

## MEDPAN CALL FOR SMALL PROJECTS



### Guidelines for the management of sea turtle nesting sites in the Western Mediterranean



Deliverable A.1.1

COMING Project

*Coordinated monitoring and management of sea turtle nesting activity in the western Mediterranean through MPA*

2021

## **Deliverable A.1.1**

### **COMING Project**

#### ***Coordinated monitoring and management of sea turtle nesting activity in the western Mediterranean through MPA***

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Author (s)	Elena Abella, Mireia Aguilera, Carlos Carreras, Marta Pascual, Lluís Cardona, Sandra Hochscheid.
Contributor (s)	CT BETA , Universitat de Barcelona, Stazione Zoologica Anton Dohrn,

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## 1. Introduction

### ***What is currently happening in the western Mediterranean?***

The Western Mediterranean (WM) has been considered as a sporadic nesting area for the loggerhead sea turtle (*Caretta caretta*) for many decades (Filella and Esteban, 1992; Llorente *et al.* 1992; Tomas *et al.* 2018; Carreras *et al.* 2018; Casale *et al.* 2018). WM includes Spanish, Italian, French, Moroccan, Algerian and Tunisian coasts. Nesting events in this region were unpredictable in time and spatially variable until the beginning of XXI century, when their frequency increased (Maffucci *et al.* 2016; Carreras *et al.* 2018; Tomás *et al.* 2018). As a result, the region supports regular nesting activity, and, although the number of nests detected per season is still low, it is increasing every year.

Current evidence suggests that these nesting events are the result of an ongoing colonisation process, potentially favoured by global warming, from individuals coming from either the Eastern Mediterranean or the Atlantic nesting beaches (Carreras *et al.* 2018). These rising nesting populations are of special conservation importance as the WM could become a refuge for turtles (together with current suboptimal areas of the eastern Mediterranean) if the current nesting beaches in the eastern Mediterranean are threatened by, for example, global warming as predictions suggest. As for now, nesting activity in the WM may have a small effect on loggerhead species population dynamics compared to established eastern Mediterranean loggerhead rookeries. However, the WM should be considered as a high priority for the survival of the species in the Mediterranean, as most eastern nesting areas have been predicted to become suboptimal on a short term due to global warming, as shown by Witt *et al.* 2010 and Pike (2013). Thus, the growing number of new nesting sites in the colder areas of the WM represent a key element to reinforce the sustainability of the species in the Mediterranean in a not-too-distant future, and it opens a new perspective for its conservation as well as the possibility to create new MPA's where sea turtle nesting can become common.

Furthermore, the green turtle (*Chelonia mydas*) is extremely rare in the western Mediterranean (Carreras *et al.* 2014) but reproduces regularly in the eastern Mediterranean (Casale *et al.* 2018). This species has expanded its nesting range in the Gulf of Mexico (Shaver *et al.* 2020) and a similar expansion might also happen in the Mediterranean in the near future. Thus, this protocol also needs to consider the potential nesting range expansion for the green turtle in the whole Mediterranean.

These facts give us a new scenario with a potential occurrence of new marine turtles nesting in the Mediterranean that requires special management actions.

Most of the nesting events in the Western Mediterranean occur on beaches with high human activity. Local people and authorities are unfamiliar with sea turtle nesting, but at the same time such events are widely publicised in the media, which may bring some change. This represents a management challenge that should be addressed, because nests may not be adequately protected resulting in damage to eggs and reduced survivorship of hatchlings. (Marco *et al.* 2016, Tomás *et al.*, 2016), and adult females may give up on nesting because of disturbance. Additionally, inappropriate management decisions result in missed opportunities to gather relevant scientific data for conservation, such as identifying the origin of breeding females and males.

Marine Protected Areas play a central role for the monitoring and management of these nesting events for several reasons. In the first place, some of the nesting events have already been located within the boundaries of an MPA and many of these MPAs are currently taking management actions to preserve loggerhead nests in the western Mediterranean. Second, some MPAs may be suitable places to relocate nests, if necessary, when the original nesting sites are not adequate for the survival of the eggs. Finally, MPAs are excellent focal points to launch information and awareness campaigns to improve the detection of nests and raise the awareness of local stakeholders and the general public that need to adapt to the presence of nesting turtles on the beaches.

### ***Common strategy for the monitoring and management of sea turtle nesting events in the Western Mediterranean***

In the context explained above, it is crucial to establish a set of common guidelines for management and conservation, to reinforce public awareness and to perform a proper scientifically based monitoring program, in order to guarantee the stabilisation of nesting events in the Western Mediterranean, as suitable habitats for the species range expansion at evolutionary scale under changing environmental conditions.

This document aims at providing a complement to the “*Marine Turtles in MPAs: a monitoring and management guide.*”, adapted to the current nesting situation in the WM and oriented to MPAs located in this region.

Considering the importance of natural nesting dispersal increase and its implication in sea turtle nesting conservation in the whole Mediterranean, this document also intends to promote a common sea turtle conservation strategy targeting MPA managers in the WM.

Colonization process will also imply changes in the presence of turtles of different life stages in the WM coastal and marine habitats. Hatchlings and adults may be observed more often in MPA's of the WM.

The main management priorities in the Mediterranean regarding marine habitats are well described in the *Marine Turtles in MPAs: a monitoring and management guide*. “*Marine protected areas are established to preserve habitats, biodiversity and specific species. In the case of marine turtles, conservation goals include ensuring their critical habitats are protected to enable successful completion of basic biological requirements, such as feeding and breeding, and the protection of individuals from threats in these locations. In this way marine turtle populations can be maintained and increased, so that they are fulfilling their ecological role in marine and coastal ecosystems. As marine turtles are highly migratory species, not recognising geopolitical boundaries, each MPA needs to contribute as best it can to the protection and preservation of the species. Using best practice and efficient deployment of resources it should bring about the most favourable results for turtles relative to the specific needs presented within the MPA. Further background on designing conservation programmes can be found in Eckert (1999)*”. But, specifically regarding nesting events in Western Mediterranean, it is crucial to guarantee successful nesting and to maximize post-hatchling survival in order to allow the establishment of new natural nesting colonies in this region.

In this document we provide information on how conservation activities should be applied, but applying them is up to every MPA, according to their resources, capacity and priorities.

## **2. Habitat-Based Monitoring Decision Tree in WM**

The Habitat-Based Monitoring Decision Tree developed on the *Methodological guide for the monitoring of marine turtles in MPAS* defines regular and sporadic nesting areas and delineates different protocols for each category. As explained before, in the WM there are still quite few nests, but nesting should begin to be considered as regular considering its annual occurrence. For this reason, the protocols to be applied specifically in the WM are the same as indicated on the Habitat-Based Monitoring Decision Tree (page 38) for regular sites, but with some modifications (see Annex I, part A of this document). Also new protocols have been specifically designed for nesting in the WM, which are developed in Annex I, part B of this document.

Therefore, the protocols to take into account in the WM are the following, together with the new ones specifically developed in this document:

### **Nest counts, threats, tagging, sampling**

Protocol H: 8- 12 -14- 15- 16 - 17 - 18 - 22- 23- 24- 33

Protocol A: 1 -2 – 4 – 5- 6- 7 –8 –9 –10-11-12-13 –16 – 25 – 26 - 27 - 33 - 36

Protocol T/M: 10 –11 – 16 – 17 – 18 - 22 - 23-24 - 25-26-27 – 33

*In blue: Essential protocols for the WM*

### 3. Key recommendations for MPA managers in WM

#### **Use of this document:**

- Use the main guide “Marine Turtles in MPAs : a monitoring and management guide” for getting information about sea turtles and managing activities in MPAs
- Use the Habitat-Based Monitoring Decision Tree found in the general guide, taking into account the adapted and new protocols of this document, where important information is included for the WM.
- Use the new protocols for the WM included in Annex I of this document when needed.

#### **Key recommendations:**

- Citizen involvement to respect and detect nesting events is essential:
  - Inform about what to do when a turtle or a track is found on the beach.
  - Inform about the "National emergency number" and/or country specific emergency protocols to facilitate nest detection and a fast management.
- The more data you collect from the nesting event, the better
- Work together with NGOs, researchers and other specialists
- Share your data with other regions



## 4. References

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## **ANNEX I. Monitoring and Research Protocols for Western Mediterranean**

## **A. Adapted protocols from the Methodological guide for the monitoring of marine turtles in Mediterranean MPAs**

In this section, some points of the protocols have been reinforced adding specific comments in the already existing protocols (General Guidelines), when they apply to WM.

**How to use this document:** In order to have the complete information to apply each protocol in the Western Mediterranean, please use together the protocols of the General Guidelines and the recommendations included in this document.

**Consideration:** Most of these recommendations must be carried out by technicians and experts with proven skills. Therefore, collaborations between MPA managers and Scientists, veterinarians, and Rescues Centers will be needed.

List of adapted protocols:

3. Sexing turtles
4. PIT tagging
5. Flipper tagging
6. Photo ID
7. Tissue sampling
9. Locating clutches
10. Relocating / Translocating clutches
11. Nest protection against predation
13. Post-hatch excavation of clutches
16. Beach surveys (ground)
36. Deploying animal mounted data gathering and tracking equipment

### **3. Sexing turtles**

**Management relevance in Western Mediterranean: levels of** hatchling production in Western Mediterranean is still unknown. Direct information about hatchlings sex ratio in this area will highlight important species information related to demographic data and its conservation needs. For rookeries in Western Mediterranean, information about hatchling sex ratio is essential to determine the number of females produced naturally in beaches recently identified as nesting areas. These data will permit us to assess how probable is the establishment of a particular new rookery in Western Mediterranean, helping to understand marine turtle population dispersion and dynamics to preserve them.

Hatchlings cannot be sexed by their carapace and tail measurements because sexual dimorphism based on external morphology is absent at this life-stage. Therefore, other methods to determine sex should be considered:

- **Actions:** In addition to interpretation of the turtle's carapace and tail measurements to infer sex, a turtle's sex can also be determined by laparoscopy or hormonal concentration blood analysis at an early stage. The collaboration with trained and qualified personnel to perform these techniques is essential. Gonadal analyses of dead embryos, dead hatchlings or headstarted juveniles (in case of headstarting program) are also highly recommended in order to have as much direct sexing data as possible for hatchlings produced in Western Mediterranean coast. However, alive and dead animals should be considered for sexing in order to avoid any mortality bias related to sex in the analyses.
- **Ethics:** Hormone determination through blood analysis and laparoscopy are a mildly and highly invasive technique, respectively, and require ethical justification and probably a permit from the relevant national authority.
- **Data to record and frequency:** Blood analysis should be made for all hatchlings when a headstarting program exists..
- **Reporting:** Sex ratio results should be shared with scientific specialist groups to infer in population dynamics.
- **Collaboration potential:** Blood analysis and laparoscopy should be only carried out by skilled experts (veterinarian, Marine Rescue Centers, etc..) in order to guarantee animal health.
- **References:**

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#### **4. PIT tagging**

Protocol 4 should be highly considered for nesting sea turtles in Western Mediterranean.

- **Management relevance:** In the context of a colonization of new nesting areas, having as much information as possible is crucial to understand nesting colonization process (eg. how many females are using Western Mediterranean beaches for nesting, identifying if they use them sporadically or if some nesting females are remigrants). PIT tagging will therefore be carried out for every nesting event where the female is able to be monitored, in order to better identify nesting females in the Western Mediterranean, and to determine nesting remigration or inter-nesting interval. In those MPAs where a headstarting program is carried out, then PIT tagging will also be applied to released juvenile sea turtles (minimum 700 g weight). This data would easily allow us to identify turtles hatched on Western Mediterranean beaches.
- **Data to record and frequency:** a PIT tag database should be updated regularly and shared with other sea turtles working groups.
- **Equipment:** Managed field teams should be equipped with PIT tag readers and PIT tags to identify and mark nesting females.

## **5. Flipper tagging**

Protocol 5 should be also highly considered for nesting sea turtles in the Western Mediterranean in order to determine remigration interval in a nesting season.

- **Management relevance:** Flipper tagging should be carried out when possible as a complement to PIT tagging in nesting females. Flipper tagging identifies remigrant nesting females visually without specific material requirements, which can be useful in beaches or areas with no monitoring teams. However, this kind of marks are not permanent and a considerable flipper tag loss has been reported in some marine turtle rookeries, but it can be a useful tool to determine inter-nesting interval for re-sighted females



## **6. Photo ID**

In Western Mediterranean, this protocol complements protocols 4 and 5 with the goal of identifying as many individuals as possible.

- **Management relevance:** Considering that sea turtles nesting in the WM come from different populations, not being a genetic unit by itself, having as many as individuals identified, both nesting females and hatchlings, is necessary. This will provide information about the number of different individuals coming to WM beaches to nest. In addition to having Photo ID from adults as described in Protocol 6 of general guide, having Photo ID of released hatchlings or juveniles will permit to identify WM hatched individuals in the future. Photos, like headshots from turtles on the beach taken by any citizen with a smartphone who happens to observe a turtle, can be collected through public awareness campaigns and citizen science programs, which the MPAs might be conducting. If a video has been made, still frames of characteristic individual features of the turtle can also be obtained. There is particular potential of this identification methods in areas where turtle nesting is rarely observed, not regularly monitored and cannot be foreseen.
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## **7. Tissue sampling**

- **Management relevance:** In the context of a colonization process, it is crucial to determine the population of origin of the colonizers as well as the number of individuals contributing to the establishment of new rookeries. Application of Protocol 7 is of high importance in the WM as it can provide important information at the individual and population level. For instance, the genetic characterization of tissue samples will allow to determine the presence of multiple paternity and to determine the minimum numbers of breeders contributing to the nests sampled in the WM. Blood sampling (individuals > 1 year) should also be considered as an important action to be carried out to gather information for specific current research topics. As nesting events in the WM are rare events, as much information as possible is needed and therefore sampling should be considered a high priority. In order to know the origin of each sporadic nest it is essential to sample the nesting female (when available) and as many hatchlings (dead or alive) as possible, as multiple samples provide additional information about progenitor origins.

## 9. Locating clutches

- **Management relevance in Western Mediterranean:** Locating clutches is essential in Western Mediterranean as 1) it is crucial for the protection of the nest; 2) will provide essential information about if a nesting rookery establishment could evolve in a specific beach or area in a colonization scenario. Locating clutches and gathering as much information as possible are of high importance on every single nest in the context of the WM because of the lack of information about sporadic nest characteristics in a species colonization scenario. Nest information such as clutch size, nest depth, distance to the sea and distance to the maximum upper limit of the beach, is more important to learn for sporadic nests detected in new nesting areas. How nest characteristics affect the Western Mediterranean colonization process is still unknown in WM nests and therefore more information is needed. In addition to the information provided in Protocol 9, the following information should also be considered when locating clutches in WM:
- **Actions:** efforts to locate clutches should be done either after egg laying or when hatchlings are observed crawling to the sea (in cases where the nest has not been previously identified). Once the clutch is located after laying, nest relocation should be considered in cases where the clutch is not in a safe place (see *Relocating / Translocating clutches* protocol). This action must be carried out only by experts and following appropriate egg management protocols (see *Relocating / Translocating clutches* protocol). If the nest is considered to be relocated, counting the eggs is a very important action in WM as it will provide more precise information about the hatchling success. During this process, different measurements should be taken (number of eggs, nest depth, distance to sea and distance to the maximum upper limit of the beach, depth to the top egg from the sand surface, beach surface characteristics –size and color of the sand, vegetation or rock presence, etc.-). Before closing the nest, a temperature data logger will also be placed in order to register nest temperature (see *Incubation Temperature Management* protocol). If the clutch is located after observing hatchlings on their way to the sea, it will be protected until apparently all the hatchlings have emerged. When the experts consider that no other hatchlings will emerge, then the nest will be opened following protocol 13. *Post-hatch excavation of clutches*
- **Data to record:** distance to sea, depth to the top egg from the sand surface, number of eggs, nest depth (it should be measured from the bottom of the egg chamber to the sand surface level using as a reference a stick placed horizontally in the sand surface)
- **Reporting:** Data will be reported each nesting season and shared at WM level.

## **10. Relocating / Translocating clutches**

- **Management relevance in Western Mediterranean:** For Western Mediterranean, where few nests exist, it is especially important to assure all nests are in a safe place, and to consider conservation actions to protect them in case of risk. Therefore, some extra special situations should be considered when trying to identify if a nest location is safe: (1) the nest is too close to the High Water Mark, this should be identified with the help of locals who know the beach dynamics and can guess if a nest is likely to be inundated; (2) the nest is in the way of possible freshwater coming from inland, which can occur in case of rainfall; (3) the nest is in a place where a conflict with human activity is highly possible (presence of an establishment, passage to arrive to boats, etc); (4) the nest is made in a place which is shaded by objects, vegetation or a building. Here it is also important to consider the seasonal changes, especially at the higher latitudes of the Western Mediterranean. The sun and temperatures will be different at the end of the season, and therefore it is important to consider all the conditions you will have all over the incubation period and take conservation actions if needed (see *Artificial Incubation protocol*)

## **11. Nest protection against predation and human disturbance**

The following information should be considered for Protocol 11 in the Western Mediterranean:

- **Management relevance in WM:** considering that Western Mediterranean nesting is recent, and the society is not used to nesting events, nest protection in Western Mediterranean should be not only against predation, but also against human disturbance. In these areas, nests are often laid within public and popular bathing beaches. Therefore, additional protection is needed in order to assure the nest is not damaged by people. The best solution is to fence the nest site with non-shading material, applying info signs and with 24h surveillance if possible (private, volunteers, etc.), who protect the nest day and night. Involvement of experts is needed in order to give advice on the better materials to be used in order to not interfere with the incubation of eggs. Public awareness campaigns to raise awareness of nesting events and nesting protocols in Western Mediterranean countries should be done to promote effective nesting protection.

### **13. Post-hatch excavation of clutches**

- **Management relevance in WM:** In the Western Mediterranean, nesting season period and weather conditions during incubation period fluctuates more than in the Eastern Mediterranean. Therefore, special situations described below should be considered before nest excavation.
- **Action:** In the Western Mediterranean there are nests that hatch after 70 days, due to late season or other weather conditions and low temperatures that can prolong incubation. The timing of excavation of the nest should be decided by an expert taking into consideration different aspects: the temperatures at which the nest has been incubated, the deterioration of weather conditions at the end of the incubation period, number of hatchlings already emerged, etc. In the WM this action is to be done case by case and with the involvement of experts.

The categorizing of nest contents will be done as explained in protocol 13, but in addition, sampling of some of this content will be done in order to gather important information: material from dead hatchlings, shells, etc. All dead hatchlings and embryos not developed will be separately stored for further analysis. The rest of the nest remains will be put back into the chamber and buried.

## **16. Beach surveys (ground)**

The following information should be considered for Protocol 16 in Western Mediterranean:

- **Management relevance in WM:** Having as much scientific information as possible about females (at least: identification, tagging, biometry, tissue sampling) is crucial in WM beaches in order to characterize those females who are breeding in WM and detect how many different animals are laying eggs in Western Mediterranean. Beach surveys will facilitate encountering the females or her tracks/nests, which will also permit nest protection and have relevant scientific information about the nesting event (*see protocols from 1 to 12*).
- **Action:** Beach surveys in the WM should be made *ad hoc* when a sea turtle is known to try to nest in the area (e.g. tracks have been detected during the previous nights that did not result in egg deposition). Patrols should be organized in these cases to maximize the possibilities to encounter the female laying eggs or whilst crawling on the beach.

### **36. Deploying animal mounted data gathering and tracking equipment**

The following information should be considered for Protocol 36 in WM:

- **Management relevance in WM:** tracking the nesting WM females will provide important information about female dispersal routes and identifying if breeders are nesting more than once in a single nesting season. Knowing migratory routes and habitats for nesting females in Western Mediterranean would aid identification of foraging habitats that may be considered new marine areas for protection if they are not already part of an MPA.
- **Action:** tracking equipment will be deployed to a few females nesting in the WM if the equipment is available and the female can be retained by trained, authorized and skilled expert personnel .

**Collaboration potential:** This action should be only carried out by skilled and trained experts (veterinarian, Marine rescue center technicians etc..) in order to guarantee animal health, and in collaboration with scientists to analyze animal dispersal data properly.



## **B. New protocols for the WM**

37

**Name:** Nest temperature monitoring and management

**Standard:** ★ ★

**Topic:** Habitat

**Reason:** Sex ratio of hatchlings produced on beaches is directly related to incubation temperature. Nest temperature monitoring in real-time facilitates quick management actions to preserve nests in risk in the Western Mediterranean due to non-adequate incubation temperature regimes, i.e. through detecting changes of nest temperature below or above the optimal range of incubation temperature (e.g. extremely cool beaches, nests laid at the end of the season, shaded nests, etc.).

**Context:** Nest incubation temperatures in the WM are still unknown, and knowing them would highlight potential differences with Eastern Mediterranean incubation temperature regimes. Natural dispersion of nesting to the Western Mediterranean needs to be well understood to detect changes in regional population dynamics. Precise data about regional nest incubation temperature range is necessary in order to determine nest viability due to temperature (essential data for managers and decision-makers), to obtain nest survival estimations related to temperature, and to estimate primary sex ratio using a simple and accurate non-invasive method. Monitoring nest incubation temperature permits to improve nest success avoiding extreme incubation temperatures (if it is needed) e.g., applying nest warming techniques on the beach or considering nest translocation (to a warmer beach or an incubator). Nest characteristics in the WM are very diverse (number of developing eggs, clutch size, nest depth, etc.) and imply variations in the amount of metabolic heating produced. Metabolic heating data in Western Mediterranean nests could be crucial to understand differences in nest incubation regimes despite knowing general beach temperatures. At the same time, it allows to make more accurate sex ratio estimations and it would allow to develop population dynamics models considering climate change scenarios not only in the Western Mediterranean but also in the whole Mediterranean.

**Associated protocols:** *Nest (and track) identification by species* [8], *Locating clutches* [9], *Re-locating / Translocating clutches* [10], *Nest protection against predation* [11], *Identification*

of nest hatching [12], *Post-hatch excavation of clutches* [13], Beach suitability assessment for nesting [14], *Beach surveys (ground)* [16], Beach survey (drone) [17].

**Equipment:** Waterproof calibrated temperature loggers (e.g. Stow Away TidbiT Onset, <http://onsetcomp.com>, with an accuracy of  $\pm 0.2$  °C)

**Manpower:** 1-2

**Cost:** €€€

**Frequency:** all nesting season

**Actions:**

Automatic temperature data loggers (Bluetooth or optical) should be deployed in the middle of the study clutch. It is important to know the clutch size, and nest and logger depth should be recorded in order to place another temperature data logger buried at the same depth to collect sand temperature at incubation depth and analyze metabolic heating.

Bluetooth temperature data loggers (with an accuracy of  $\pm 0.2$  °C) permits to monitor real - time incubation temperature as often as it would be required. This is the best option to monitor nest incubation temperature in those areas where extreme temperature regimens are suspected to negatively affect nest incubation and viability, because managers can take quick decisions applying different nest protection techniques in order to protect them from extremely low or high temperatures. To monitor incubation temperature of a nest in case it was not possible to acquire Bluetooth loggers, data loggers should be deployed to monitor sand temperature at incubation chamber depth. A third option is to use temperature loggers probes buried in the clutch with a wire running to the surface (temperature can be recorded any time by connecting the probe to the data reader. These data would be a rough estimation of real incubation temperature, but temperature recorded in the sand indicates that incubation temperature is at least 0.2°C to 0.5°C higher than sand temperatures from the second third of incubation period (during the 1st third of incubation both temperatures should be very similar and in the 3rd third of incubation, incubation temperature should be even higher than in the middle third).

Automatic temperature data loggers should be programmed to record temperature every 30 min. Data loggers (factory calibrated at purchase) should be checked for accuracy by deploying them in beach sand, simultaneously, for at least 48 h before and after data collection period, in order to find differences among them.

To monitor incubation temperatures, there should be at least one logger per nest and at least two loggers per nest if metabolic heating is to be analyzed. GPS location of each logger should be taken. The logger to record sand temperature at incubation chamber depth should be placed at least 70 cm from the nest, parallel to the sea line.

**Ethics:** As deployment of data loggers in the middle of the nest involves altering, to some degree, the incubation condition of the eggs and clutch size sampling implies egg management, it should only be carried out by trained and authorized personnel. Further actions to protect nests against extreme temperatures, such as nest translocation or installation of warming equipment on the beach, should also be undertaken by trained and authorized personnel. Incubation temperature monitoring should be carried out as frequently as needed by trained people able to interpret data.

**Data to record:** Raw temperature data from incubation temperature loggers should be processed to provide mean daily temperature. Each temperature profile should be associated with a nest and GPS coordinate.

**Management relevance:** Monitoring incubation temperature facilitates additional nest management actions, improve nest emergence success and assess a better understanding of natural primary sex ratio in beaches of current nesting areas of colonization. Knowing precise incubation temperature regimes and profiles rather than only sand beach temperatures is important as the proportion of females' hatchlings produced in those areas may vary over time and space and hence colonization time estimations based only on beach sand temperatures can be excessively inaccurate.

**Reporting:** At minimum, the daily incubation nest temperature profiles across the season per temperature logger should be presented in graphical form to show at a basic level if incubation temperature of each beach or beach section experiences temperatures above and below the optimal range of temperature or the pivotal temperature, which can be assumed to be ~29°C. Extreme incubation temperature based on these observations can be detected and discussed in order to improve nest hatching success. At the next level, incubation temperatures for the periods of the middle thirds of individual nest incubations (the thermosensitive period for sex determination) and the amount of metabolic heating produced can be extracted to give more detail on primary sex ratio produced or skewing of sex ratios with more detailed discussion on spatial and temporal variation in likely sex ratios.

**Collaboration potential:**

Deploying temperature loggers inside nests can be carried out by NGO or University teams in close coordination with the MPA authority, however the manager may want to share temperature logger and nest data with research scientists who can fully investigate likely nest incubation temperature regimes, assess nest protection techniques against extreme incubation temperatures, sex ratios produced within the MPA and compare this seasonally with other locations.

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**Name: Artificial incubation and monitoring**

**Standard: ★ ★ ★**

**Topic: Animal and Threat/Management**

**Reason:** Artificial incubation and monitoring is a conservation tool that permits to protect eggs from predation, poaching and climatic uncertainty (e.g., natural low or high-temperature incubation conditions), and permits to gather additional scientific data (egg development stages, clutch size, etc.), for a better quality assessment of WM nests in a recently detected nesting area. Thus, artificial incubation is an alternative to on-beach clutch relocation when the original conditions of the nest jeopardize its viability or for scientific reasons without compromising the success of the nest.

**Context:** There is little literature about the quality of nests in the Western Mediterranean. Furthermore, nest quality has a direct impact on its viability (eggshell calcification, eggs development rate, fertilization rate, etc), hatchling fitness, and the establishment of a new rookery. Artificial nest incubation is a common initiative to protect eggs from risk undertaken by conservation authorities, parks, zoos, and other management stakeholders that involves incubation of eggs in an *ex situ* setting followed by hatchling or juvenile release (Eckert et al. 1999), and ultimately increases nest survival. This practice typically involves removing eggs from natural nests, placing the eggs in boxes or incubators, and allowing eggs to hatch under a specified incubation regime (Páez et al. 2015), permitting to evaluate reproductive parameters as fertilization rate or embryo development stages. After hatching, juveniles are either released immediately, or are ‘head-started’, which involves raising individuals to larger body sizes before release to theoretically increase survival in the wild (Mullin 2019). Artificial incubation regimes should be performed responsibly and resemble natural incubation regimes in order to avoid degradation of quality of hatchlings released to the wild, because incubation temperatures not only affect sex ratio determination (Mrosovsky & Pieau 1991) or survival (Bobyne & Brooks 1994) but also have profound impacts on many aspects of hatchling phenotypes and performance (Sim et al. 2015, Kobayashi et al. 2018).

In order to have bigger guarantees of embryo development (incubation setting diversification) under risky incubation scenarios, splitting the nest and retiring a small percentage of eggs (10%-20% always considering if it is appropriate according to clutch size) from the natural clutch on the beach should be considered. This conservation measure should be applied only

in places with a small number of nests per year (e.g. Spain, France) in order to assure the recruitment of individuals and the acquisition of scientific data.

**Associated protocols:** *Nest (and track) identification by species* [8], Locating clutches [9], Re-locating / Translocating clutches [10], Nest protection against predation [11], Identification of nest hatching [12], *Post-hatch excavation of clutches* [13], Beach suitability assessment for nesting [14], *Beach surveys (ground)* [16], Beach survey (drone) [17].

**Equipment:** Incubator, vermiculite, waterproof calibrated temperature loggers (with an accuracy of  $\pm 0.2$  °C)

**Manpower:** 1-2

**Cost:** €€€

**Frequency:** On encountering a nest

**Actions:**

Before the arrival of eggs in the laboratory, the incubators where the eggs will be placed must be checked for correct calibration. Depending on incubator characteristics, it would be essential to place the incubator in an acclimatized room to avoid temperature fluctuations during incubation due to environmental temperature. In hot regions, it is possible to have air temperature up to 30°C modifying the temperature regime desired inside the incubator. So, the incubator should be programmed and checked to see if it reaches the desired range of incubation temperatures. For accurate data, temperature should be registered in the incubator by waterproof temperature data loggers placed inside boxes containing moist vermiculite (water potential -150kPa) as done for natural egg incubation. To ensure good hatching success, it is important to ensure there are no extreme temperature or moisture level deviations during the incubation. The most adequate temperature regime of incubation would depend on the conservation strategy for individual nests but in any case, artificial incubation regimes would fall within a normal range of incubation temperatures naturally experienced by the species, ideally predicting production of a proportion of both sex hatchlings from each clutch. Data loggers should be maintained recording incubation temperature until egg hatching. Egg transportation from the beach to incubator should ideally take place within 6 hours of nesting, to minimize movement induced injury to embryos (Boulon Jr, 1999), but if care is taken to avoid rotation and jarring of the eggs, the practice can be undertaken within first four days of nesting and result in negligible impact on hatching success (Abella et al. 2007) (see *Relocating / Translocating Clutches* protocol). Once in the laboratory, eggs should be placed in sealed plastic boxes with small ventilation holes in their lids. Inside the boxes the

eggs should be separated without contact with one another (e.g., 10 per box), and surrounded and slightly covered by moist vermiculite (water potential -150kPa). Immediately after that, every single box must be weighed in order to monitor and replace water lost during incubation. Every box and egg is identified by writing an identification code on the box. During incubation, eggs should be checked periodically. 48 hours after oviposition, it is possible to observe a white spot at the top of the eggs indicating embryo development has commenced and allowing a rough estimation of egg viability short time after oviposition. If fungal infestation is detected in any egg, they should be isolated from healthy eggs. If water loss is detected through weighing the boxes, mineral water would be added until the box recovers its initial weight. If changes in incubation temperature are suspected, temperature data logger would be launched as frequently as necessary. It is recommended to check egg appearance once per week without taking or moving the eggs (just removing a little bit of the vermiculite to observe the top of the shell). Towards the end of incubation, boxes must be inspected daily to determine day of pipping and day of hatch. On hatching, hatchlings should be isolated at 25°C to 27°C in jars lined with a wet paper towel for further days (1 to 3 days maximum) to allow the carapace and plastron to take on their proper shape (Fujimoto et al. 2020). Once hatchlings have matured, they would be measured and sampled (see *Protocol 2. Measuring turtles*; *Protocol 7. Tissue sampling*). In order to study the egg contents and take samples of non-hatched eggs see *Protocol 13. Post-hatch excavation of clutches*.

**Ethics:** Artificial incubation is a moderately invasive technique and requires ethical justification and probably a permit from the relevant national authority. Incorrect egg management during artificial incubation procedures can have a harmful impact on their hatching success, sex ratio and hence recruitment of new turtles to the wild, thus this practice should only be carried out by trained personnel. Permits will be required to transport and manipulate the eggs.

**Data to record:** Number of eggs developed, hatching success, incubation duration until pipping and hatching, raw temperature data from incubation temperature loggers should be processed to provide mean daily temperature during incubation.

**Management relevance:**

Artificial incubation and monitoring permit to protect those risky nests that are unable to be incubated naturally on the beach. Although the first option should always be natural incubation, artificial incubation will be considered in some cases: e.g., if a nest is laid late in the nesting season (end of August or September) having a high probability that incubation temperature would be under the optimal range of temperature for hatching success; or a general storm coastal flooding affecting overall specific region. Higher clutch size should have lower hatching

success than smaller nests (Tavares et. al 2021). Splitting those nests retiring some eggs to incubator it could improve hatching success on the beach, diversifying incubation setting to avoid stochastic mortality and allowing to get relevant scientific data from those primary nests. This conservation measure should be only considered for places with a low number of nests per year (e.g. Spain, France).

**Reporting:** Annual summary data presented on artificial incubation results (number of developed eggs, number of hatched eggs, day of pipping and day of hatching, hatchling weight, length and width , fungal infection monitoring) should include mean, Std Dev, range, sample size for both pipping and hatching success for every nest. Data on threats that are likely to have impacted artificial incubation success should be listed and discussed to highlight the potential need to revise management actions. At minimum, the daily incubation temperature profiles per temperature logger should be presented in graphical form to show, at a basic level, the artificial incubation temperature regimes and see if eggs are experiencing temperatures above and below the optimal range of temperature or the pivotal temperature, which can be assumed to be ~29°C. Deviations on incubation temperature based on these observations can be detected and discussed in order to improve artificial hatching success. At the next level, incubation temperatures for the periods of the middle thirds of individual nest incubation (the thermosensitive period for sex determination) can be extracted to give more detail on sex ratio produced.

**Collaboration potential:**

Artificial incubation and monitoring can be carried out by zoos, parks, marine rescue centers, NGO or University teams which have appropriated equipment and personal trained in close coordination with the MPA authority, however the manager may want to share temperature logger and nest data with research scientists who can fully investigate likely artificial incubation temperature regimes, sex ratios produced and compare this among natural nests and diversity of equipment.

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**39**

**Name: Headstarting**

**Standard: ♦**

**Topic: Animal, Habitat, Threats/Management**

**Reason:** Headstarting is a conservation tool that assumes rearing hatchlings to a larger size will give these individual juveniles survival advantage compared to their non-reared counterparts. However, headstarting programs would also permit tagging and identification of juveniles hatched from primary nests on Western Mediterranean beaches using PIT tag or satellite tracking. Having dispersal movements data of juveniles born in Western Mediterranean beaches is essential to know migratory routes and feeding habitats of juveniles in Western Mediterranean MPAs to promote conservation. Identification of female recruitment by identifying headstarted tagged animals would help to understand and protect new nesting rookeries established in Western Mediterranean.

**Context:** Headstarting is a broad term for the captive hatching and rearing of turtles through an early part of their life cycle. After reaching a prescribed age or size, headstarted turtles are released into their native habitat where they are assumed to have improved survivorship (Heppel et al. 1996). Headstarting programs have been established on an experimental basis for several endangered sea turtle species (Heppel et al. 1996; Burke 2015, Shaver et al. 2015; Barbanti et al. 2019). Beyond being a conservation tool, headstarting programs offer the opportunity to tag animals in order to study dispersal movements of sea turtles during their first stages (newborn, lost years and juvenile survival), and also permits to diminish sample collection impacts in small animals due to becoming bigger size (e.g. genetic sampling). Abalo-Morla et al. 2018 monitored 19 headstarted post-hatchlings born in Spain dispersed over large areas using variable routes, mainly off the continental shelf. Nonetheless, post-hatchlings dispersed to high-productivity warmer areas during the coldest months of monitoring. These areas might be optimum for their survival and development. There were observed differences regarding dispersal orientation and routes among individuals, even from the same nest, release date, and location. They observed a high probability of survival in head-started individuals during the first months after release, usually the most critical period after reintroduction, suggesting that such hatchling management measures may contribute to the conservation and range expansion of the loggerhead turtle population in the western Mediterranean.

Carreras et al. (2018) showed that nesting events in Western Mediterranean are carried out by both non-philopatric Atlantic and Mediterranean females exhibiting colonization behaviors. Nowadays, it remains unknown how many individuals that nest in Western Mediterranean are colonizers or the first generation of hatchlings that were hatched in that region, but according to Carreras et al. (2018), Lampedusa (Italy) seems to hold a second nesting female generation. In next decades, headstarted juvenile PIT tagging and genetic analyses would permit identify and estimate female recruitment to Western Mediterranean nesting areas.

**Associated protocols:** *Nest (and track) identification by species* [8], Locating clutches [9], Re-locating / Translocating clutches [10], Nest protection against predation [11], Identification of nest hatching [12], Beach suitability assessment for nesting [14], *Beach surveys (ground)* [16], Beach survey (drone) [17].

**Equipment:** Marine turtle headstarting installations should be structured and organized to carry out year-long activity, and it should be closely linked with a scientific institute, university or research center. Headstarting is expensive and for this reason, it usually involves the collaboration of marine turtle rescue centers, aquariums or marine research centers that already have specific equipment to maintain and care for turtles adequately. Requirements imply high cost of equipment and technical installations (tanks of several dimensions depending on turtle size, clean water source, individual seawater distribution circuit per tank, water filtration and thermostatic systems, UV light, adequate electrical installations, etc.), veterinarians and skilled and trained staff with herpetological experience, logistical organization (feeding, caring and turtle environment requirements), and operational protocols to be used when handling animals (animal transportation, rearing, husbandry, rehabilitation and tagging procedures) and data collection standards.

**Manpower:** 2 minimum for every group of 30 headstarted hatchlings

**Cost:** €€€€€

**Frequency:** All-year-round after encountering a nest or hatchlings

**Actions:**

Hatchlings would be kept and reared for at least 9-12 months, and minimum weight and size recommended for releasing should be 700 g and 15 cm curve carapace length. To accomplish this, it is advisable to have pools of various dimensions to permit different-sized animals to move freely and come to the surface to breathe, dive or remain at the bottom. Sea turtles are, generally, solitary animals, thus turtles housed together must be prevented from injuring each other. The number of animals per tank should be limited (Bluvias & Eckert 2010,

Kanghae et al. 2016), and daily observations should be carried out to identify incidents of biting other individuals housed in the same tank. Ideally, there should be one turtle per tank (or per complete system) to avoid aggression, reduce contamination, and simplify feed monitoring. Sick or injured turtles should be held in isolation tanks for medical treatment to reduce the risk of disease transfer and health complications. However, it is sometimes unavoidable to accommodate multiple turtles together in a single tank. The seawater that supplies the pools should be filtered if there is a closed, semi-open or open circulation system. Water circulation system should be independent for each tank in order to avoid a broad pathogenic microorganism infestation. Water quality should be controlled with special instruments in order to strictly respect the salinity and pH values of the Mediterranean. The pools should be supplied with a continuous circulation of water. Each pool should be equipped with one or two inflowing water faucets, a drainage hole in the upper portion and another drainage with a faucet on the bottom. Furthermore, the edges of the pools should be smooth and rounded, with no inside protrusions. The materials should be seawater resistant, non-toxic and easily cleanable. To reduce the risk of contagion each pool should have its own cleaning equipment (abrasive cloth, sponges, siphons, etc.). Water temperature should be monitored (automatic data logger). It should never fall below 19°C, and a constant temperature range between 22°C and 25°C is desirable. Thermostatic systems should be always present because temperature fluctuations could produce stress shock to the animals. Analyses of the water quality should be done periodically. It is recommended to monitor (weight and length) the animals once per week. The scientific literature can provide several examples of a correct dietary plan (fresh high protein diet of varying fish, squid, etc.) for hatchling and juvenile sea turtles (Bluvias & Eckert 2010). The amount of food may vary with each turtle, but the feeding strategy is generally to provide approximately 7% to 10% of the turtle's body weight in grams of fresh food per day (Cambell, 1996) in 2 to 3 meals, and supplemented with essential vitamins and minerals. Rigorous feed monitoring has to be done, and weight gains and losses can be used as a guide for dietary management. Turtles should undergo periodic veterinary check-ups in order to detect physical or biochemical problems during husbandry.

Environmental enriched tanks are highly recommended for better rearing results. Enrichment items are excellent for tactile stimulation and natural exploration of healthy turtles. Hiding places simulating algae or floating objects are essential for hatchlings.

Before releasing, an assessment must be conducted by a veterinarian in order to confirm an optimal health condition, physically and chemically. Also, turtles should be sampled (see *Tissue Sampling* protocol) and PIT tagging (see *PIT tagging* protocol). At this moment, an appropriate satellite tracking equipment would be deployed to a major number of turtles as

possible in order to follow them and determine their post-captivity behavior, dispersal movements, feeding areas used, and estimate juvenile survival rates (see *Deploying animal mounted data gathering and tracking equipment* protocol).

Once a turtle is cleared for release and tagged, the animal should return to a safe and non-polluted area at sea, ideally the site where the nest was found or in an area where other nesting events of its species are known to occur. The most common protocol is to allow the turtle to crawl on the beach simulating beach imprinting, into the sea.

**Ethics:** Headstarting is an invasive conservation technique and requires ethical justification and probably a permit from the relevant national authority. Incorrect animal management during husbandry can have a harmful impact on their survival, and hence recruitment of new turtles to the wild, thus this practice should only be carried out by skilled and trained personnel. Permits will be required to transport and manipulate turtles.

**Data to record:** From hatching, rigorous monitoring of individual hatchling growth (mass and length), feeding, rearing behavior, periodic health assessments and medical history (if it is the case), as well as survival or death date of every animal should be recorded.

**Management relevance:**

According to Carreras et al. 2018 *“general warming would favour the colonisation of the western Mediterranean during the following decades, while the eastern Mediterranean nesting areas would become too hot thus decreasing hatchlings survival as predicted in previous studies. The combination of contiguous range expansion and long distance dispersal mechanisms has proved to be effective in an evolutionary scale, as these animals have survived the drastic climate changes of the last 110 million years. However, whether or not these mechanisms would be fast enough to counterbalance some of the effects of the current climate change is something that remains to be tested”*. Headstarting turtles would only contribute greatly to colonization of the Western Mediterranean if undertaken on a massive scale. The most important contribution of this management technique for the WM management and knowledge is i) the use of headstarting individuals for research (eg. telemetry to study post-hatchling dispersal routes, sex-ratio, post-hatchling tagging, etc.); ii) increase new born survival when releasing is risky (eg. Late nests emerging in cold conditions). Research actions related to this potential conservation tool could elucidate decisive information of these rare events through monitoring on potential marine habitats. This,

coupled with its protection and conservation may be crucial to facilitate the possible expansion and long-term survival of the species.

**Reporting:** Annual summary data presented on headstarting results (hatching date, individual hatchling growing (mass and length), feeding strategy, animal rearing behavior, health assessments and medical history, as well as survival or death date) should include mean, Std Dev, range, sample size for at least hatchling mass and length during husbandry and survival rate. Data on threats or methodology techniques used that are likely to have impacted headstarting success should be listed and discussed to highlight the potential need to revise management actions or protocols. At minimum, the weekly biometry data should be presented in graphical form to show at a basic level hatchling growing and development during husbandry. Deviations on data can be detected and discussed in order to improve headstarting success. At the next level, scientific data collected can be analyzed to give more detail on genetics, dispersal movements, or post-headstarting survival.

**Collaboration potential:**

Headstarting can be carried out by zoos, parks, marine rescue centers, NGO or University teams which have appropriated installations, equipment and skilled and trained personnel in close coordination with the MPA authority. However, the manager may want to share headstarting results with research scientists who can fully investigate benefits of headstarting program results regarding species and population dynamics in the Mediterranean under a colonization scenario.

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