can only rarely be determined) (Eckert *et al.* 1999) (See also the ‘Sea Turtle Necropsy Data Sheet’ in Annex VIII, UNEP(DEPI)/MED IG.22/Inf.7). Body-size data should be collected to generate curves of somatic growth. Bones (humerus and eye ossicle) should be collected from dead animals for skeletochronology (age class) analyses. Skeletochronology is based on the counting of the lines of arrested growth (LAG). In sea turtles, the humerus is generally used, and this technique has been validated for sea turtles using turtles of known age (Snover *et al.* 2010), the use of LAGs labelling (e.g. injection of fluorescent tetracycline intrabone marker; Klinger and Musick, 1992) and the comparison of results of skeletochronology and mark-recapture records (Van Houtan *et al.* 2014). The bone should be cut into 2−3 mm thick sections using a low speed saw. Sections are fixed in 10% formalin, decalcified and sectioned using a freezing microtome (25μ), stained and mounted on slides.

5.2. Existing telemetry information

A database for all existing satellite tracking, GPS/GSM tracking, radio tracking and logger information should be established. Within this framework, all owners remain ownership, with the general use for the program being permitted. For other publications, all owners must be included as collaborating authors unless otherwise agreed. In this way, researchers/organisations may be willing to share their data, which for the Mediterranean, is extensive (e.g. UNEP(DEPI)/MED. 2011; Luschi and Casale 2014). The UNEP(DEPI)/MEDWG.359/Inf.8 rev1. 2011 should be updated within this framework.

5.3. Opportunistic Data Collection

Opportunistic observations on non-dedicated platforms (ferries, merchant marine ships or amateurs/yachts, use of citizen science) should record the GPS location and date of the sighting and species, and approximate size class where possible (and even photographs), to add supplementary information to standardised surveys. This could be added as a supplementary layer, for inclusion/exclusion, depending on the information being sought. To encourage the involvement of these sectors, a webpage should be developed that is made available in regularly accessed shipping materials. Trained observers could also be placed on host ships and aircraft to survey remote pelagic waters (e.g. Platforms-of-opportunity (POP) surveys). In such cases, data must be extrapolated to infer trends in abundance, as sightings become opportunistic.

5.4. Bibliographic Sources and Multi-Media

Personnel should be dedicated towards assimilating all bibliographic and online sources on the location of sea turtle nesting beaches, wintering, feeding and developmental areas surveys by different groups (fishermen, NGOs, guides, articles) of already known sites, probability of occurrence models (that indicate areas where a species is likely to occur based on statistical models that relate habitat variables to the presence/absence of a species) and regional expert knowledge.

6. Data Management

A central database maintained by a central body needs to be established where all data (tracking, tagging, genetic, stable isotope, skeletochronological, counts etc.) from all Mediterranean countries are assimilated and inter-linked, providing a means of connecting nesting sites with foraging/wintering sites. Online database for recording opportunistic sightings at breeding and general marine area by laypersons/fishers, including stranded and rehabilitated animals.

Ideally, a central body for genetic, blood, stable isotope, skeletochronological sample analysis from all countries; this approach would overcome financial constraints of individual countries and facilitate the development of a Mediterranean wide database delineating the connectivity among all sites.

Following the suggestions of the NRC (2010), a similar framework is recommended for the Mediterranean (see also **Section 1.4**) whereby:

- To integrate all data sources, a metadatabase should be established, identifying as many of the sea-turtle datasets in the Mediterranean as possible. The online database should be updated at least once every 6-months. The database would provide information on available data, status of each dataset (e.g., computerized, hard-copy only, lost), and contact information, etc.

- Develop a mechanism to obtain, computerize, maintain, link and make accessible as many sea-turtle databases as possible. Issues, such as data ownership, authorship requirements, and ensuring appropriate use of data, will need to be addressed through data safeguards, extensive outreach, and participant incentives. Priorities for selecting which databases to conserve should be based on the integrity of the data, the amount and type of data, and risk of loss.

- Improve coordination among data holders. Incentives should be developed to encourage data sharing; these may include providing participating researchers with data-analysis services and data products, regional data summaries, data backup assurance, assistance with publication of results, and facilitation of collaborative relationships.

- A Mediterranean wide strandings network database should be established to make information on all stranded turtles available for evaluation and to link stranded turtles with nesting origin areas.

- Establish standard research and data-collection protocols, with emphasis on techniques that have recently emerged. Develop incentives for researchers to adopt the protocols and outline a plan for continuing training in methods and analytical techniques.

- Establish and maintain long-term blood, tissue, stable isotope, humerus banks. Develop effective incentives to encourage participation in these banks, such as collecting humeri from turtle carcasses and tissue samples from turtles captured incidentally in fisheries, from which the data can be used to establish the connectivity of turtles among breeding, foraging and wintering areas.

7. Concluding Statement

This Guideline describes and suggests improvement on the methodology for the long-term standardized collection and assimilation of data on adult and juvenile loggerhead (*Caretta caretta*) and green (*Chelonia mydas*) sea turtles at nesting, foraging and wintering areas throughout the Mediterranean. Different levels of monitoring are suggested to facilitate the different capacities of different countries around the Mediterranean, ensuring that (1) uniform baseline information is obtained from all collaborating parties throughout the region, along with (2) focal surveys requiring greater effort at sites representing different habitat, size class, and numbers of turtles, with the aim to reduce uncertainty in demographic modelling. The combined use of a variety of assessment techniques is suggested to facilitate demographic analyses, which should be covered in the '*Standardization of methodologies to estimate demographic parameters for population dynamics analysis, such as population modelling*'.

The proposed approach depends on the willingness of the main research groups and independent researchers working in the Mediterranean to collaborate. Ideally, a central unit of researchers should be assigned to collect, collate and analyse the data to identify key gaps in information that need to be addressed, and to develop effective models on population demography, along with the potential impacts of existing and newly identified pressures. A central, dynamic and interactive database needs to be established that can be accessed by researchers from different disciplines, which will include all existing, ongoing and future spatial and non spatial data (including existing and potential nesting, foraging and wintering areas, nesting data, data from foraging areas, tagging data, genetic data, etc.), including the need for a GIS open platform on which to place the different layers of information for integrated use. However, to accomplish this, clear benefits for groups/researchers that contribute with information must exist, including clarity on data ownership and permissions for data use beyond the owners, along with data quality checks to ensure that data are representative, along with clarification on the limitations of datasets.

By standardising methods, the integrated use of data becomes possible, allowing existing published and unpublished information to be combined using different tools from different sites over different time periods. Access to such information would allow researchers from different disciplines and with different skill sets to develop robust estimates and predictions, which would improve the protection effort of sea turtles as well as ensuring the delineation of appropriate networks of sites to maximise protection. In conclusion, a Mediterranean wide database of green and loggerhead turtle datasets is essential to understand how nesting, foraging and wintering sites connect and protect these areas appropriately. Such an approach also promotes collaboration at an international scale, drawing together people with similar objectives and different skill sets, whose expertise combined could help determine how best to protect sea turtles and ensure their persistence into the future.

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