EMFF 8.3.1 Marine environmental monitoring:

Towards effective management of Malta's marine waters

Long Term Monitoring Strategy for the Marine Mammals and Marine Reptiles in Maltese Waters

Result 3

Report by EcoMarine Malta Ltd

TENDER FOR THE ESTABLISHMENT AND PILOT

IMPLEMENTATION OF A LONG-TERM MONITORING STRATEGY FOR

MARINE MAMMALS AND MARINE REPTILES IN MALTESE WATERS

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Investing in sustainable fisheries and aquaculture

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Introduction

This report is the final deliverable of the “Tender for the establishment and pilot implmentation of a lon-term monitoring strategy for marine mammals and marine reptiles in Maltese waters”, funded as part of the project “EMFF 8.3.1 Marine environmental monitoring: Towards effective management of Malta’s marine waters”. The report outlines the long-term monitoring strategy for marine reptiles and mammals and is based on the Data Analysis Report of the Pilot Project Implementation. Several of the monitoring approaches that are considered for the purpose of the long-term monitoring strategy were applied and tested in the 2021 as part of the pilot survey in order to comply with the policy requirements stipulated by the EU Habitats Directive 92/43/EEC (HD), the EU Marine Strategy Framework Directive 2008/56/EC (MSFD) and the Barcelona Convention. The approaches employed in the pilot survey were:

- Aerial-based survey
- Vessel-based survey
- Photo-identification
- Behaviour
- Drone videos
- Acoustic
- Satellite tracking
- eDNA
- Local ecological knowledge (LEK)
- Literature review

Most of these approaches have been successfully used in the past 50 years to estimate animal abundance and distribution, to investigate habitat and demographic parameters, and to assess anthropogenic pressures of marine mammals and marine turtles. Other methods have only been recently developed (i.e., drones, eDNA) and, in some areas, are becoming very promising.

For Malta’s waters, survey-based methods using different platforms applied in the pilot survey, have been successful for collecting data as required by the MSFD. In particular the aerial-based survey allowed the coverage of a relatively large area of Malta’s waters in a few hours at a relatively little cost. The vessel-based survey allowed the collection of other essential data, such as pictures for photo-identification, animal behaviour (visual and by using drones), reaction to the research platform, presence of calves and sounds (from dolphins and their environment). All these approaches have been included in the present strategy.

Approaches based on acoustic surveys used during the pilot implementtion process were less efficient than line- transect methods to estimate abundance. Acoustic methods were included in this strategy in the form of sound trap and bottom recorder to investigate dolphins’ presence and their interactions with fishery, aquaculture and tourism boating activities.

In recent years, the identification of dolphin vocalizations using acoustic datasets has improved the ability to study these animals in the wild. Acoustic recordings have also been utilized to investigate the occurrence and distribution of cetacean species, such as resident coastal dolphins. Moreover, the correlation between dolphin acoustic behaviour and ambient noise (natural, anthropogenic, and other biological sources) can provide additional insights into their ecology and habitats (Lammers et al., 2017; Caruso et al., 2020) In particular, the monitoring of coastal dolphins is essential to identify critical areas of habitat use and mitigate the impacts of anthropogenic activities (Ingram and Rogan, 2002).

Satellite tracking and eDNA gave some interesting results but have not been considered as a priority for this long-term monitoring strategy.
Approaches based on LEK and historical data are not the focus of this strategy and therefore have not been included.

The detailed protocols for data collection and research equipment needed for most of the monitoring approaches included in the present strategy are fully detailed in the pilot survey report (ERA, 2022). In this strategy a description of the monitoring methodologies, locations, frequencies and analysis of the data is reported for each monitoring method. In the analysis section, statistical methods, software and packages, important references and websites are provided.

Formulas and assumptions of the cited methods are not reported in the present strategy. This is because the many methods cited would have made the document incomplete. In fact, for each method there is not only one formula but rather a family of formulas of which the best use depends on the data collected and on the scientific question aimed to be answered. Instead, the sources cited for each method allow the policy implementer to easily and quickly navigate through the different techniques.

EU and regional monitoring and reporting requirements for marine mammals and reptiles

Marine Strategy Framework Directive

The **Marine Strategy Framework Directive 2008/56/EC (MSFD), 2008,** aims to achieve or maintain a Good Environmental Status (GES) of the EU marine waters, through the development and implementation of marine strategies by each member state. In accordance with Article 17 of the Directive, the monitoring strategies must be kept up-to-date and reviewed every six years, with the current monitoring cycle covering the period 2020 to 2025. Criteria and methodological requirements, as well as specifications and standardised monitoring and assessment methodologies are established by Commission Decision (EU) 2017/848. For marine mammals and reptiles, the species status assessment requires data in accordance with the criteria identified as primary or secondary for the Descriptor 1 (D1) - Biodiversity and described below:

- **D1C1** — Primary - "The mortality rate per species from incidental by-catch is below levels which threaten the species such that its long-term viability is ensured".
- **D1C2** — Primary - "The population abundance of the species is not adversely affected due to anthropogenic pressures, such that its long-term viability is ensured".
- **D1C3** — Secondary - "The population demographic characteristics (e.g., body size or age class structure, sex ratio, fecundity, and survival rates) of the species are indicative of a healthy population which is not adversely affected due to anthropogenic pressures".
- **D1C4** — Primary - "The species distributional range and, where relevant, pattern is in line with prevailing physiographic, geographic and climatic conditions".
- **D1C5** — Primary - "The habitat for the species has the necessary extent and condition to support the different stages in the life history of the species".

Habitats Directive

The **Habitats Directive 92/43/EEC (HD), 1992,** represents the main European legislation for the conservation and protection of species of community interest listed in its annexes. The main goal of the directive is to maintain and restore natural habitats and species of wild fauna and flora of Community interest at a Favourable Conservation Status (FCS), which is achieved when:

- Population dynamics data on the species concerned indicate that it is maintaining itself on a long-term basis as a viable component of its natural habitats.
- The natural range of the species is neither being reduced nor is likely to be reduced for the foreseeable future, and
- For habitat, there is, and will probably continue to be, a sufficiently large habitat to maintain its populations on a long-term basis.

Range, Population, Habitat and Future Prospects are the parameters used for the assessment of the conservation status of the species, within a 6-yearly cycle. The current monitoring cycle covers the period 2019 to 2024.

All species of marine mammals and reptiles found in Maltese waters are listed in Annex IV of the Habitats Directive, meaning they require a strict protection regime within and outside Natura 2000 sites. The bottlenose dolphin and the loggerhead turtle are listed in Annex II that lists animal and plant species of Community interest of which conservation requires the designation of SACs forming part of the Natura 2000 network.

Barcelona Convention and IMAP

The **Integrated Monitoring and Assessment Programme and related Assessment Criteria (IMAP), 2016,** was established under the Barcelona Convention. This programme results from the EcAp process, which brings together the MAP's measure under an integrated framework for the achievement of the Good Environmental Status (GES) of the Mediterranean Sea. Among the five common indicators (CI) related to biodiversity (EO1) fixed by IMAP, three are about marine mammals including the species regularly occurring in Maltese waters:

- **CI3** - Species distributional range,
- **CI4** - Population abundance of selected species,

- **CI5** - Population demographic characteristics.
- **CI12** - Bycatch of vulnerable and non-target species

The situation is similar for marine turtles which require data for two to three CI to determine if GES is achieved, these are: CI3 on the distribution of turtles; where turtles need to remain present across their known distribution, CI4 on the abundance of turtles; where turtles numbers need to be stable or increasing to ensure GES, and to a lesser extent CI5 on the demography of turtles; which in this situation relates to identifying proportions of turtles in different size classes that relate to maturity status and to the maintenance of suitable sex ratios.

Comparison between the EU monitoring requirements

The assessment system represented by the MSFD and HD significantly overlaps with the one represented by the Ecosystem Approach (EcAp) and the IMAP process of the Barcelona Convention. Both assessment systems intend to report on the conservation status of the species through the elaboration of criteria/indicators to determine GES and/or FCS. Monitoring strategies are therefore needed to address such criteria/indicators and findings need to be compared to baselines or threshold values.

The equivalence between criteria and indicators required for the assessment of GES are reported in Table 1.

Table 1 - Comparison between criteria for the MSFD and HD Criteria, and IMAP Common Indicators

Monitoring requirements	Criteria in accordance to the Commission Decision (EU) 2017/848 of 17 May 2017	Criteria in accordance to the Habitats Directive 92/43/EEC	EO1 Common Indicator (CI) Under the IMAP
Bycatch mortality	**D1C1:** Primary - The mortality rate per species from incidental by-catch is below levels which threaten the species such that its long-term viability is ensured	**n/a**	**CI 12**: Bycatch of vulnerable and non-target species
Population	**D1C2:** Primary - The Population Abundance of the species is not adversely affected due to anthropogenic pressures, such that its long-term viability is ensured.	**Population**	**CI 4**: Population abundance of selected species

	D1C3: Secondary - The population demographic characteristics (e.g. body size or age class structure, sex ratio, fecundity and survival rates) of the species are indicative of a healthy population which is not adversely affected due to anthropogenic pressures.		**CI 5**: Population Demographic Characteristics (e.g. body size or age class structure, sex ratio, fecundity rates, survival/mortality rates related to marine mammals, seabirds, marine reptiles)
Distributional Range	**D1C4:** Primary**-** The species distributional range and where relevant, pattern, is in line with prevailing physiographic, geographic and climatic conditions	**Range**	**CI 3**: Species Distributional Range (related to marine mammals, seabirds and marine reptiles)
Habitat extent and condition	**D1C5:** Primary - The habitat for the species has the necessary extent and condition to support the different stages in the life history of the species.	**Habitat for the Species**	Partially related to **CI5**

Marine Mammals and Reptiles Considered for the Long Term Monitoring Strategy

Twenty-seven species and subspecies of cetaceans have been recorded in the Mediterranean Sea (ACCOBAMS, 2021), among which eight (fin whale, sperm whale, Cuvier's beaked whale, long-finned pilot whale, Risso's dolphin, common bottlenose dolphin, striped dolphin, and common dolphin) have been recorded in Maltese waters (Notarbartolo and Mifsud, 2002). However, only the common bottlenose dolphin (*Tursiops truncatus)*, the common dolphin (*Delphinus delphis)*, and striped dolphin (*Stenella coeruleoalba)* are regularly encountered in Malta.

Among marine turtles, six species have been recorded in the Mediterranean (Casale *et al*., 2020), with the loggerhead turtle (*Caretta caretta*) being the most common in Maltese waters.

Only the species that are regularly encountered in Malta, as mentioned above, will be the focus of this Long Term Monitoring Strategy. The selection of the species, as reported in Table 2, follows the updated assessment of status of marine mammals and reptiles (ERA, 2020), and is based on the requirements and criteria listed in the Commission Decision (EU) 2017/848 and the Guidance for Assessments under Article 8 of the Marine Strategy Framework Directive (Walmsley *et al*., 2017).

Table 2 - Target species for the LTMS, to be assessed under Descriptor 1 of the EU MSFD

Ecosystem Component	Species group	Species
Mammals	Small toothed cetaceans	Common bottlenose dolphin (*Tursiops truncatus)*
		Common dolphin (*Delphinus delphis)*
		Striped dolphin (*Stenella coeruleoalba)*
Reptiles	Marine turtles	Loggerhead turtle (*Caretta caretta)*

The **common bottlenose dolphin** (hereafter 'bottlenose dolphin') is one of the best known cetaceans worldwide and one of the most frequently observed in the Mediterranean Sea, with records of sightings reported in all the Mediterranean countries. The subpopulation of the Mediterranean Sea, although considered as one, includes genetically but also socially and culturally differentiated populations across its range (Gaspari *et al*., 2015; Natoli *et al.*, 2005). The recent down listing from Vulnerable (2009) to Least Concern in the IUCN Red List does not exclude that some local populations might be particularly vulnerable due to intense pressures from anthropogenic activities. This species is in fact listed in Annex II of the HD (listing species whose conservation requires the designation of Special Areas of Conservation), in Annex II of the Protocol concerning Specially Protected Areas and Biological Diversity (SPA/BD Protocol) and requires protection according to other national and international legislation.

The **common dolphin** was abundant and widespread in the Mediterranean Sea (Bearzi *et al*., 2003) until the 1960s, but declined rapidly in the following decades due to intentional killing and conflicts with fisheries, causing subsequent demographic fragmentation. Due to this decline, the Mediterranean subpopulation has been listed as Endangered in the IUCN Red List since 2003. Although killing practices have ceased since then, important threats are still affecting the species, such as prey depletion, bycatch, habitat degradation, chemical pollutants, and underwater noise, resulting in scattered occurrences and small group sizes.

The **striped dolphin** is the most sighted and abundant species of cetacean in the Mediterranean Sea, where the Western Basin seems to host the most suitable habitat for this species (Mannocci *et al*., 2018). This dolphin is found predominantly in oceanic waters (Azzelino *et al*., 2008) in large groups.
Like the above-mentioned delphinids, the Mediterranean striped dolphin is genetically distinct from its Northeast Atlantic conspecific and represents a subpopulation that is listed as Least Concern in the IUCN Red LIst. However, anthropogenic activities represent significant threats for the species, especially in terms of bycatch, chemical pollution, microplastic ingestion and disease (Braulik *et al*., 2021).

The **loggerhead turtle** is a long-lived reptile which is relatively well understood (Casale *et al*., 2018). The species has established an endemic regional management unit in the Mediterranean but also loggerhead turtles originating from the Atlantic are present in the western Mediterranean (Wallace *et al*., 2010), with recent evidence indicating they are now breeding there (Carreras *et al*., 2018). In the Mediterranean the loggerhead regional management unit is listed as Least Concern, but with the caveat that this population status is largely conservation dependent (Casale, 2015).

Monitoring Requirements

Bycatch mortality

As part of the requirements of the Common Fisheries Policy (Regulation 1380/2013), Malta collects data on incidental by-catch of marine mammals and reptiles per métier through the Data Collection Multi-Annual Programme (DC-MAP), as set by the Commission Delegated Decision (EU) 2019/910 and Commission Implementing Decision (EU) 2019/909, and required by (EU) Regulation 2017/1004 and (EC) Commission Regulation 665/2008.
According to the results of previous assessments, the artisanal fishing methods primarily used in Malta by the local fisheries, do not seem to represent substantial threats to small odontocetes nor turtles in terms of bycatch, as evidenced by the absence of records (Terribile *et al*., 2020, ERA, 2020). However, this does not necessarily exclude bycatch impacts, and the vulnerability of marine mammals and reptiles to such threat must be taken in consideration. Accurate information collected through improved and independent data collection processes are therefore necessary to ensure that the current data available reflect the real scenario.

Monitoring methodologies for assessing bycatch mortality of small toothed cetaceans

To quantify and spatially describe fisheries bycatch, two types of information are required: a measure of fishing effort (total number of fishing days) and a bycatch rate (number of individuals taken per unit of fishing effort). Data shall therefore be provided per population per fishing métier to identify particular fisheries and fishing gears most contributing to incidental catches for each species.

1. **Assessment of bycatch rates using fishery-dependent and independent data**

Different and complementary methodologies, summarised below, are proposed to provide estimates of bycatch mortality.
Onboard observations should be carried out independently by trained observers to record incidental bycatches in accordance with standardised methods.
Interviews should be used as an alternative but also as a complementary tool for the collection of quantitative information when onboard observations cannot be carried out. Standardised questionnaires should be used to conduct the interviews in the ports or wherever fishers can best be gathered and approached.

Logbooks should be completed by fishers while at sea. For this methodology, correct species identification is fundamental for correct reporting, thus training and workshops should be provided.

Protocols for the data collection to be used for each of the methods described above are presented in the Annexes of the technical paper "Monitoring the incidental catch of vulnerable species in Mediterranean and Black Sea fisheries: Methodology for data collection" by FAO (2019).

Strandings provide additional data on bycatch, especially whenever the cause of death can be detected (e.g. entanglement in fishing gears). However this type of data can be only considered qualitative and may not be directly related to the number of animals by-caught in the area.

Acoustic monitoring carried out using SoundTraps, a relatively low-cost PAM (Passive Acoustic Monitoring) system (https://oceansonics.com/products/) deployed on actively fishing nets during fishing operations. The acoustic system provides high-resolution information of toothed whales presence, movements and acoustic behaviour (Macaulay *et al.* 2022) near the nets. The use of the SoundTraps across broad spatial and temporal scales will generate an extensive dataset on dolphins behaviour near nets, which might be used to develop a model of bycatch risk. Towed array can be employed during ship based acoustic monitoring to estimate the interaction of the dolphins presence and interaction with trawling fishing activity where the use of sound traps is less recommended.

Monitoring locations. Observations, interviews and logbooks should cover the entire fishery by random sampling in order to obtain unbiased estimate of bycatch mortality, ensuring that fishing trips originating from all fishing ports are sampled.

The suggested acoustic monitoring system can be installed by a researcher during routine fishing operations and requires only 15 minutes of additional boat time to find the PAM devices on the bottom. By installing the PAM System on different fishing gears, it is possible to study different areas and thus identify those where there is significant presence and interaction between dolphins and fisheries. The locations are represented by the fishing zone, in the FMZ, assigned to each fisherman participating in the process.

Monitoring Timing and Frequency. Sampling, including onboard observations, interviews and logbooks and acoustic monitoring should be carried out annually and cover all seasons, in order to allow annual and seasonal estimates of bycatch mortality. According to FAO (2009) sampling coverage should range from 2% to 7% of the fishing effort. Proposed sampling include:

- 20 onboard observations per year (5 per season) to be carried out by fishery-independent observers
- 120 interviews per year (40 fishers to be interviewed every 3 months)
- One logbook follow-up every 3 months

Acoustic devices should be employed annually and cover all seasons on different fishing gears (i.e. purse seine and long-lining). A towed array can be employed to collect acoustic data in presence of trawling fishing gears. Acoustic sampling should be performed yearly by collecting 1 week or recordings per month.

Analysis. Simple ratio estimators are sufficient in a properly designed study. The bycatch rate is given by the number of individuals dying for bycatch per observed fishing trip. When estimating the total bycatch, the bycatch rate should be calculated per métier per year (bycatch rate x fishing effort), as described by the FAO (2019). Acoustic spectrograms should be analysed by manually identifying the presence of the animal vocalisations and extracting the associated acoustic parameters [i.e. frequency (Hz), durations (seconds)] through dedicated acoustic software such as PamGuard (University of St. Andrews - https://www.pamguard.org/), RavenPro (Cornell University - https://ravensoundsoftware.com/software/raven-pro/) or Sound Emission Analyzer Pro SeaPro (http://www-3.unipv.it/cibra/seapro.html- developed by University of Pavia, CIBRA) to detect dolphins' presence within recordings and also classify call types (whistles, tonal sounds).

Monitoring methodologies for assessing bycatch mortality of the loggerhead turtle

1. Assessment of bycatch rates using fishery-dependent and - independent data

The same methods proposed for small toothed cetaceans apply for turtles, excluding acoustic monitoring. However, it has to be noted that, since mortality estimates can only be based on the individuals declared dead at observation, the mortality rates obtained are to be considered underestimated due to high post-release mortality [see de Quevedo *et al*., (2013)]. In the case of the Mediterranean loggerhead turtle population, individuals larger than 20 cm are considered most at risk of bycatch (de Quevedo *et al*., 2013) therefore representing the section of population to be considered in the estimation of mortality rates. In addition, the relevant parameters should be determined separately for juveniles and adults.

Monitoring locations. As for small toothed cetaceans

Monitoring Timing and Frequency. As for small toothed cetaceans

Analysis. As for small toothed cetaceans

Costs

Table 3 - Estimated costs for monitoring dolphins and turtles Bycatch under D1C1

Item	Unit type	Number per monitoring cycle[1]	Unit cost (€)	Total (€)
Questionnaires with fishers (1)	person hours	1,920	15	28,800.00

[1] 6 years

On-board observations (2)	person hours	1,200	15	18,000.00
Logbook follow up with fishers (3)	person hours	1,920	15	28,800.00
Compilation of data	person hours	288	12	3,456.00
Analysis in relation to national fishing effort	person hours	60	15	900.00
Deployment & Retrieval of Sound Trap (4)	person hours	540	10	16,200.00
Mapping, plotting, areas more susceptible to dolphins' presence	person hours	480	18	8,640.00
Characterization and Mapping fishing fleets and their métiers	person hours	320	12	3,840.00
Acoustic data analysis and assessment	person hours	540	18	4,860.00
Interaction with fisheries, bycatch data analysis and assessment	person hours	540	18	9,720.00
Footage analysis and assesment	person hours	1,080	15	3,240.00
Bycatch database development/maintenance	person hours	288	12	3,456.00
Stranding database development/maintenance	person hours	288	12	3,456.00
SoundTraps (5)	equipment	3	6,000.00	18,000.00
Batteries for SoundTraps	consumables	18	10.00	180.00
Total D1C1				€ 151,548.00

(1) Assuming 1 questionnaire (every 3 months), overestimated at taking 2 hours because some visits will not be successful, with 40 fishers (vessels >12m), annually throughout the 6-year cycle
(2) Assuming 20 fishing boat on-board surveys per year 2 person working 10 hours per day
(3) Assuming follow up every 3 months with 40 fishers (<12m vessels), overestimated at taking 2 hours because some visits will not be successful, annually throughout the 6-year cycle
(4) Assuming 1 person working 2 hours per deployment and retrieval, 15 days per 3 fishing boat with different gears in different areas
(5) Sound traps have been allocated 100% in D1C1and indicated as cost 0 in overlapping methodologies tables

Population Abundance

Two methods are mainly used to estimate animal abundance. Data on population abundance are essential to understand the trends in populations over time. Presence and density of large fish (such as sharks, tunas, swordfishes, mobulas, sunfishes, etc.) and litter can also be estimated through such methodologies.

Method 1 - Line-transect
Line transect methods sample space where animals live providing the density of animals in a defined area at a particular time or over a period. Line transects were selected as they represent the most widely used methodology to estimate abundance and assess the density of marine animals occurring at low densities and over large or relatively areas. Line transect methods are based on the idea of estimating the abundance of animals in systematically or randomly placed strips, surveyed by transects. The abundance is then extrapolated to the entire study area. Because not every animal is expected to be spotted in the area, the measurements of perpendicular distance of the animal (or the group) from the transect line is used to estimate the probabilities of detection. The detection probability will be then used to correct the abundance estimations.

Visual effort should be carried out following a survey design. In order to be efficient, survey design must be compatible with the logistics (budget available, cost of the platform, platform autonomy, etc.) and the characteristics of the region. Survey designs can be easily generated using dedicated softwares. Currently the most used is *Distance* (Thomas *et al.,* 2010) that has the advantage to be open-access, to be continually updated by the developers and to provide technical and analytical support by a worldwide mailing list. Line-transect surveys can be carried out using aircraft or boats. For aerial survey parallel/line designs are generally used. For vessel-based surveys, systematic continuous zigzag designs are preferred to parallel-line designs to decrease navigation off effort between transects.

Line transect methods assume that all animals present on the transect line will be detected with certainty. For cetaceans, this is clearly not the case, with the result that estimates of abundance will be biased downwards by an unknown amount. There are several methods to account for the proportion of animals missed on the transect (i.e., availability bias). Method details for aerial surveys are provided by Forney et al. (1995), Hiby & Lovell (1998), and Hiby (1999), and for vessel surveys by Palka (1995), Buckland & Turnock (1992) and Borchers et al. (1998). Every detail related to transects methods is available by Buckland et al. (2001, 2004).

Method 2 - Mark-recapture
Using photo-identification, mark-recapture methods sample and re-sample distinct individuals rather than areas. These methods provide an estimate of the number of animals using a defined area during the study period (not the density of animals in the defined area). Mark-recapture methods have proven to be a successful way to estimate demographic parameters of the cetacean populations such as abundance, survival probability, growth and recruitment rates as well as residency and movement patterns.

Mark-recapture methods are generally based on photo-identification techniques that allow the capture and recapture of individuals without physical handling. The combination of multiple recapture occasions results in individual capture histories that are used by mark-recapture models to estimate the demographic parameters. Mark-recapture methods are very effective for coastal relatively small “resident” populations, such as bottlenose dolphins.

Mark-recapture estimates refer to the population of animals that have appropriate marks. Marks need to be unique, cannot be lost, and must be correctly recorded and reported. For dolphins, photo-identification is based on long-term natural marks such as notches and nicks in the dolphins' dorsal fins (Würsig and Würsig, 1979; Würsig and Jefferson, 1990; Wilson et al., 1997), as well as on any additional mark in other parts of the body. During sightings, all dolphins in the group should be photographed, regardless of how distinct their markings are because this reduces the heterogeneity of capture probabilities resulting from differences in distinctiveness of natural markings. If not all animals in the population have sufficient natural markings, data on the proportion of well-marked animals is used to estimate the proportion of markable animals in the population (Wilson et al., 1999).

Picture processing is based on the identification of matches between photographs of individuals (i.e., the recaptures). This can be done manually, using the photographic catalogue of existing individuals or using dedicated softwares (e.g. Hiby and Lovell 1990; Hillman et al. 2002).

Monitoring methodologies for assessing the abundance of small toothed cetaceans

1. Aerial-based surveys using line-transect methods for small toothed cetaceans

Aerial surveys provide coverage for large areas in a relatively short time, maximising the effort in good weather conditions, and allowing high accuracy and precision in the collection of important data such as the group size and the animal position (e.g., measure angle abeam is more precise than measure animal distances at sea). Aeroplanes also do not affect potential animal movement at sightings (one of the conditions of the line transect methods). Finally, aerial surveys are relatively less expensive than vessel surveys in terms of budget expenses and personnel (i.e., three-four researchers for a few days instead of eight-nine people for several weeks). Aerial survey allows estimates of density and abundance for all species encountered.
A detailed protocol for visual aerial-based data collection is available through the Deliverable 2a of the project [ERA (2022)].

Monitoring locations. Due to their speed (typically 90–100 knots) aircrafts allow little time for animals to surface. This is an issue in particular for cetacean species that spend most of their time underwater. To increase the probability that dolphins are available at the surface when the survey occurs, the size of the study area must be large (at least 10,000 km2). Malta's Fisheries Management Zone (FMZ MIC-MT-MS-01 25 NM, 11,700 km2 - hereafter FMZ) was identified as the correct scale of monitoring for the status assessment of very mobile species such as cetaceans (UNEP/MAP, 2011). All target species are regularly and commonly found within the FMZ (Figure 1). The Natura 2000 sites specifically identified for the cetaceans are included in the FMZ (MT0000113, MT0000115 and MT0000116).

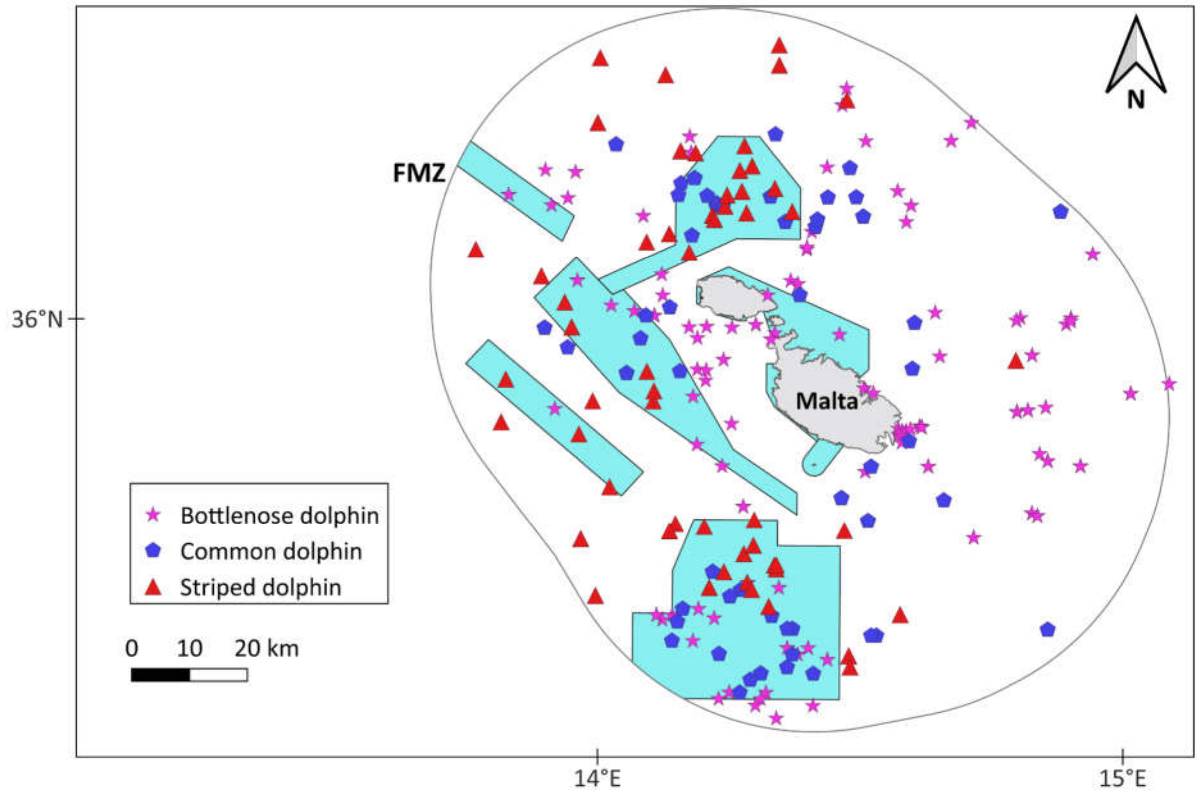

Figure 1 - Sighting distribution for the three target species bottlenose dolphin (pink star), common dolphin (blue pentagon), and striped dolphin (red triangle) within the Malta Fishery Management Zone (FMZ). Sightings were made during the following projects: ERA GF/Admin/40/2020, LIFE10 NAT/MT/090, LIFE11 NAT/MT/1070, BirdLife Malta, and LIFE12 NAT/MT/000845. The light blue polygons represent the Natura 2000 sites including those designated for conservation of *Tursiops truncatus* (MT0000113, MT0000115 and MT0000116).

Monitoring Timing and Frequency. At least one summer survey every six years. Depending on the budget, a winter survey in the same year would provide seasonal information about abundance and distribution.

Analysis. Design-based abundance of dolphins will be estimated using the equations in Buckland et al. (2001). The detection probability, i.e. the probability that a group of dolphins in the covered area is detected, can be estimated using the software Distance or the free and open-source software R with dedicated R packages such as *Distance*, *mrds*, *dsm* and *dsims*, all available on the CRAN website page (https://cran.r-project.org/web/packages/Distance/index.html). Detection probability models can assume that the probability to detect dolphins is only dependent on their perpendicular distance from the trackline, or they can include heterogeneity by incorporating covariates affecting the detectability in the detection function (such as sea state, observer, platform, etc.).

2. **Systematic and regular vessel-based surveys using line-transect methods small toothed cetaceans**

Coastal line-transect surveys can be carried out using different kinds of boats. Sailing boats have the advantage of providing shelter and facilities for the researchers allowing navigation in open waters. On the contrary they are generally slower (5-8 knots) and more challenging to manoeuvring around animals than rigid-hull inflatable boats - hereafter RIBS - (14-18 knots). Survey average speed should be at least two or three times faster than the average speed of the animals (2-3 knots) being surveyed in order to avoid biases related to animal movement (i.e., recapture of the same individuals on the same transect). Platform height is also a factor to be taken into account, as higher are located the observer's eyes, easier and more accurate is the measurement of the animals distances. A detailed protocol for visual vessel-based data collection is available through the Deliverable 2a of the project [ERA (2022)].

Monitoring locations. Data on abundance are among the most basic in ecology and conservation biology. However, this information is quite challenging to obtain for large, (often) opportunistic, and very mobile animals such as cetaceans. One of the main issues is to establish the appropriate size for the study area when estimating abundance using line transect methods. Representative study areas should in fact include at least all essential habitats for the target population (e.g., feeding, reproductive/mating, resting, socialising areas). The waters within the 12 NM from the coastline represents a good compromise between the probability to find all the three target species and the feasibility of visual vessel-based surveys. Studies from the past show that bottlenose, common and striped dolphins are all regularly and commonly encountered within the 12 NM (see Figure 2). For safety and logistic reasons, surveys within 12 NM from the coast are preferred when using platforms such as small sailing boats or RIBs. Furthermore, the 12 NM represents a good space-unit to understand if abundance is or will be adversely affected due to anthropogenic pressures. It is In this area in fact that most human activities such as artisanal fishing, aquaculture, and leisure boating occur. Malta's 12 NM include most of the Natura 2000 sites, identified for the conservation of *Tursiops truncatus* (MT0000113, MT0000115 andMT0000116).

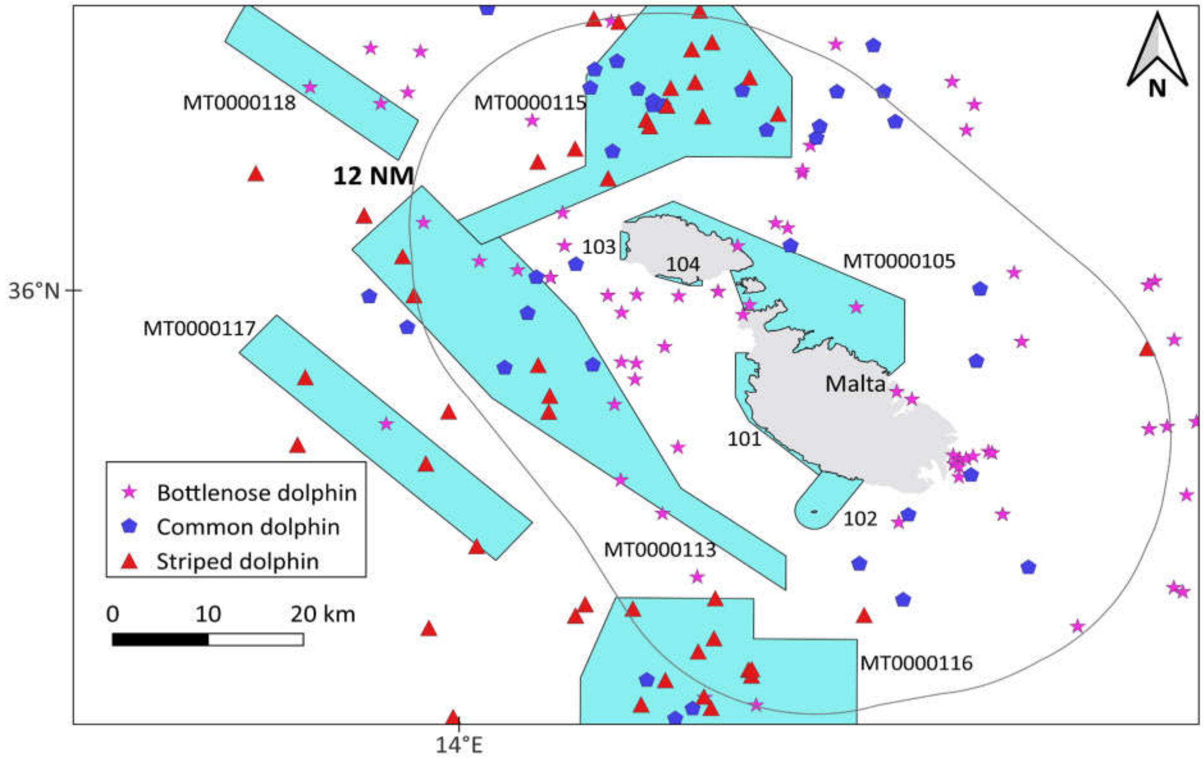

Figure 2 - Sighting distribution for the three target species bottlenose dolphin (pink star), common dolphin (blue pentagon), and striped dolphin (red triangle) within the 12 NM (the black line represents the offshore boundary of the 12 NM). Sightings were made during the following projects: ERA GF/Admin/40/2020, LIFE10 NAT/MT/090, LIFE11 NAT/MT/1070, BirdLife Malta, and LIFE12 NAT/MT/000845. The light blue polygons represent the Natura 2000 sites including those designated for the conservation of *Tursiops truncatus* (MT0000113, MT0000115 and MT0000116); 101=MT0000101, 102=MT0000102, 103=MT0000103, and 104=MT0000104.

Monitoring Timing and Frequency. To date, there is no regular cetacean monitoring of the 12 NM in Malta. The period to cover is suggested to span over two months, with at least 15 days to spend at sea, twice per year. Periods could be January-February and July-August, the latter important to monitor bottlenose dolphin calving season. Data collection winter will also provide useful information about dolphin behaviour with low traffic pressure from leisure vessels. Such monitoring should be undertaken at least once during the six-year reporting cycle.

Analysis. Same as analysis for aerial-based surveys.

3. Abundance using mark-recapture methods for common bottlenose dolphin and common dolphin

Photo-identification of bottlenose and common dolphins should be carried out from two platforms: a sailing vessel, following the vessel-based survey outlined in the chapters above, and a rigid-hull inflatable boat (hereafter RIB). Mark-recapture methods are rarely used with striped dolphins because of their large number (several thousands) and a preferred offshore habitat.

The RIB-based survey will follow navigation *Ad libitum*, (meaning that no predefined routes will be followed) to investigate dolphin presence around the fish farms or within other hotspots such as Marine Protected Areas. The main objective of the RIB-based survey around fish farms is to collect photo-identification of dolphins interacting with the cages, mainly common bottlenose dolphins that have been reported feeding around the cages.

Protocols for data collection during the RIB-based survey are the same as the vessel-based survey with the exception that navigation, environmental, and sightings data will be tape-recorded. Data on geographical position and time will be automatically collected by a GPS and, later, combined with tape-recorded data.

Using digital cameras with appropriate zoom lenses (e.g., 200 or 300 mm focal length) pictures of the dorsal fin of each individual will be taken. Pictures of the body can also be useful for individual identification. During the sighting, pictures of all individuals must be taken, irrespectively of their distinctiveness. A detailed protocol for picture processing is available by ERA (2022), including the categories used for dolphin-fin distinctiveness, and the categories used for the picture quality.

It is important to note that good quality pictures of dolphin dorsal fins from opportunistic platforms can be also used for photo-identification.

Monitoring locations. Two areas have been identified for photo-identification studies: the 12 NM (see Figure 2) and the coastal area around the fish farms. This area, called fish-farms corridor (FFC), can be generated by digitising the fish farms visible from Google Map, joining them in a single polygon (using the tool "Minimum Bounding Geometry" and geometry Type "Convex hull" available in the free and open-source software QGIS), and generating a buffer of 0.001 degrees around the convex hull obtained (see Figure 3).

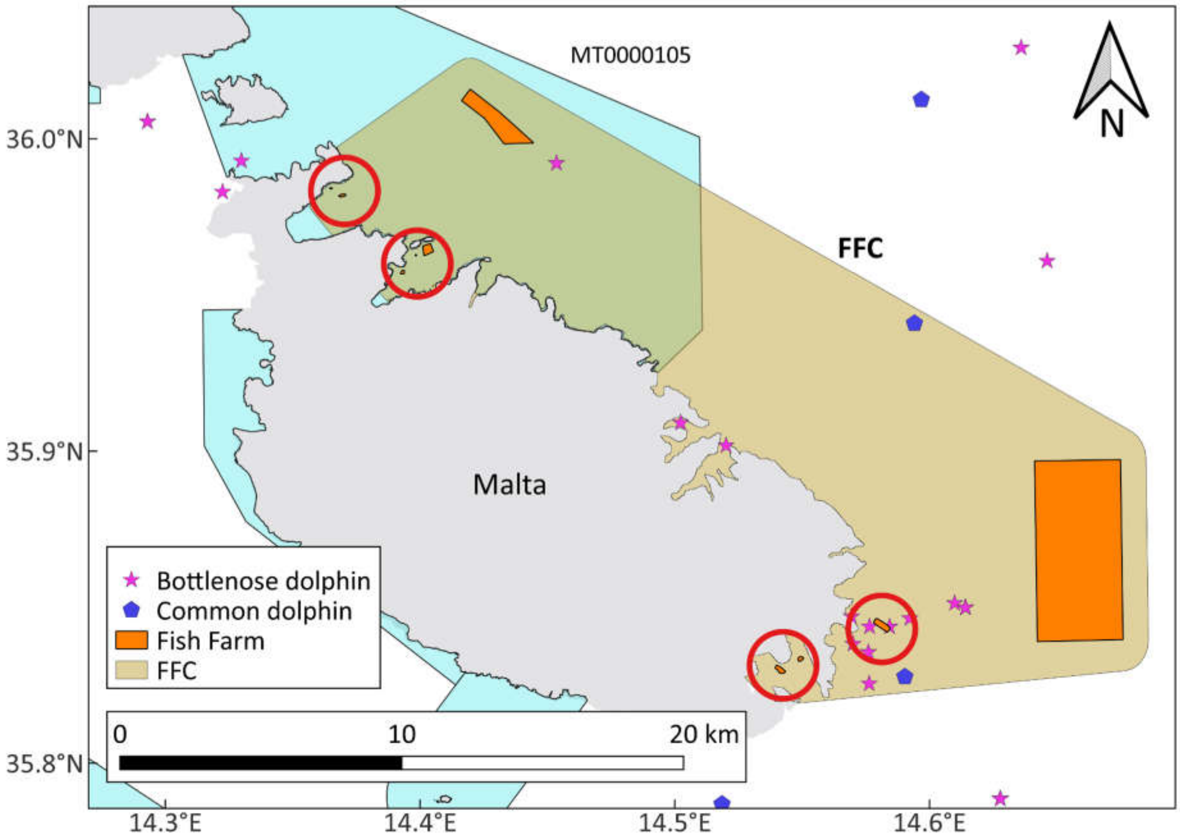

Figure 3 - Map showing the study area, fish-farms corridor (FFC) selected to carry out the RIB-based survey. Malta's fish farms are also shown: the orange polygons represent the two tuna farms, North and South of Malta; the four red circles represent areas where the sea bream, sea bass and meagre cages are positioned. The map also shows the sightings distribution of bottlenose and common dolphins from previous projects: ERA GF/Admin/40/2020, LIFE10 NAT/MT/090, LIFE11 NAT/MT/1070, BirdLife Malta, and LIFE12 NAT/MT/000845.

Monitoring Timing and Frequency. 15 days twice per year (summer and winter) within the 12 NM, during the vessel-based survey and 5 days per month per year (i.e., 60 days per year) within the FFC during the RIB-based survey. Such monitoring should be undertaken at least once during the six-year reporting cycle.

Analysis. Photo processing precedes statistical analysis in mark-recapture studies using photo-identification. Each new photograph should be graded for quality and then matched to the existing catalogue of marked animals (matches are recaptures). Mark-recapture methods require at least two sampling occasions. The Petersen estimator (Hammond, 2010), or a variant of it, is used for closed population models where it is assumed that population size is closed to births, deaths, immigration and emigration (i.e. it does not change over the period of study). This can also be assumed when a study is sufficiently short in time. If the population is not closed open population models might be used to estimate abundance although these models are very data hangry.

The most used free and open source softwares are CAPTURE, and MARK (both downloadable at the website page www.phidot.org/software/mark/downloads). In particular MARK provides an extensive range of models that also provide estimates of survival, recruitment, and population growth rates. The "Rcapture" package in the free and open-source software R also provides models for mark-recapture analysis.

Monitoring methodologies for assessing the abundance of the loggerhead turtle

1. Aerial-based surveys using line-transect methods

The same standardised methods (aerial-based and vessel-based surveys using line-transect methods) described above for cetaceans are suitable for estimating the abundance at sea for the loggerhead turtle.

<u>Monitoring locations</u>. As for small toothed cetaceans i.e. 25 nautical miles. **Figure 4** shows the sighting distribution for the loggerhead turtles from past research projects.

<u>Monitoring Timing and Frequency</u>. As for small toothed cetaceans.

<u>Analysis</u>. As for small toothed cetaceans.

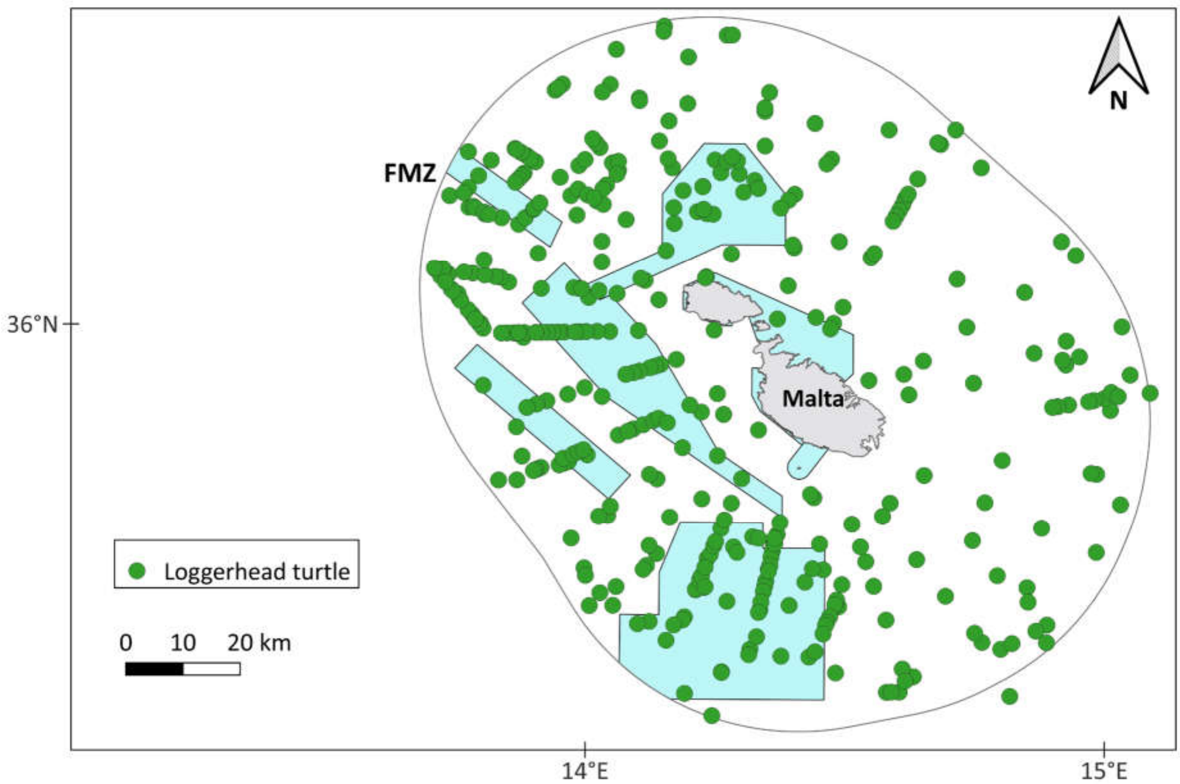

Figure 4 - Sighting distribution for the loggerhead turtle (green dots) within the Malta Fishery Management Zone (FMZ). Sightings were made during the following projects: ERA GF/Admin/40/2020, LIFE10 NAT/MT/090, LIFE11 NAT/MT/1070, BirdLife Malta, and LIFE12 NAT/MT/000845. The light blue polygons represent the Natura 2000 sites including those designated for marine reptiles conservation (MT0000113, MT0000115 and MT0000116)..

2. **Systematic and regular vessel-based surveys using line-transect methods**

As for small toothed cetaceans. However, inclusion of an additional observer would be beneficial to support multi-taxon surveying.

Monitoring locations. As for small toothed cetaceans.

Monitoring Timing and Frequency. As for small toothed cetaceans.

Analysis. As for small toothed cetaceans.

3. **Nesting activity monitoring**

Loggerhead turtle nesting in Malta, after being absent for several decades has returned and in recent times nests have been recorded on Malta's beaches yearly. Nesting is however still at very low levels and dispersed over different sandy beaches so that specific 'nesting beaches' cannot yet be defined. Because of the emerging breeding population, turtle nesting monitoring should be carried out, and in doing so nationally the methods described below should be used for the collection of the data proposed.

First of all, reporting of nesting turtles or of their tracks should be ensured through the establishment of a 24-hours hotline. Once a nest has been identified, the site should be promptly attended by a trained response team to safeguard the turtle (if present) and protect the nest if eggs are deposited. Additionally, foot patrols or UAS surveys should be carried out over non-frequented beaches to assess for missed nesting activity. Relevant monitoring and management actions from the MedPAN guide (Rees, 2020) and its Annex relating to sporadic and emerging nesting sites (Abella *et al*., 2021) should be followed. The data to be collected should include date and location of each adult female emergence onto a beach and whether it resulted in clutch deposition or not. Negative results from speculative nesting activity surveys need to also be recorded.

Monitoring locations.
All sandy beaches across Malta's three main islands. Each beach's suitability for nesting can be assessed using the *Sea Turtle Nesting Beach Indicator Tool* (Cousins *et al*., 2017) to aid identification of likely turtle nesting sites.

Monitoring Timing and Frequency. Loggerhead nesting in the Mediterranean generally occurs from late May until August, so June and July are the months when nesting on Malta's beaches can be expected to be reported and beaches need to be surveyed. Monitoring efforts in response to reports of turtle nesting should be carried out immediately. Non-people frequented beaches should be monitored for nesting activity by UAS at least once per month, in late June and late July. National quantification of nesting on all potential nesting sites should be carried out at least once every six years.

<u>Analysis</u>. Abundance of turtles present at a breeding site can be inferred from the numbers of nests deposited on the monitored nesting beaches and subsequently divided by the number of 10 km cells to provide a density value. However, nest numbers do not provide an irrefutable direct indication of the number of adults breeding annually in a population. This is because adult females deposit between one and five clutches in a given breeding season, and successive breeding seasons may be two or more years apart for the nesting turtles (Casale *et al*., 2018). Nevertheless, the use of nest count trend data is generally accepted as the most practical way of determining population abundance. Therefore the number of nests recorded per year is the metric to be reported, but to avoid misinterpretation caused by interannual variation, a time series of at least six years of nest count data should be used to infer any real trends in turtle abundance.

Costs

Table 4 - Estimated costs for monitoring dolphins and turtles Population Abundance under D1C2 (1)(2)(3)

Item	Unit type	Number per monitoring cycle	Unit cost (€)	Total (€)
Visual Observer Aerial - 2 Senior Researchers (4)	person hours	40	18	1,440.00
Real time data entry Aerial Survey - 1 Senior Researcher (4)	person hours	40	18	720.00
Boat and RIB Skipper - 1 person	person hours	1080	15	16,200.00
Boat Crew/coskipper - 1 person	person hours	450	10	4,500.00
Boat Environmental Effort data collection, equipment responsible and Visual observer coordinator - 1 Researcher	person hours	450	15	6,750.00
Boat Photo-id field data collection and equipment responsible 1 Researcher	person hours	1468.8	15	22,032.00
Boat Visual observer and data collection assistant - 6 trained persons	person hours	1080	10	64,800.00
Boat and land Drone footage - 1 trained person	person hours	472.5	15	7,087.50
Population and Abundance data analysis and assessment 1 Senior Researcher	person hours	270	18	9,720.00
Photo-Id data analysis and assessment 1 Senior Researcher	person hours	118.8	18	2,138.40
Footage analysis and assessment 1 Researcher	person hours	216	15	3,240.00
Mapping, plotting 1 Senior Researcher	person hours	244.8	18	4,406.40
Drone	equipment	1	3,800	3,800.00
Digital camera	equipment	2	1,450	2,900.00
Lens	equipment	2	1,800	3,600.00
Waterproof, underwater Camera with accessories	equipment	2	580	1,160.00
Clinometer	equipment	2	150	300.00
Drone Batteries	consumables	3	250	750.00

Go Pro Batteries extra	consumables	2	50	100.00
Total D1C2				€ 155,644.30

(1) Boat/RHIB expenses, personnel, and other expenses shared for different monitoring methodologies have been allocated by percentage annually throughout the 6-year cycle. Photo camera, GoPro, Drone, Lenses have been allocated 100% in D1C2 and indicated as cost 0 in overlapping methodologies tables

(2) Assuming boat Researchers, skipper, crew, and visual observers, working 10 hours per day per 30 days yearly through the 6-year cycle

(3) Assuming RIB Researchers, skipper, drone operator, working 7 hours per day per 60 days yearly through the 6-year cycle

(4) Assuming Aerial Researchers working 8 hours per day per 5 days 1 year through the 6-year cycle

Demographic characteristics

Monitoring methodologies for assessing the demographic characteristics of small toothed cetaceans

1. Identification of unit to conserve using biopsy sampling for small toothed cetaceans

Understanding dolphin population structure is critical to correctly estimate trends in abundance, to assess the impact of by-catch, or to model the role of marine mammals in an ecosystem. Laws and treaties make an implicit assumption that information about the structure of the cetacean populations is already available. For dolphins commonly inhabiting Malta's waters, genetic studies aimed to identify the presence of demographically independent populations have never been attempted.

Biopsy sampling can be conducted during the vessel-based and/or the surveys to investigate genetic diversity and local population structure. The work will allow us to understand if there is a genetic separation from conspecific populations in the Mediterranean Sea. If present, the degree of interchange or isolation among local populations within the Mediterranean Sea will permit us to assess the scale of management units for Maltese populations. Furthermore, skin and blubber samples, collected with a small biopsy dart will be stored and archived to allow subsequent analysis for natural isotope ratios (C/N), pollutant contaminants, etc. in future associated research. As expertise for data collection and data analysis is not present in Malta, a collaboration with an external university is needed. Training in biopsy sampling should also be carried out during the first 15 days of survey to at least two people living in the Island. If the expected number of sampling is not reached, biopsy sampling can continue in the second 15 days of survey carried out by personnel who have been trained. Samples should be sent out to the chosen laboratory for the analyses after obtaining the CITES permits. Conservation methods for samples must be agreed with the contractor.

Monitoring locations. FMZ, although the vessel-surveys will be mainly conducted within the 12NM.

<u>Monitoring Timing and Frequency</u>. Biopsy collection should be conducted in one year during the vessel-based surveys (30 days twice per year, summer and winter). For bottlenose dolphins, biopsy samples can also be collected from the RIB surveys described in section 3.1 for criterion D1C2. The target sample is 50 samples per species collected from different groups encountered in different areas (e.g., not all samples from the same group/area). For bottlenose dolphins 25 samples should be collected from individuals encountered offshore and another 25 from individuals encountered further inshore.

<u>Analysis</u>. To be agreed with the contractor.

2. **Acquiring of demographic parameters using mark-recapture methods for common bottlenose dolphin and common dolphin**

Photo-identification data collected during the abundance vessel-based and RIB-based surveys will also provide demographic parameters of the cetacean populations. Mark-recapture methods have proven to be a successful way to estimate demographic parameters such as survival probability, growth and recruitment rates. Moverover, at sighting, data on group size, group composition (age classes include newborn, calf, juvenile (only for bottlenose and common dolphin), adult), and sex based on visual observations will be also collected. Sex is usually determined using available genital photographs (underwater or during jumps) or if adults are seen in repeated associations with a known calf.

<u>Monitoring locations</u>. 12NM and Fish Farm Corridors. High quality pictures of dolphin dorsal fin collected from opportunistic platforms and from land can also be used if made available on a public database for reporting cetacean sightings.

<u>Monitoring Timing and Frequency</u>. 15 days twice per year (summer and winter) during the vessel-based survey and 5 days per month per year (60 days per year) during the fish farm monitoring.

<u>Analysis</u>. Reproduction and survival rate can be studied using either longitudinal or cross-sectional approaches. Because this latter requires one large collection and direct takes it will not be considered further for cetaceans. Longitudinal studies rely on re-capture histories of uniquely marked individuals over a long time, ideally their entire life. These studies, based on photo-identification methods, have provided the most comprehensive estimates of reproductive parameters. Protocol for picture processing has already been addressed in the mark-recapture section above. For the estimates of survival, recruitment, and population growth rates, the software MARK provides an extensive range of "live recapture" models (i.e., models whereby live animals are re-sighted) such as the Cormack-Jolly-Seber and its broader applications. Details of these models can be found in the open-source online book "Program Mark: a gentle Introduction…" (www.phidot.org/software/mark/docs/book).

3. **Body size, age class, sex ratio and reproductive success using unoccupied aerial system (UAS) for small toothed cetaceans**

Data on body size, age classes, sex ratios, reproductive success as well as health status can be collected by unoccupied aerial system (UAS) using photogrammetry to non-invasively measure body morphometrics of individuals with known life history information. UASs allow the collection of whole-body measurements from above. These pictures once they are calibrated with data on altitude can be converted to body measurements (Bierlich et al., 2021, Dawson et al., 2017). Potentially, UAS techniques also provide information about pregnancy, with photographs of pregnant individuals being visually wider. Measurements taken at increments along the body axis from the lateral edges of the animals' body have been shown to be a reliable measure of body width (Cheney et al., 2022). This increment has been successfully applied to study body condition, nutritional status and health (Christiansen et al., 2018; Christiansen et al., 2019; Durban et al., 2021). Potentially, metrics such as body width, that are more sensitive to changes in reproductive success or nutritional stress, could enable the identification of potential population level changes and therefore the implementation of adaptive management approaches.
Laser photogrammetry photographs can be taken concurrently with photo-identification on vessel-based and RIB surveys. This technique involves two parallel laser sights fixed horizontally to a camera which are projected onto the dolphin to provide a scale on the image. Body lengths are then estimated using the relationship between this scale and the distance from the dolphins' blowhole to dorsal fin. This latter is then estimated using the relationship with the body length derived from measurements of stranded individuals in the area or, if not available, from the literature.

<u>Monitoring locations</u>. 12NM. UASs can also be used from land when animals are spotted.

<u>Monitoring Timing and Frequency</u>. 15 days twice per year (summer and winter) during the vessel-based survey and 5 days per month per year during the fish farm monitoring.

<u>Analysis</u>. Several software, depending on the UAS brand, are available to download altitude data from the drone. Body measurements (i.e., length and width) of known individuals could be used to investigate growth, pregnancy status and health status. Differences in body width–length ratio between years, and seasons can be investigated used regression model (with the width–length ratio as the dependant variable). Other analysis could include methods such as ANOVA, regression and linear models, linear discriminant analysis, etc. The open-source software R provides plenty of packages to run such analysis (e.g., "MASS").

4. **Body size, age class, sex-ratio and more from necropsy of stranded small toothed cetaceans**

Dolphin carcasses washed ashore are an essential source of information. To be available, stranding data need a formal stranding response scheme, including: widespread repeated campaigns to encourage stranding reporting; the ability to perform a full examination (necropsy) of the carcasses using standard procedures; and, the presence of a national database to store the data, including the historical one. Full necropsies of stranded species

should be carried out in order to collect data such as age (by collecting teeth), sex and fecundity status (by collecting reproductive organs), body measurement for life history, and reveal possible cause of death, with special regard to contaminant loads, stomach contents, disease, incidents and any physiological or anatomical abnormalities. This last will be also used to assess bycatch and measure anthropogenic impacts on cetacean populations.
If not already available *in loco*, training should be made available to a local veterinarian and to personnel from organisations working with cetaceans.

Monitoring locations. Malta's coast.

Monitoring Timing and Frequency. Ad hoc.

Analysis. Many protocols have been developed for marine mammals necropsy and sample collections, some for taxonomic groups and others aimed to reveal any human interaction. Two of the most recents are "Handbook for Cetaceans' Strandings" by Mazzariol et al. (2015), and "Best practice on cetacean post mortem investigation and tissue sampling" by IJsseldijk et al. (2019).

Monitoring methodologies for assessing the demographic characteristics of the loggerhead turtle

1. In-water monitoring

All aerial and boat abundance/distribution surveys should be regarded as multi-taxon marine surveys in that observations of cetaceans and marine turtles (and sea birds) should all be recorded during the surveys, as detailed in the sections above on cetacean in-water monitoring. To inform the population demographic characteristics for marine turtles, turtle size should be assessed where possible, assigning observed individuals into life-history size classes. Turtles with shell length less than 30cm should be classified as oceanic juveniles, those with shell length 31-69cm juveniles/subadults and those over 70cm as adults (Dimitriadis *et al.*, 2022). Adult-sized turtles may also be sexed, males have long thick tails that extend well past the rear margin of the carapace, and should be recorded according to sex where possible.

Monitoring locations. As for small toothed cetaceans i.e. 12 nautical miles for vessel surveys and FMZ for the aerial surveys.

Monitoring Timing and Frequency. As for small toothed cetaceans.

Analysis. Data to be used in reporting will determine population structure based on the proportions of oceanic juveniles, juveniles/subadults and adults with further classification of adults as males or likely females in the observed sample. Remembering that Malta's own contribution to the at-sea turtle population from nesting is negligible, the demographic characteristics recorded will be dependent on conditions at distant nesting areas and threats and pressures present across the wider eastern Mediterranean, and as such national determination of good status would be concluded if all life stages and both sexes of turtles continue to be recorded in Malta's waters, however region-wide data would need to be analysed for a determination of GES at species level.sample.

2. Nesting activity monitoring

Monitoring marine turtle nesting activity and active nest management are two cornerstones of marine turtle conservation strategies, widely used around the world. Increasing the success of reproductive output from turtle nests is foundational in increasing annual recruitment to turtle populations and recording related data provide insights into the breeding demographics, which is Malta's case related to a small, emerging, fragile population.

Monitoring locations. See monitoring proposed for nesting turtle abundance

Monitoring Timing and Frequency. See monitoring proposed for nesting turtle abundance. However, demographic characteristics data required for marine turtles necessitate monitoring nests until hatching to determine incubation duration and then carrying out post-hatch excavation of the nests. Nests may take around 60 days or even longer to incubate so nest monitoring may potentially need to continue from August through to late September, depending on when the nests were created.

Analysis. Incubation durations can be used as a proxy for nest temperature and nest temperature affects the sex ratio of hatchlings produced in a nest. Using data obtained from Greece (Mrosovsky *et al*., 2002), Incubation periods of 57 days produce balanced sex ratio of hatchlings, shorter incubation periods (implying warmer nests) produce predominantly female hatchlings and longer incubation periods (implying colder nests) produce predominantly male hatchlings. Post-hatch excavation of nests allows determination of clutch size (number of eggs in the nest), hatching success (percentage of the eggs that produced hatchlings) and hatchling emergence success (percentage of the eggs that produced viable hatchlings that were able to escape the nest and head to the sea). These data are important to identify population recruitment each nesting season, and incorporating the incubation duration for each specific nest a rough estimation as to the overall sex ratio of hatchlings recruited to the population can be determined each summer.
Data to be reported should include mean clutch size, mean hatching success, mean hatching emergence success, the estimated hatching recruitment which is given by the sum of eggs that hatched and the hatchlings left the nest, and the estimated sex ratio of hatchlings recruited to population, given by ratio of the sum of females produced from each nest based on hatchling emergence success and incubation duration, to the sum of males based on the same criteria.

3. Body size, age class and sex-ratio autopsies of stranded turtles

As for marine mammals, marine turtle carcasses washed ashore are an essential source of information. To be available, stranding data need a formal stranding response scheme, including: widespread repeated campaigns to encourage stranding reporting; the ability to perform a full examination (necropsy) of the carcasses using standard procedures; and, the presence of a national database to store the data, including the historical one. Full necropsies

of stranded species should be carried out in order to collect data such as body sex and fecundity status (by observing reproductive organs), body measurement for life history, diet (from stomach contents), contaminant loads etc. and also reveal the possible cause of death, with special regard to assess bycatch incidents and measure anthropogenic impacts on turtle populations.
If not already available *in loco*, training should be made available to a local veterinarian and to personnel from organisations working with marine turtles.

Monitoring locations. Malta's coast.

Monitoring Timing and Frequency. Ad hoc.

Analysis. Data collected to supply information on turtle size distribution, sex ratio (from tail length or inspection of gonads in dead individuals), cause of death and quantification of debris ingestion (where possible to determine). Any turtles that are not loggerheads should be reported on separately.

Costs

Table 5 Estimated costs for monitoring dolphins and turtles' population Demographic characteristics under D1C3 (1)(2)

Item	Unit type	Number per monitoring cycle	Unit cost (€)	Total (€)
Sailing boat at sea for biopsy sampling	external assistance	15	400	6,000.00
Biopsy sampling training held by professional expert	external assistance	1	5,000	5,000.00
Biopsy sampling analysis and assessment	external assistance	1	10,000	10,000.00
Professional support (Mentoring) 1 person(3)	external assistance	360	15.00	5,400.00
Travel abroad fees for one Researcher to be trained	external assistance	2	5,000.00	20,000.00
Training (Online and/or in person)	external assistance	180	15.00	5,400.00
Fieldwork training abroad (Direct experience in Research Institutes/Universities)	external assistance	120	40.00	9,600.00
Training/workshops by professional expert	external assistance	1	10000	10,000.00
Boat and RIB Skipper	person hours	1080	15	16,200.00
Boat Skipper for first year biopsy sampling collection	person hours	150	15	2,250.00

Boat Crew/coskipper	person hours	450	10	4,500.00
Boat Crew/coskipper for one year in the cycle for biopsy sampling collection	person hours	150	10	1,500.00
Boat Environmental, effort data collection and equipment responsible. Visual observer coordinator 1 Researcher	person hours	450	15	6,750.00
Boat Environmental, effort data collection and equipment responsible. Visual observer coordinator for one year /biopsy 1 Researcher	person hours	150	15	2,250.00
Boat Photo-id field data collection and equipment responsible 1 Researcher	person hours	1425.6	15	21,384.00
Boat Photo-id field data collection and equipment responsible for one year /biopsy 1 Researcher	person hours	150	15	2,250.00
Boat Visual observer and data collection assistant 6 trained persons	person hours	1080	10	64,800.00
Boat visual observer biopsy collection 2 trained person	person hours	120	10	2,400.00
Boat and land Drone footage 1 trained person	person hours	472.5	15	7,087.50
Turtle Nesting site Patrolling and monitoring coordinators 2 persons	person hours	720	12	17,280.00
Photo-Id data analysis and assessment 1 Senior Researcher	person hours	118.8	18	2,138.40
Behaviour data analysis and assessment 1 Senior Researcher	person hours	360	18	6,480.00
Footage analysis and assessment 1 Researcher	person hours	216	15	3,240.00
Mapping, plotting 1 Senior Researcher	person hours	237.6	18	4,276.80
Drone	equipment	1	0	0.00
Digital camera	equipment	2	0	0.00
Lens	equipment	2	0	0.00
Waterproof, underwater Camera with accessories	equipment	2	0	0.00
Crossbow	equipment	1	5,000	5,000.00
Powdered-free nitriles gloves	consumables	2	9	18.00

Crossbow dolphin darts	consumables	5	200	1,000.00
Small containers for biopsy sampling	consumables	50	3	150.00
Stainel steel Scalpel	consumables	1	50	50.00
Fuel for sailing boat for 15 days for one year in the cycle	Consumables	15	100	1,500.00
CITES permits one for the first year	Other costs	1	500	500.00
Mail delivery gentic samples	Other costs	1	200	200.00
Total D1C3				€ 244,604.70

(1) Boat/RIB expenses, personnel, and other expenses shared for different monitoring methodologies have been allocated by percentage annually throughout the 6-year cycle. Photo camera, GoPro, Drone, Lenses have been allocated 100% in D1C2 and indicated as cost 0 in this table. Assuming boat Researchers, skipper, crew, and visual observers, working 10 hours per day per 30 days yearly through the 6-year cycle Assuming RHIB Researcher, skipper, drone operator, working 7 hours per day per 60 days yearly through the 6-year cycle

(2) Boat and personnel Expenses related to the biopsy collection with professional expert for genetical studies 1 year over the 6-year cycle Assuming Skipper, crew and researcher working 15 days 10 hours per day

(3) Assuming one external professional mentoring and updating/sharing information new technologies and techniques the National responsible yearly per 10 days throughout the 6-year monitoring cycle

Distributional Range

Range is a rather theoretical concept, derived from the distribution (population) map. Range covers: actual distribution and suitable and/or potential localities within the area of included gaps. For cetaceans distributional range is particularly difficult to estimate and, to date, there are no threshold values established.

Monitoring methodologies for assessing the distributional range of small toothed cetaceans

1. Predicting presence/absence (and abundance, biomass, etc.) using survey-based methods for small toothed cetaceans

Knowing where the dolphins are, what environmental characteristics influence their choice of habitat and how this choice changes with time is crucial to understanding the species' ecology, identifying the areas of critical importance, assessing the overlap with human activities and, ultimately, guiding appropriate conservation efforts (Redfern et al. 2006). Many factors influence a species' space-use, and those considered important for cetacean species include food availability (Benoit-Bird & Au, 2003; Hastie et al., 2004; Frederiksen et al., 2006), predation risk (Corkeron et al., 1987; Heithaus & Dill, 2006), competition and interspecific interactions (Shane, 1995; Weir et al., 2009), mating, reproduction and care for calves (Cañadas & Hammond, 2008). Thanks to the development of geographic information systems (GIS) and spatial modelling methods, data from survey based methods (effort and sightings) can be related with environmental data collected by different sources (remote-sensing systems, oceanografic ships, buoys, etc.). This has greatly improved the ability to describe and quantify the processes that determine species distribution and provide powerful tools to predict spatial and temporal occurrence of species. Navigation and sighting data will be collected during aerial and vessel surveys described in the sections above.

Monitoring locations. FMZ.

Monitoring Timing and Frequency. Distributional range information is provided by data collected with survey-based methods. Therefore timing and frequency overlap with the survey ones: once (or twice, if budget allows for a winter survey) every six years for the aerial survey; 15 days twice per year (summer and winter) for the vessel-based survey; and 5 days per month per year (60 days per year) for the RIB-based survey.

Analysis. Data preparation involves the use of GIS software. The free and open-source software QGIS (qgis.org) provides the core of functions and plugins needed to carry out habitat modelling, as well as a continuously growing number of capabilities. Efforts and sightings are usually organised in grids (i.e., 10x10 km^2) and combined with physiographic, oceanographic, and biological variables from different sources (usually satellites).
There are several analysis techniques to study the spatial distribution of animals. Logistic regression analysis, generalised linear models (GLMs) (McCullagh & Nelder, 1989), generalised additive models (GAMs) (Hastie & Tibshirani, 1999) and, generalised estimating equations (GEEs) (Liang & Zeger, 1986) are among the most commonly used statistical models (Guisan & Zimmermann, 2000). The use of one technique or the other usually depends on data available. A good synthesis is provided by Boyd et al. (2010).

2. **Space-use using mark-recapture techniques for common bottlenose dolphins and common dolphins**

Photo-identification data collected during the vessel-based and RIB-based abundance surveys will also increase knowledge about distribution and movements of marked individuals and therefore of the cetacean populations.

Monitoring locations. 12NM. High quality pictures of dolphin dorsal fin collected from opportunistic platforms and from land can also be used if made available on a public database for reporting cetacean sightings.

Monitoring Timing and Frequency. 15 days twice per year (summer and winter) during the vessel-based survey and 5 days per month per year (60 days per year) during the RIB-based survey.

Analysis. See sections above.

Monitoring methodologies for assessing the distributional range of the loggerhead turtle

1. Aerial and boat based surveys

Loggerhead turtles are a pervasive species in the Mediterranean and as such their distributional range (distribution) is assumed to cover all parts of the Mediterranean, but with some areas showing higher abundances/densities than others. Consequently aerial and boat-based surveys to determine turtle abundance are effectively also surveys to confirm distribution. Furthermore, given Malta's location in the central part of the central Mediterranean, it is highly likely that turtles will not be present in its waters and consequently specific surveys to determine distribution are not necessary.

Monitoring locations. As for small toothed cetaceans.

Monitoring Timing and Frequency. As for small toothed cetaceans.

Analysis. No special analysis required for distributional range as Malta is in the centre of the loggerhead's distribution in the Mediterranean.

Costs

Table 6 - Estimated costs for monitoring dolphins and turtles' population Distributional Range under D1C4 (1)

Item	Unit type	Number per monitoring cycle	Unit cost (€)	Total (€)
Deployment & Retrieval of Bottom recorder	person hours	384	12	4,608.00
Boat and RIB Skipper	person hours	1080	15	16,200.00
Boat Crew/coskipper	person hours	450	10	4,500.00
Boat Environmental, effort data collection and equipment responsible. Visual observer coordinator 1 Researcher	person hours	450	15	6,750.00
Boat Acoustic field data collection and instrumentation responsible 1 Researcher	person hours	1800	15	27,000.00
Boat Photo-id field data collection and equipment responsible 1 Researcher	person hours	1425.6	15	21,384.00
Boat Visual observer and data collection assistant 6 trained persons	person hours	1080	10	64,800.00

Boat and land Drone footage 1 trained person	person hours	472.5	15	7,087.50
Distribution data analysis and assessment	person hours	540	18	19,440.00
Photo-Id data analysis and assessment 1 Senior Researcher	person hours	122.4	18	2,203.20
Acoustic data analysis and assessment 1 Senior Researcher	person hours	270	18	4,860.00
Footage analysis and assessment 1 Researcher	person hours	216	15	3,240.00
Mapping, plotting 1 Senior Researcher	person hours	237.6	18	4,276.80
Towed Hydrophone (2)	equipment	2	3,000	6,000.00
Audio interface	equipment	1	190	190.00
Digital recorder	equipment	1	510	510.00
Bottom Recorder	equipment	3	2,500	7,500.00
Headphones	equipment	3	280	840.00
Drone	equipment	1	0	0.00
Digital camera	equipment	2	0	0.00
Lens	equipment	2	0	0.00
Waterproof, underwater Camera with accessories	equipment	2	0	0.00
Total D1C4				€ 201,389.50

(1) Boat/RHIB expenses, personnel, and other expenses shared for different monitoring methodologies have been allocated by percentage annually throughout the 6-year cycle. Photo camera, GoPro, Drone, Lenses have been allocated 100% in D1C2 and indicated as cost 0 in this table. Assuming boat Researchers, skipper, crew, and visual observers, working 10 hours per day per 30 days yearly through the 6-year cycle Assuming RHIB Researcher, skipper, drone operator, working 7 hours per day per 60 days yearly through the 6-year cycle

(2) Towed hydrophone Audio interface, Digital recorder, Bottom Recorder, Headphones have been allocated 100% in D1C4

Habitats for the species

Monitoring methodologies for assessing habitat extent and conditions for small toothed cetaceans

1. Predicting species habitat using habitat modelling for small toothed cetaceans

Knowing what and how environmental characteristics influence dolphins habitats with time, is crucial to understanding the species' ecology, identifying the areas of critical importance, assessing the overlap with human activities and, ultimately, guiding appropriate conservation efforts (Redfern et al. 2006). Results from the spatial analysis highlighted in the section "Distributional range", will provide information about the species preferred range of physiographic (e.g. depth, slope, aspect), oceanographic (e.g. sea surface temperature), and biological (e.g. chlorophyll a surface concentration) variables. From the variables found relevant for cetacean populations, habitat maps could also be generated. It is important to understand that because variables affecting cetaceans (e.g. prey abundance) are often hard to measure directly, other easier-to-obtain variables have been used as proxies even if they are not always directly and causally related with animal presence. Therefore habitat maps based on dynamic environmental variables need to consider natural (e.g. circadian cycles, seasons) and anthropogenic (e.g. climate change) variations need to be often updated. Monitoring information on space-use from behaviour and acoustic methods explained in the sections above and collected during the vessel-based survey, could also be used as variable to model habitat for the species.

<u>Monitoring locations</u>. FMZ.

<u>Monitoring Timing and Frequency</u>. Distributional range information is provided by data collected with survey-based methods. Therefore timing and frequency overlap with the survey ones: once (or twice, if budget allows for a winter survey) every six years for the aerial survey; 15 days twice per year (summer and winter) for the vessel-based survey; and 5 days per month per year (60 days per year) for the fish farm monitoring.

<u>Analysis</u>. Same analysis reported in the analysis section "Predicting presence/absence using survey-based methods".

Monitoring methodologies for assessing habitat extent and condition for the loggerhead turtle

1. Assessing habitat condition through autopsies on stranded turtles

As indicated above, loggerhead turtles are pervasive across the Mediterranean and as such their presence in Malta is generally not a useful indicator for habitat extent or condition. However, their omnivorous nature means they consume all manner of non-food items and assessment of debris found in their digestive tracts is a useful indicator of prevalence of pollution.

Monitoring locations. Malta's coast.

Monitoring Timing and Frequency. Ad hoc.

Analysis. Amount and characteristics of any debris ingested as determined from autopsy to be reported as a proxy for habitat condition.

Costs

Table 7 - Estimated costs for monitoring dolphins and turtles' Habitat for the Species under D1C5 (1)

Item	Unit type	Number per monitoring cycle	Unit cost (€)	Total (€)
Boat and RIB Skipper	person hours	1080	15	16,200.00
Boat Crew/coskipper	person hours	450	10	4,500.00
Boat Environmental, effort data collection and equipment responsible. Visual observer coordinator 1 Researcher	person hours	450	15	6,750.00
RIB Behaviour data collection 1Researcher	person hours	2520	15	37,800.00
Boat Visual observer and data collection assistants 6 persons	person hours	1080	10	64,800.00
Boat and land Drone footage 1 trained person	person hours	472.5	15	7,087.50
Turtle Nesting site Patrolling nest monitoring and post-hatch excavation coordinators 2 persons	person hours	720	12	17,280.00
Population and Abundance data analysis and assessment 1 Senior Researcher	person hours	270	18	9,720.00
Footage analysis and assessment 1 Researcher	person hours	216	15	3,240.00
Towed Hydrophone (2)	equipment	2	0	0.00
Audio interface	equipment	1	0	0.00
Digital recorder	equipment	1	0	0.00
Bottom Recorder	equipment	3	0	0.00

Headphones	equipment	3	0	0.00
Total D1C5				€ 167,377.50

(1) Boat/RHIB expenses, personnel, and other expenses shared for different monitoring methodologies have been allocated by percentage annually throughout the 6-year cycle. Photo camera, GoPro, Drone, Lenses have been allocated 100% in D1C2 and indicated as cost 0 in this table. Assuming boat Researchers, skipper, crew, and visual observers, working 10 hours per day per 30 days yearly through the 6-year cycle Assuming RHIB Researcher, skipper, drone operator, working 7 hours per day per 60 days yearly through the 6-year cycle

(2) Towed hydrophone Audio interface, Digital recorder, Bottom Recorder, Headphones have been allocated 100% in D1C4 D1C2 and indicated as cost 0 in this table

Pressures

Cetaceans and turtles are subject to a wide range of threats, mostly caused by direct or indirect anthropogenic factors. The populations that live in enclosed seas appear to be particularly affected by human activities (Avila *et al*., 2018). The Mediterranean Sea in general is highly exploited for fisheries, oil and gas extraction, offshore renewable energy and also for shipping, tourism and recreation (UNEP, 1996). Such activities can cause critical environmental degradation of the marine environment (Pace *et al*., 2015) and pose particularly severe threats to cetacean survival, especially on those species inhabiting offshore and coastal waters, in particular the bottlenose dolphin, the common dolphin and the striped dolphin. The main threats known to affect small toothed cetaceans include bycatch, habitat loss and degradation, pollution, chemical contamination and disturbance (Oceancare, 2021). Coastal development and associated activities together with non-human predation are most prevalent threats to turtle breeding sites whereas incidental capture in fishing gear and intentional killing are the most important factors affecting marine turtle survival when at sea (Casale *et al.,* 2018). Climate changes are having an effect over the distributions of many species, including cetaceans and marine turtles, and these effects are expected to increase in the future. Moreover, for marine turtles, the worst effect is expected at the breeding areas where nesting beaches may be lost due to sea level rise and increased storminess as well as extreme feminisation of turtle offspring sex ratios (Casale *et al.,* 2018). Other threats known to affect marine animals and in particular the loggerhead turtle are: marine litter (such as plastic bags) that can be ingested and eventually lead to blockages or perforations of the digestive tract; and traffic that can increase the probability of boat collisions, in particular for the turtles that spend periods of time basking at the sea surface.

Information supporting pressure assessments can be gathered from various sources and does not always require specific monitoring activities. For example, relevant data can be collected during surveys aimed to assess abundance or distribution or can also be obtained from existing datasets or from various stakeholders or authorities.

Below, there is a list of monitoring actions that would also allow us to understand the effect that anthropogenic pressure could have on small toothed cetaceans and marine turtles in Malta's waters.

1. Assessing marine debris through aerial surveys (cetaceans and turtles)

Marine debris, notably constituted by plastic, represents a serious threat worldwide, having been found in every ocean and all along the water column, from the surface to the seabed. Macroplastic can be ingested by certain species, especially deep divers, such as the sperm whale and pilot whales, due to their feeding habits, but may also represent a major cause for entanglement, leading to injuries and death. In Malta, derelict fishing gear, mainly FAD ropes, represent the main source of marine debris, contributing 96.2% to the overall litter (Consoli *et al.*, 2020).

Presence, abundance and distribution of floating marine litter can be monitored simultaneously during aerial surveys carried out for the monitoring of megafauna (Lambert *et al.*, 2020). Data collection on marine debris can be easily included in the surveys' monitoring protocols with observers actively searching also for debris, following the line-transect methods described in the chapter "Population Abundance". Debris distribution can later be related with distribution maps of cetaceans and marine turtles to investigate the level of overlapping. Results can be used for the implementation of adequate conservative strategies for the megafauna.

Monitoring locations. FMZ.

Monitoring Timing and Frequency. Once (or twice, if budget allows for a winter survey) every six years.

Analysis. Density, abundance and presence probability of marine debris can be estimated using the same analysis described in aerial surveys methodologies for dolphins and turtles.

2. Sampling behaviour to assess the impact of leisure boating activities on small toothed cetaceans

Behavioural disruption occurs when cetaceans are approached whether intentionally or unintentionally. Although it may be confined in space and time, it may be regionally significant when it occurs for coastal populations. Both short and long term effects can be induced by boating activities, ranging from masking their communication due to noise to alternation of animal behaviour and disturbance. Boat disturbance can produce short-term effects, such as for instance reduction in foraging activity (Pirotta *et al.*, 2014). High levels of disturbance by boats, notably motorboats, could bring cetaceans to leave that area (La Manna *et al.*, 2013). Tourism and leisure activities can therefore represent a major pressure if not regulated.

Recreational boating in Malta is particularly intense, especially in summer months and in coastal zones. When spotted, animals are often immediately surrounded by small boats willing to have a close look and often unaware of the disturbance they can cause with their behaviour. When sightings will be made during the vessel-based and RIB-based surveys, short term behavioural responses of dolphins will be visually and acoustically collected in presence and absence of leisure boats to investigate whether the presence, magnitude and timing of tourism operations affect dolphin natural behavioural.

Visual behavioural parameters should be collected before, during and after the disturbance event and should include: group size, group cohesion, dispersion, speed, directionality, ventilation patterns, and presence and amplitude of particular behaviour (i.e. aerial). Unit observation samples depend on species and for common bottlenose dolphins is usually 5 minutes.

<u>Monitoring locations</u>. Visual monitoring**:** 12NM. Acoustic monitoring: a selected area intensively used by dolphins and influenced by human activity within the MT0000105.

<u>Monitoring Timing and Frequency</u>. Visual monitoring: 15 days twice per year (summer and winter) for the vessel-based survey; and 5 days per month per year (60 days per year) for the fish farm monitoring. Acoustic monitoring: Acoustic sampling should be performed yearly by collecting 1 week (night and day) of recordings per month.

<u>Analysis.</u> Visual data: behavioural parameters can be analysed using statistical methods such as ANOVA, regression and linear models (GLM) performed by using the free and open-source software R. Acoustic data: the soundscape obtained firstly needs to be evaluated. Acoustic spectrograms should be analysed to study the bio-, geo- and antrophony through dedicated acoustic software such as PamGuard (University of St. Andrews - https://www.pamguard.org/), RavenPro (Cornell University - https://ravensoundsoftware.com/software/raven-pro/) or Sound Emission Analyzer Pro SeaPro (http://www-3.unipv.it/cibra/seapro.html- developed by University of Pavia, CIBRA). Changes in acoustic parameters (i.e. increase in frequency in presence of vessel) could provide information about a change in the short-term behaviour of dolphins in relation to tourism and leisure boating activity.

3. Changes in abundance and distributional range as consequences of climate change for small toothed cetaceans and loggerhead turtle

Habitat alteration may derive from climate-related changes in sea level, temperature and acidification, directly and indirectly affecting cetacean and turtles populations. Changes in abundance, distribution (especially for those species with restricted geographical distribution), migration ranges, food availability, reproduction success and survival due to climate change represent increased risk factors, especially for vulnerable species (Simmonds and Isaac, 2007). Data provided by regular survey-based monitoring and modelled in relation to physiographic, oceanographic, and biological variables can provide a baseline against which to measure the impacts of climate change.

<u>Monitoring locations</u>. FMZ.

<u>Monitoring Timing and Frequency</u>. Distributional range information is provided by data collected with survey-based methods. Therefore timing and frequency overlap with the survey ones: once (or twice, if budget allows for a winter survey) every six years for the aerial survey; 15 days twice per year (summer and winter) for the vessel-based survey; and 5 days per month per year (60 days per year) for the fish farm monitoring.

<u>Analysis</u>. Same analysis reported in the analysis section “Predicting presence/absence using survey-based methods” for small toothed cetaceans and “Monitoring methodologies for assessing the distributional range of the loggerhead turtle” for loggerhead turtles.

4. Chemical contamination through necropsies for small toothed cetaceans and loggerhead turtle

Top predators, including cetaceans and marine turtles are subject to accumulation of high concentrations of anthropogenic contaminants, which are Persistent, Bioaccumulative and Toxic (PBT). In cetaceans' tissues organochlorine compounds (OCs), such as polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB) and dioxins, polycyclic aromatic hydrocarbons (PAHs), are known to have immunosuppressive effects and to cause immune and reproductive dysfunction. Due to its enclosed and oligotrophic nature and the intense anthropogenic activities, the Mediterranean sea is highly polluted, especially in coastal areas.
Cetaceans and turtle carcasses that wash ashore are an extremely valuable source of information about the exposure to chemical contaminants that would not be easily acquired from live animals. The ability to perform a full necropsy of the carcasses stranded ashore will provide information regarding contaminant loads and the relation to contaminant presence and the cause of death.

Monitoring locations. Malta's coast.

Monitoring Timing and Frequency. Ad hoc.

Analysis. As for analysis in the necropsy of stranded specimens.

5. Assessing the interaction of bottlenose dolphins with fish farms

The habitat of bottlenose dolphins often overlaps with aquaculture in several coastal areas around the world (Watson-Capps & Mann (2005). In the Mediterranean Sea, bottlenose dolphins have been observed foraging in the proximity of fish farm cages on a regular basis (Würsig and Gailey, 2002). Complex substrate, increased nutrient levels and provision of fish-feed produce trophic enrichment and can attract dolphin prey (Bearzi et al., 2008; Piroddi et al., 2010; Pace et al., 2012). Clarifying the ecological role played by fish farms, as well as the factors that make individual fish farms attractive or unattractive for bottlenose dolphins, can help allocate coastal areas designated for marine aquaculture, so that marine mammal occurrence is taken into account and any potential conflict is minimised (Bonizzoni et al., 2013).
Interaction with cages can be visually evaluated during RIB surveys. In addition, the use of UASs/drones can maximise the evaluation of dolphins presence in the proximity of the cages. Acoustic recordings, collected through Bottom Records, can allow evaluating day and night presence of the dolphins at the fish farms locations. In addition the analysis of the vocalisations can provide information to the dolphins predominant activity.

Monitoring locations. Fish farms. 1 inshore and 1 offshore

Monitoring Timing and Frequency. Visual monitoring: 5 days per month per year (60 days per year) for the fish farm monitoring. Acoustic monitoring: 1 week (night and day) of recordings per month per year.

Analysis. Visual: statistical analysis includes descriptive statistical methods, ANOVA and GLM performed by using the free and open-source software R. Acoustic: spectrograms should be analysed by manually identifying the presence of the animal vocalisations within the recordings and extracting the associated acoustic parameters [i.e. frequency (Hz), durations (seconds)] through dedicated acoustic software such as PamGuard (University of St. Andrews - https://www.pamguard.org/), RavenPro (Cornell University - https://ravensoundsoftware.com/software/raven-pro/) or Sound Emission Analyzer Pro SeaPro (http://www-3.unipv.it/cibra/seapro.html- developed by University of Pavia, CIBRA) to detect dolphins' presence and also classify call types (whistles, tonal sounds) performed in presence of fish farms.

Costs

Pressure costs are already included in the monitoring methodology per criteria since data would be collected during the monitoring surveys suggested in this document.

Baseline and threshold values

Although threshold and baseline values are considered crucial in the assessment of GES for the species under Commission Decision (EU) 2017/848, no thresholds or baselines have been adopted at the regional level to date. Until such values are established, each Member State may consider national baselines, threshold values, or trends, and is required to do so by the first review of the initial assessment and determination of GES under MSFD Article 17. Since Malta has yet to establish specific baseline and threshold values for any of the monitoring requirements for small toothed cetaceans and turtles, the recommendation is to establish scientifically sound and realistic baseline values as a result of the six-years implementation of the proposed strategy.
It should be noted that baseline and threshold values for marine turtle GES monitoring have been drafted for the MSFD and IMAP (UNEP/MAP 2021b) processes, whilst baseline and threshold values for cetaceans GES monitoring have also been drafted for the IMAP process (UNEP/MAP 2021a) and hence it would be beneficial for Malta to note the recommendations in these draft documents when determining its national assessments.

Conclusions and recommendations

Systematic monitoring programmes are a legal requirement under the EU Habitats Directive (HD), the Marine Strategy Framework Directive (MSFD) and the Barcelona Convention. The monitoring approaches outlined here are the minimum requirements for monitoring key species such as marine mammals and marine turtles.

Designing appropriate monitoring for marine mammals and marine turtles is highly technically and costly demanding. For small toothed cetaceans the following monitoring priority to be applied within the is suggested: genetic diversity and local population structure; RIB-based and vessel-based survey using line-transect and photo-identifications methods;

Aerial surveys have proved to be an effective and more efficient method to obtain abundance estimates and record species distributions than traditional sea level platforms. And vessel platforms allow for the collection of many ancillary tough essential data (i.e. videos and pictures, and behaviour). Capture-recapture methods are frequently employed to estimate the abundance of cetaceans using photographic techniques. Long term monitoring programs that include photo identification and behavioural data collection could provide reliable information about demographic parameters of populations. UASs also could contribute to data collection on animal health, behaviour and interaction with aquaculture. Acoustic methods will provide further information about cetacean population structure and interaction with human activities such as fisher, aquaculture and tourism.

Although not critical in determining data to fulfil the monitoring requirements of the MSFD and IMAP mechanisms, use of depth recording tracking devices to assess 3D use of marine habitat around Malta by loggerhead turtles, that may be resident or transient individuals, is important. In this way we may fully understand the overlap between fisheries and marine turtles and hence more precisely manage and protect this endangered species. However, tracking of nesting individuals should be prioritised for three reasons: 1) they are the ones using Maltese waters as critical habitats, not having only a transient association, 2) they are the ones re-establishing Malta as a regular breeding habitat and determining their overwintering locations can strengthen calls for conservation at these locations, 3) tracking these individuals can direct conservationists to identify locations of nests that may otherwise be missed and left unprotected.

To be implemented, the present strategy requires the coordination and implementation of monitoring. ERA is the prime agency responsible for marine mammals and marine turtles and the health of their ecosystem and therefore has the responsibility of monitoring. It is recommended to establish an organised and coordinated network of stakeholders with the priority objective of ensuring this strategy is implemented to achieve MSFD Good Environmental Status (GES) in accordance with the identified criteria. Network actions include providing the financial support and expert personnel to create a full-time team for establishing and managing the network; carrying out specific monitoring surveys, in collaboration with fishers, in fishing areas to understand the relationship between marine mammals, marine reptiles and fisheries. Engaging in relations with fishers that accidentally catch turtles can lead to better outcomes for the animals, either through safe release on the spot carried out by the fishers or from the bycaught turtles being brought to shore for assessment and rehabilitation before release; developing dedicated tools to facilitate the exchange and sharing of data between stakeholders, scientists and authorities to provide policymakers with the most accurate information to base their decisions; providing proper guidelines for standardised and shareable data collection that allows sharing data at the national and regional levels.

It is also recommended that staff is ensured to monitor that protocols and data from different monitoring is consistently collected and stored in dedicated databases, that reporting meets agency/policy requirements, and that data is available for analysis. Regular reporting should be also made available including annual population estimates. Furthermore, research should be undertaken by appropriate researchers and, if needed, extra funding is sought by cooperating with other Research Institutes and Universities. Monitoring of relevant parameters including chlorophyll-a and sea surface temperature should be undertaken as part of a wider monitoring framework for the marine environment, in consideration of the monitoring strategy for cetaceans. These parameters should be used in developing habitat models. Monitoring of such parameters is not being included as part of the costings provided by this report.

Estimates of trends are more likely to be statistically robust over more extended time periods. Therefore, although the HD and MSFD reporting period is six years, it is recommended to estimate at least trends over two reporting cycles (i.e. 12 years). After the first six-years cycle, a revision based on the data collected during the first 6-year cycle is recommended to update and where appropriate modify the monitoring strategy introducing new technologies and methodologies developed in the meantime; to set up a long-term monitoring program every year in larger areas and in different seasons involving other organisations already studying marine animal populations in Maltese waters resulting in a more cost-effective surveys strategy. It is also recommended to enhance collaboration and coordination with adjacent countries. Marine vertebrates such as marine turtles and dolphins are known to move hundreds (if not thousands) of kilometres that cross numerous borders; therefore, it is crucial to develop monitoring programs comparable to those in bordering countries integrated into the region's established networks of information sharing and decision-making.

Total costing of the Long Term Monitoring Strategy

Table 8 - Costs allocated across all Criteria.

Item	Unit type	Number per monitoring cycle	Unit cost (€)	Total (€)
Cloud computing space to share data Gsuite per year	external assistance	6	1,200	7,200.00
Sailing boat at sea every year per 6 years	external assistance	180	400	72,000.00
RIB at sea per 6 years	external assistance	360	350	126,000.00
First Aid course for 6 persons	external assistance	3	45	810.00

RYA Sea Survival course for boat Research responsible for 3 persons	external assistance	1	270	810.00
Development of baseline and threshold values for GES assessment	person hours	360	18	6,480.00
Writing up reports	person hours	720	18	12,960.00
writing up articles	person hours	360	18	6,480.00
Hard disk 1TB SSD photo, video, sound backup per year per 6-years cycle	equipment	3	130	390.00
Laptop	equipment	2	1,400	2,800.00
Water-Resistant Tablet	equipment	2	550	1,100.00
usb-GPS	equipment	2	50	100.00
NMEA 2000 Wi-Fi Gateway	equipment	1	180	180.00
Monitor	equipment	1	300	300.00
Batteries (AAx4) 16 items pack	consumables	6	16	96.00
n.10 SD cards 128Gb per year	consumables	12	30	360.00
Audio cables	consumables	12	6	72.00
Power banks	consumables	2	40	80.00
A4 Clipboard Writing Pad File	consumables	24	3	72.00
Printed data collection forms	consumables	600	0	240.00
Marker pens	consumables	30	3	90.00
Fuel for sailing boat for 30 days per year	consumables	180	100	18,000.00
Fuel for RIB	consumables	360	150	54,000.00
Travels	other costs	6	1,000	6,000.00
ERA PERMIT one per year per 6 years	other costs	6	0	0.00
CSD Permit boat survey one per year per 6 years	other costs	6	300	1,800.00

CSD Permit aerial survey one per cycle	other costs	1	300	300.00
Skipper Liability insurance	other costs	6	370	2,220.00
Crew insurance	other costs	6	250	1,500.00
Drone insurance	other costs	6	750	4,500.00
Total shared costs for all Criteria				€ 326,940.00

Table 9 -Total costing

Summary	
Category	**Total**
Personnel	820,836
Travel	6,000
Ext. Assistance	278,220
Durable Goods	54,670
Consumables	76,758
Other Costs	11,020
Overheads	87,325
Total	**1,334,829**
The budget figures are calculated with the prices known as realistic in year 2022. It is wise to keep this in mind as prices tend to inflate by 3% per annum.	
The figures in these budgets are exclusive of vat	
these budgets do not include a contingency which would be calculated between 10 to 15% of total amount	

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