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Guidelines to standardize methodologies to estimate demographic parameters for marine turtles populations in the Mediterranean

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SUMMARY

Sea turtles have been living on Earth for millions of years. People are always attracted to sea turtles with their long-life spans, covering long migration distances, mysteriously appearing at night on sandy beaches to lay their eggs and leave quietly. People are also a threat to turtles through direct consumption of meat and eggs, and the use of their shells. Along with many other species, increasing human activities is threatening sea turtles over the past two centuries. Today, all sea turtle species are listed as endangered or threatened and are protected by various legislations in their habitats. The main threats to sea turtles in the Mediterranean, as well as the rest of the world, can be summarized as; degradation of the nesting, feeding and wintering habitats; incidental capture in fisheries; intentional killing; eggs exploitation, collision with marine vehicles and pollution.

Monitoring an endangered species population (such as sea turtles) for conservation measures is a necessary step to achieve the conservation goals. However, these species' biological characteristics (such as migrating between breeding and foraging grounds, spending different life stages in different marine habitats) would make it difficult to monitor them. Population status and trends should be precisely assessed for effective conservation. Apart from these, the study of demography provides a mathematical description of how such population parameters change over time. Particularly size and density of a population, age structure, fecundity, mortality and sex ratio.

There are a few population modelling studies on sea turtles in the Mediterranean which used limited demographic parameters. In contrast to the general belief, Mazaris et al. (2005) explained the effect of various reproductive patterns, they suggested that adult females' fecundity and survival rate of early juveniles are highly important to population dynamics of loggerhead turtle in the Mediterranean. Mazaris et al. (2009) also suggested a greater importance of Hatching and Hatchling Emergence Success over the Nesting Success for the overall hatchling production. Casale and Heppell (2016) derived a theoretical demographic structure of the Mediterranean population, assuming a stationary age distribution, and provided a likely order of magnitude of population abundance as whole and in different life stages. Casale and Heppell (2016) investigated the sea turtle bycatch effect on the population by a Stationary Age Distribution model for simulating population abundance and potential biological removal in the Mediterranean. They estimated the population abundance range to be 0.81–3.38 million loggerheads (the mean number of adults 15,843), and 0.26–2.21 million green turtles (the mean number of adults 3,390) in the Mediterranean.

The aim of this document is to a). Summarise the information about population status and b) To guide the Mediterranean countries to implement the Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean with its related regional action plans and c). Provide the needed tools for those procedures.

This document describes the standardized methodologies to estimate demographic parameters for population dynamic analysis, such as population modeling. This document also contains the guidelines on; species distribution range, population abundance and population demographic parameters.

SEA TURTLES OF THE MEDITERRANEAN

1.1. Population Status of Mediterranean Sea Turtles and Recent Studies

The leatherback turtle (*Dermochelys coriacea*), green turtle (*Chelonia mydas*), and loggerhead turtle (*Caretta caretta*) are regularly encountered in the Mediterranean (Groombridge 1990, Casale & Margaritoulis 2010). Loggerhead and green turtles are recognized as regional management units (Wallace et al. 2011) while leatherback turtles considered as a visitor species from the Atlantic and breeding is not reported yet (Casale et al. 2003). Two other species (*Eretmochelys imbricata*, Hawksbill sea turtle *Lepidochelys kempii*, Kemp's ridley sea turtle) are very rarely encountered within the basin while only one record of Olive Ridley turtle (*Lepidochelys olivacea*) reported recently (Revuelta et al., 2015). In this report, only data on loggerhead turtle and green turtle are presented.

The most abundant sea turtle species in the Mediterranean is the loggerhead turtle. Main breeding areas are listed as Greece, Turkey, Cyprus, and Libya (Casale & Margaritoulis 2010). Loggerhead turtle is mainly encountered in the oceanic zones of the western Mediterranean basin, the Strait of Sicily, and the Ionian Sea. Being a migratory species, the loggerhead turtle uses different areas for foraging. Main foraging areas in the western basin are used by both the Mediterranean and the Atlantic loggerhead populations (Carreras et al. 2011). Neritic zones are also frequently used as foraging areas by loggerhead turtle such as the Adriatic, Tunisia, Libya, Egypt, and the southern coasts of Turkey (Casale & Margaritoulis 2010). Green turtles mainly use neritic areas in the eastern Mediterranean basin and nest on beaches in Turkey, Cyprus, and Syria (Casale & Margaritoulis 2010).. Considering the methodology, It is necessary to choose a suitable sampling method (i.e., aerial surveys, nesting and stranding monitorings, diving/snorkeling transects and capture-mark-recapture studies, satellite tracking, fisheries bycatch data...etc) that allow adequate knowledge of the distribution range of each species. Such sampling involves high effort for areas that have not been fully surveyed yet. Monitoring effort should be long termed and should cover all seasons to ensure that the information obtained is sufficient. With these techniques used, a gridded map of 10 x 10 km cells showing presence/absence of;breeding,wintering or feeding/developmental areas would be a good indicator. This method will be applied along the entire basin or in subregions and in all pelagic marine areas. The quality of the range calculation could consist of a scaling system, combining the reliability of the distribution at the time it was mapped, how recently it was mapped, and the method used to map it. The results would be classified in three categories: 3 = reliable (accurate to within 10%); 2 = incomplete (accurate to within 50%); or 1 = poor (definitely not accurate to within 50%) and should be presented in the same style by the partner countries.

1.2. Threats to the Sea Turtle Populations in the Mediterranean

There are two breeding sea turtle species in the Mediterranean, loggerhead turtles and green turtles. Both species are affected by several anthropogenic threats, such as degradation of nesting habitats, incidental catch in fisheries, collision with marine vehicles, and intentional killing (Tomás et al. 2008, Aureggi & Khalil, 2010, Demetropoulos & Hadjichristophorou 2010, Casale & Margaritoulis 2010, Casale et al. 2010, Casale 2011, Domènech et al. 2014). Incidental catch of sea turtles in fishing gears is considered the main threat for the Mediterranean Sea turtles (Casale, 2011). There are several methods to estimate the number of deaths using fisheries bycatch data but unless the total number of turtles in the population is known, the decrease in overall survival probability will be difficult to estimate. Annually, 44,000 sea turtle deaths were estimated in 132,000 bycatch in the Mediterranean (Casale, 2011). These numbers seem to be very high but the effects on a population is unpredictable without having a population size estimate.

We do not have enough information on the turtle populations in the Mediterranean including different sexes and age classes. Broderick et al. (2002) estimated that 2280 to 2787 loggerhead females and 339 to 360 green turtle females are nesting in the Mediterranean. There is insufficient information on males to date. Based on the available data from monitored beaches in the Mediterranean, the total number of nests was estimated at 7250 and 1600 for loggerhead and green turtles, respectively (Casale & Margaritoulis, 2010). These numbers are considered to be a very small proportion of the total populations (Heppel et al. 2002). It is clear that understanding the actual effects of fisheries on sea turtle populations in the Mediterranean is not possible without having estimates of the whole population size. In addition, the previous results were only based on bycatch data. Therefore, it is expected that the mortality rates will increase when the other factors are taken into account (Casale et al. 2015), and as a whole, will represent a high level of threat for both species (Wallace et al. 2011). Therefore, more studies on population structure of sea turtle populations are needed in the future.

2. CRITERIA OF ASSESSMENTS

To assess a population status of a species, various variables are required to do so such as lifespan, sex-ratio, distribution, habitat preferences, growth rates, age at maturity, fecundity and survival rate. Conversely, such parameters are very difficult to obtain for a long-lived and migratory species. Although sea turtles have been well researched for years, there is still a gap in estimating sea turtles populations. Sea turtles are spending almost all their life in the sea. However, despite knowing almost all their activities on nesting beaches, there is a great lack of information in their activity in sea habitats. For instance, neonate sea turtles do not reappear until they have grown to juveniles and their life history for this period is unknown for most areas in the world. This period is known as the lost years. Simultaneously, there is also a lack of information about adult turtles' life-cycle in marine habitats, for instance foraging areas, migratory corridors. Therefore, we can obtain more precise and integrated approach by gathering different demographic parameters instead of focusing only on one parameter in population estimation. Demographic parameters, such as clutch frequency, interannual nesting intervals, and growth rates show variation among species and different populations of the same species. In addition, genetic structure and individual behavior may vary among individuals in the same nesting population. Therefore, to get an accurate estimate of the available data, it is necessary to take into account individual differences, and to work with large data sets.

Within this scope, areas of priority for research are presented. This is along with the assumptions for how to obtain high accuracy inferences by using methods in population estimation. Therefore, the aim of this manuscript is to bring together and synthesized methods that are frequently used around the world and in the Mediterranean countries.

2.1. Genetic Structure

Current knowledge on genetic structure of loggerhead turtle in the Mediterranean is based on published studies from Calabria, Crete, Cyprus, Libya, Western Greece and Turkey. Results of these studies suggest the existence of six distinct nesting populations of loggerhead turtle (Kaska, 2000, Saied et al. 2012; Yilmaz et al. 2012; Clusa et al. 2013; LaCasella et al. 2013). Loggerhead turtles nesting in Egypt have not been documented yet, while the loggerhead turtle population nesting in Tunisia is not genetically distinct from other Mediterranean populations (Chaieb et al. 2010).

As adults, maternal philopatry to breeding areas has created several reproductive clades across the eastern Mediterranean as suggested through mtDNA analysis (Carreras et al. 2007; Clusa et al. 2013; Garofalo et al. 2009; Saied et al. 2012; Yilmaz et al. 2011), but some male mediated gene flow may reduce the overall depth of reproductive isolation (Carreras et al.

2007; Yilmaz et al. 2011). Furthermore, it has been recently proposed that the genetic structuring found in the Mediterranean could be also driven by regional and thermal adaptation (Novelletto et al. 2016) or water circulation (Carreras et al., 2006). Despite this marked genetic structuring at the nesting beaches, individuals hatched at different sites have high mobility within the Mediterranean and mostly share foraging grounds. The neritic area of North Africa is an important foraging habitat even for distant breeding grounds, it is used by adult turtles from Greece (Margaritoulis et al. 2003; Schofield et al. 2013; Zbinden et al. 2011), Cyprus (Broderick et al. 2007; Snape et al. 2016) and Libya (Casale et al. 2013) with no published data for loggerhead turtles nesting in Turkey. The Adriatic Sea is also an important foraging/overwintering area for turtles that breed in Greece (Margaritoulis et al. 2003; Zbinden et al. 2011; Schofield et al. 2013; Giovannotti et al. 2010) with lower numbers of that group foraging elsewhere around the Mediterranean (the Aegean Sea, the Levant coast and Cyprus) (Margaritoulis et al. 2003; Margaritoulis and Rees, 2011; Snape et al. 2016). As for the Mediterranean green turtle, several studies have highlighted the lack of resolution in the mitochondrial DNA markers due to the over dominant presence of a single haplotype. Consequently, this leads to a failure in detecting the structuring expected for this philopatric species (Kaska 2000; Bagda et al. 2012; Naro-Maciel et al. 2014). Coupled with possible male-mediated gene flow between reproductive populations (Wright et al. 2012b), this may leads to a failure in detecting the structuring expected for this philopatric species. Tikochinski et al. (2012) stated that although traditional mitochondrial markers have revealed no major genetic structuring of green turtles in the Mediterranean, , more polymorphic may reveal some structure in the future, especially with the fast technological development of this method. In fact, a more recent regional study has shown a clear structuring among the north-eastern Mediterranean populations that contradicts the worldwide mitochondrial markers assessment. This was done through using a larger set of markers (Bagda et al. 2012), thus indicating that a deep structuring may exist in the region.

Recently, and using genetic analyses, Sari et al. (2017) studied the multiple paternity (MP) on Dalyan beach, Turkey. They collected tissue samples (from 522 hatchlings) from two to three successive clutches (a total of 25 clutches) of ten nesting females. Evidence of MP in seven out of 10 females (70%) was found, and it was detected that four out of these seven females mated with at least two males, whereas the remaining three females mated with at least three males. They explained the high frequency of MP as a result of high genetic diversity within the population, or because most of the mating took place in a narrow area in Dalyan. Similar studies are needed in other index sites in order to have an estimate on the male population in the Mediterranean Sea.

In conclusion, the detailed knowledge of population structuring of the green turtle in the Mediterranean is still incomplete due to the lack of resolution in most of the nuclear and mitochondrial genetic markers used, but also due to an incomplete analysis of all known nesting areas.

2.1.1. How Sub-Populations Can Be Identified via Genetic Studies: Regional Management Units

In the Mediterranean Sea, there are three independent Regional Management Units (RMUs) for loggerhead turtles (Wallace et al. 2010): the Mediterranean, the North West Atlantic and the North East Atlantic (Monzon-Arguello et al. 2010, Wallace et al. 2010) of which, by definition, only individuals from the Mediterranean RMU are supposed to breed in the region. The Mediterranean Sea hosts an independent loggerhead turtle RMU but is also visited by individuals from two Atlantic RMUs (Wallace et al. 2010), with individuals from the Atlantic occurring in the western Mediterranean basin mostly (Clusa et al. 2014). Nesting populations are well structured, due to the female philopatry, and seven independent Management Units (MUs) have been identified within regions using mitochondrial DNA (mtDNA) markers (Shamblin et al. 2014) as following: (a) Calabria, Italy, (b) western Greece (Zakynthos + Kyparissia + Lakonikos), (c) Rethymno (Crete, Greece), (d) Dalyan + Dalaman (Turkey), (e) western Turkey (Fethiye to Çirali), (f) eastern Mediterranean (middle + eastern Turkey + Lebanon + Israel + Cyprus), (g) Libya + Tunisia. Mediterranean loggerhead turtles are considered to form an isolated meta-population (Carreras et al. 2011; Clusa et al. 2013) with most nesting occurring in Greece, Turkey, Cyprus and Libya (Casale and Margaritoulis 2010). The life-history for this metapopulation has been presented in the literature as following; hatchlings enter oceanographic currents in which they disperse to varied locations away from the breeding area (Hays et al. 2010a; Casale and Mariani 2014), as the turtles increase in size they are capable of more directed movements and may undertake long migrations through oceanic and neritic waters (Bentivegna 2002; Casale et al. 2007a; Hochscheid et al. 2010; Casale et al. 2012a, 2012b). During this period, foraging is switched protractedly from pelagic to benthic (Laurent et al. 1998; Casale et al. 2008). In general, they complete maturation in locations closer to their natal origin rather than being complete by random dispersal (Laurent et al. 1998, Maffucci et al. 2006, Garofalo et al. 2013).

2.2. Growth and Age at Maturity

Growth rates and age at maturity are important parameters in age-based population modelling. However, the lack of information on age-specific rates in sea turtles, especially in a distinct population, is an obstacle for age-based population models.

Sea turtle carapace length is an important parameter to understand their lifecycle. Carapace length is used to classify age classes and more importantly estimate the age at maturity. As a longliving species, age at maturity and longevity are an important parameter for demographic and population structuring studies. Sampling of nesting females on nesting beaches is an easy method to assess the range of adult female carapace length. However, this method gives limited data for assessing the whole population structure because it does not provide information about males, sub-adults and juveniles. Direct sampling of other age classes and males in foraging and breeding areas by various capture–mark–recapture methods (CMR) at-sea is applicable but time consuming and needs laboring. Conversely, hundreds of

dead or alive specimens are stranded in the Mediterranean coasts. Stranded animals are an important data source for demography studies as well as for molecular studies, stable isotope analysis and skeletochronology analysis.

Skeletochronology is an excellent tool in evaluating pattern of population age structure in wild animals, it allows us to study life history strategies of species in a more reliable way especially more than categorization. When available, age dependent estimations can be obtained for parameters such as survival, mortality, growth and reproductive traits (i.e. age of sexual maturity and fecundity) (Cardona et al. 2015). Moreover, skeletochronology provides the historical information that might be utilized in charting the rise and decline of reptilian populations in relation to climate change and the impact of human activities. Skeletochronology relies on the analysis of the Lines of Arrested Growth (LAGs) in bones, and is a rapid and reliable chronological tool already successfully used in many reptilian species.

Previous studies showed that sea turtles have slow growth and late sexual maturity (Avens & Snover 2013, Scott et al. 2012). The relationship between age and carapace length of sea turtles can be addressed through capture-tagging-recapture studies or through skeletochronology.

Validation of skeletochronology methods can be done via a known age of turtle (Snover & Hohn 2004), the use of Lines of Arrested Growth (LAGs) labelling via injection of fluorescent tetracycline intrabone marker (Klinger & Musick, 1992; Coles et al., 2001) and comparing results of skeletochronology and mark-recapture records can also be used together (Van Houtan et al., 2014).

The Humerus bone (the longest digit from the phalanxes usually fixed in 70% ethanol) has been used for skeletochronological studies in sea turtles. During histological studies, tissue processing are followed validated methods for sea turtles (Casale et al. 2011a, 2011b; Limpus 1990; Piovano et al. 2011).

Age at Sexual Maturity (ASM) has been estimated through growth models, it is ranging between 14.9-18.6 years for small nesters of 66 cm CCL (Curved Carapace Length) and 26.3-34.9 years for larger reproductive females of 84.7 cm CCL. However, the mean size of female loggerhead turtles nesting in the Mediterranean is 79.5 cm CCL and males appear to reach maturity at a similar size (Casale et al. 2005, Casale et al. 2014). The average ASM for the Mediterranean loggerhead population was estimated at 25 years (range: 21-34 years). This estimate was extracted from the mean values of eight age-at-length relationships obtained by the above studies and applied to a size at maturity of 80 cm CCL (Casale & Heppell 2016).

2.2.1. Growth Curves

Information on the growth rates based upon different methodological approaches became available only in recent years. Mediterranean loggerheads appear to reach 28 cm CCL when 3.5 years old. their growth rate ranges from 11.8 cm year⁻¹ in the first months of life to 3.6 cm year⁻¹ at the age of 2.5-3.5 years, similar to the Atlantic turtles (Casale et al. 2009a).

Different foraging grounds appear to affect carapace length and clutch size (Zbinden et al. 2011, Patel et al. 2015) and this suggests an effect of food availability on growth. The growth rates will not just depend upon the environmental conditions and the time of recruitment of different habitats, but are also influenced by the origin of the individuals (Piovano et al. 2011). Mature females nesting in the Mediterranean are not only smaller than their equivalent from the western North Atlantic, but they may also be younger (Piovano et al. 2011). Within the Mediterranean, substantial differences exist in terms of clutch size, with the smallest females and clutch sizes observed in Cyprus and the largest females and clutch sizes observed in Greece.

2.3. Mortality Rates

Annually, over 132 000 captures per year, with probably over 44 000 incidental deaths per year in the Mediterranean (Casale, 2011), more than 39,000 turtles are captured by bottom trawlers, more than 13,000 turtles by demersal longlines, and over 23,000 turtles by gillnets (Casale, 2011). Stranded turtles are used to estimate the number of deaths and it is considered that mortality rates are higher than estimated. This is mainly due to the fact that the number of stranded turtles represents small proportion of the total deaths (Epperly et al., 1996; Hart et al. 2006).

It is not surprising that there are many different types of surveys that can be used to address mortality rates. However, it urgently needs updated estimates that take in consideration a sub-basin approach. This approach should be derived from common survey methods with onboard observers and questionnaire based surveys (Carreras et al. 2004; Álvarez de Quevedo et al. 2010).

Casale and Heppell (2016) derived a theoretical demographic structure of the Mediterranean population, assuming a constant age distribution, and provided a likely order of magnitude of population abundance as a whole and in different life stages. Population abundance range was estimated as 0.81–3.38 million loggerheads and 0.26–2.21 million green turtles in the Mediterranean in the same study as previously addressed in this report.

2.4. Survival Probabilities

So far, two studies have investigated survival probabilities of Mediterranean loggerhead turtles. Annual survival probability based upon capture-mark-recapture data of loggerheads of 25-88 cm CCL was estimated at 0.73 and was considered as an underestimate by at least 0.1 because of tag loss (Casale et al. 2007b). Annual survival probabilities of large juveniles at four different foraging areas were estimated through a Catch Curve analysis, resulting in values ranging 0.71-0.86 depending on the area (Casale et al. 2015). These values were considered to be lower than expected from a healthy population and possibly due to anthropogenic mortality like bycatch, especially in some areas like the south Adriatic (Casale et al. 2015).

2.5. Sex Ratio Studies

The mean of sex ratio is the ratio between the number of males and females within a population and across all age (size) classes. It plays a major role of the target species life stages, and may help researchers predict population growth or decline and population status of the global decline or extinctions. Especially, knowing the sex ratio in species, which sex depends on environmental factors, is very important for the conservation and protection of the species. These can be done in several ways:

- Sex identification of adults in census and transects (juveniles and sub-adults) requires other techniques such as laparoscopy, blood analysis, genetic analysis.
- Sexing of stranded specimens (size, blood or genetic analysis, laparoscopy).
- Sexing of tagged (capture and recapture) (size, blood or genetic analysis, laparoscopy).
- Sexing of offsprings before leaving the nest, and at different growth stages until maturity (blood or genetic analysis).

For Mediterranean loggerhead turtle, The pivotal temperature (egg incubation temperature at which both sexes are produced at equal numbers) is about 29-29.3°C under laboratory conditions, and the pivotal incubation period (at which both sexes are produced at equal numbers) is 53 days (Kaska et al. 1998; Mrosovsky et al. 2002). It is similar to other populations around the world.. Other studies at natural conditions (Fuller et al. 2013) found the pivotal temperatures to be lower (28.9°C) and the incubation duration to be longer as expected (56.3 days) due to the effect of metabolic heating generated by the whole nest.

By applying different indirect sex determination methods, loggerhead hatchling production at most Mediterranean beaches was suggested to be highly female-biased, with the major rookeries in Greece, Turkey, Libya and Cyprus producing 60-99% females (Casale et al. 1998, 2000, Erzin et al. 2006, Kaska et al. 2006 Godley et al. 2001a, Katselidis et al. 2012, Jiribi et al. 2013, Kılıç & Candan, 2014). Interestingly, gonadal histology as a direct sexing method showed less skewed loggerhead hatchling sex ratios (55.6-79% females) although the method is possibly biased by the field sampling protocols and it is only applied in limited number of cases. Male-biased hatchling production might be possible in some years at least in few sites (e.g. Zakynthos and Lakonikos Bay in Greece; Dalyan, Kizilot and Patara in Turkey) (Godley et al. 2001; Kaska et al. 2006), while a small loggerhead nesting aggregation on the Kuriat Island in Tunisia seems to have dominant male production (Jribi & Bradai 2014).

Spatio-temporal variations of sex ratios were also reported (Kaska et al. 2006, Fuller et al. 2013; Yalcin-Ozdilek et. al., 2016; Uçar et al., 2012), with more male hatchlings being produced from the nests laid at the beginning of nesting season (nests laid in May) and the end of nesting season (nests laid in August), than from those laid in the middle of nesting season (June-July). The eggs at the top of a nest are also producing relatively more females than those at the bottom of a nest (Kaska et al. 1998). The location of a nest on the open beach and close to the vegetation is also another factor affecting the sex ratio of hatchlings

produced. The beach sand color (albedo), sand grain size, shading of vegetation etc seems important factors in determining the hatchling sex ratios as well (e.g., Kaska et al. 1998, Zbinden et al. 2007, Fuller et al. 2013).

Surprisingly, and opposite to the predominant female-biased hatchling production, the sex ratios of juvenile loggerhead turtles in most Mediterranean marine habitats showed no departure from 1:1 ratio, with the proportion of females between 52 and 56%. Initially, a discrepancy between strong-female biased hatchling production (and even sex ratios) in juvenile loggerheads was explained by a strong male-biased immigration of Atlantic juveniles to the Mediterranean Sea (Casale et al. 2002, Casale et al. 2006). However, equal sex ratios are found in the north-central Adriatic Sea, an area with no Atlantic contributors (Lazar et al. 2008, Garofalo et al. 2013, Maffucci et al. 2013). However, strong female-bias (2:1) in juvenile assemblages from the Atlantic Ocean (Wibbels 2003, Delgado et al. 2010) provided little support to this hypothesis. Overall, female bias in juvenile sex ratio (1.56:1) was recorded in the long-term study in the Tyrrhenian Sea, although in some years this ratio showed little diversion from 1:1 ratio (Maffucci et al. 2013). As juvenile populations represent a condensation of different cohorts with different sex ratios, originating from different source populations, there are several plausible and probably interconnected explanations for such sex ratio dynamics in the Mediterranean. For example, generally speaking, a female bias in hatchling production may be greatly overestimated (Delgado et al. 2010), as TSD mechanism remains unclear (Wibbels 2003), and hatchling production in the Mediterranean may exhibit significant intra- and inter-annual variations in sex ratios (Godley et al. 2001b). Contribution of different source populations to the juvenile population in marine habitats may also change between years, as well as sex-specific death rates, behaviour and spatial distribution (Maffucci et al. 2013). At present, it seems that the sex ratio of juvenile loggerheads in the Mediterranean could be female biased, although to a lesser extent than the Atlantic stock. However, long-term assessments in other marine areas are needed to mitigate the effect on inter-annual variability and to get an estimate of the actual sex ratios in the juvenile populations (Maffucci et al. 2013).

Adult sex ratios at different loggerhead's foraging grounds range from female to male biased (Chaloupka & Limpus C 2005). Although sex determination in adults is possible by external sexual characteristics (long tail of around 20 cm for male adults), their low abundance makes such studies challenging. Moreover, sex-specific behaviour and breeding periodicity may affect the results of such studies. Male-bias in the Amvrakikos Gulf is present between May and September (Rees et al. 2013), and in the central Mediterranean during the period from June to September (Casale et al. 2014). This can be explained by fewer females present at foraging grounds during the reproductive season. Inversion of the sex ratios from female-biased in juveniles to male-biased in adults in the Tyrrhenian Sea is intriguing and deserves further investigation (Casale et al. 2014). Operational sex ratio was estimated just in the Laganas Bay (Zahynthos, Greece) and has suggested an even sex ratio (Hays et al. 2010b).

Primary sex ratios of green turtles tend to be female-biased (70-96% females). Currently, no information is available for juvenile and adult sex ratios of green turtles at foraging areas. An operational sex ratio of 1.4M:1F is estimated from genotyping of hatchlings from Cyprus (Wright et al. 2012b).

2.6. Nesting Female Parameters and Clutch Parameters

The number of individuals within a population (population size) is usually defined as the number of individuals present in an animal aggregation (permanent or transient) in a subjectively designated geographical range. The index of population abundance is a single species indicator that reflects the temporal variation in the breeding or the non-breeding (wintering/feeding/developmental) populations of a target species compared to a base year or referenced level.

There are various methods to estimate the population size. One of the most common, and simplest, estimation methods is calculation of nesting females. The information on sea turtle populations in the Mediterranean is mainly based on nesting females, eggs, and bycatch data. Also, sea turtles demonstrate natal homing and occasionally nest on more than one nesting beach (Bowen et al. 2004). This allows us to easily estimate the population size of a specific nesting female population. In order to estimate the population size of nesting females in a particular area, each nesting female should be captured and tagged by the same methodology at each occasion. These occasions repeated throughout a nesting season and each successive nesting year. This method is then repeated with constant labor force and sampling period for each season. In addition to this method, the number of nests and tracks are counted to estimate the nesting population. Subsequently, collected data are pooled for each nesting beach, average number of nests and nesting density per kilometer are estimated with mathematical models or population estimation software such as MARK (White & Burnham 1999, Cooch & White 2016).

Beach monitoring studies are conducted mainly through beach patrols to detect sea turtle tracks on the nesting beaches in the Mediterranean (Kaska, 1993, Kasperek et al. 2001; Tomas et al., 2002; Bentivegna et. al., 2011; Bradai and Jiribi, 2010; Broderick & Godley, 1996; Broderick et. al., 2000, 2003; Cambell et al., 2001; Cardona et al., 2009; Carreras and Tomas, 2010; Casale et al., 2007a; Margaritoulis, 1988, 1998; Yalcin-Ozdilek & Yerli, 2006; Kaska et al. 2010; Durmuş et al. 2011; Ergene et al. 2013; Yılmaz et al., 2015; Cardona et al., 2015 [Demography Working Group of the Conference, 2015]). Aerial surveys are also started to be conducted using drones for monitoring beaches in distinct nesting areas in recent years (McClellan 1996, Cardona et. al. 2005, Lauriano et. al. 2011, Bevan et al. 2016). Most of the major nesting beaches in the Mediterranean have been monitored more than 25 years continuously, while some others have been monitored discontinuously. Although individual nesting beaches might be showing an increase or decrease in nesting, it is still not possible to put a general trend for the entire Mediterranean basin (Ilgaz et al. 2007, Türkozan & Yılmaz 2008, Casale & Margaritoulis 2010, Margaritoulis et al. 2011). Sea turtles are long-lived animals and it takes decades to mature. Nest counts are not a reliable indicator to estimate a whole population because juveniles compose the largest part of the population and nest counts give only information about adults. (Heppell et al. 2003, 1996, 2002, National Research Council 2010). It requires monitoring a particular population for years to assess the

population trend. For instance, no significant trend was observed in the annual nest numbers at the Dalyan Beach, Turkey but a dramatic increase in the annual nest numbers was observed in recent years (Kaska et al., 2015). In addition, assessing the trends based on the number of nests can be misleading as various environmental factors can affect the frequency of reproduction and eventually the annual nest numbers (Hays 2000, Solow et al. 2002, Mazaris et al. 2004; Olgun et al., 2016; Öz et al., 2004; Özdemir et al., 2008, 2011; Rees & Margaritoli 2004; Sönmez et al., 2013; Taşkın & Baran, 2001; Türkozan, 2000; Türkozan & Kaska, 2010).

The incubation period is negatively correlated with the temperature of the nests (Kaska et al. 2006). For example, nest temperatures as low as 27.2 °C with an incubation duration up to 66 days have been recorded in Dalyan- Turkey (Kaska et al. 1998), while nest temperatures as high as 31.9°C and with an incubation duration as low as 47 days have been observed on Göksu, Turkey (Sari & Kaska 2015).

The mean clutch size for green turtles among different Mediterranean sites is 84 eggs (Cardona et al., 2015). The number of clutches per season ranged between 1.9–3.5 clutches per season for green turtles in Alagadi, Cyprus (Broderick et al. 2002). The available information indicates recruitment at sea (hatchlings at sea per egg) of 0.58-0.85 (Türkozan et al. 2011). The nest temperatures ranged from 28.3 °C with an incubation period of 59 days (Candan & Kolankaya 2016) to 32.2 °C with an incubation period of 50 days in Cyprus (Kaska et al. 1998).

2.6.1. Reproductive Output, Development, Growth and Age at Sexual Maturity

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

- Age class identification in censuses and transects (based on size class estimates).
- Aging of stranded specimens (skeletochronology and/or age-size correlation sea turtles).
- Aging of beached specimens (skeletochronology and/or age-size correlation sea turtles).
- Aging of tagged (during capture and recapture) specimens: size correlation for sea turtles.

Nesting beaches and the number of nests in each were summarized by Casale and Margaritoulis (2010). Great variation in clutch size may exist within a single rookery and has been attributed to a different migration and foraging behaviour of the nesting females (Zbinden et al. 2011, Cardona et al. 2014). The number of clutches per season ranged between

1.2–4.6 clutches per season for loggerheads in Alagadi, Cyprus (Broderick et al. 2002). It has also shown that clutch size increased with body length in the Mediterranean (Broderick et al., 2003). Curved carapace length (CCL) and curved carapace width (CCW) are used for identifying body size. The mean CCL ranged between 66.5-84.7 cm and CCW from 50-88 cm (range = 50-88). Mean clutch sizes ranged from 65-130.4 (range = 6-211) in the Mediterranean. Morphometric measurements and clutch sizes (CS) are well known for all the major nesting sites in the Mediterranean except Libya (Cardona et al., 2015).

Another characteristic is inter (Re)-Nesting Intervals (RNI) which means the time between two successive nests. RNI estimations are well reported for Turkey, Greece and Cyprus, the mean RNI ranges from 12.7 to 19.9 (referenced in Cardona et al., 2015).

Sea turtles are migratory species and they migrate between foraging and breeding grounds. Migration and reproduction consume a substantial amount of energy. Therefore, female turtles are not able to nest every year and they skip at least one nesting season. The time between two successive nesting seasons is named Re-Migration Intervals (RMI). RMI is estimated approximately 2 years for Greece and Cyprus and 3.35 years for Turkey. A female turtle deposits more than one clutch per nesting season which is called clutch frequency (CF). The mean clutch frequency ranges from 1.2-2.2 clutches per season for Greece, Turkey and N. Cyprus.

The available information available indicates recruitment at sea (hatchlings at sea per egg) of 0.58-0.67 (Turkozan et al. 2003, Margaritoulis 2005, Turkozan & Yilmaz 2008).

2.7. Telemetry Work on Sea Turtles

Identifying the foraging areas and migratory corridors of adult turtles has a considerable importance for the conservation of sea turtles (Patel, 2013; Jeffers & Godley 2016). Most common methodology for identifying the foraging areas and migratory corridors is satellite telemetry of adult turtles. Capture, Mark and Recapture studies and combination of different methods have been used recently by Rees et al (2017)., they used flipper tagging, satellite tracking and genetics to identify the origin of loggerhead turtles living in Amvrakikos Gulf, Greece. Satellite telemetry is also used to assess the surfacing time for turtles. The data delivered from satellite telemetry is critical for aerial surveys to derive absolute population estimates (Coyne & Godley 2005).

Tracking data for loggerhead and green turtles were published in various studies in the Mediterranean (Godley et al. 2002; Luschi et al. 2013). Luschi and Casale (2014) reviewed both published and unpublished tracking studies for both species. Loggerhead turtles were tracked from Cephalonia (Hays et al. 1991), Crete (Margaritoulis & Rees 2011; Patel et al. 2015), and Zakynthos (Zbinden et al. 2011; Schofield et al. 2013). Cardona et al (2015) also reported unpublished tracking data for a total of 92 loggerhead turtles, of which 55 were juveniles and 37 were adult sized. Stokes et. al 2015, also published green turtle tracking data from Levantine basin, two nesting female green turtles were also tracked from Akyatan, Turkey (Turkecan & Yerli 2011). In Cyprus, only tracked male green turtle was reported from Cyprus (Wright et al 2012a).

Cardona et. al., 2015 reported all turtles' tracking information from the published data for the Mediterranean. In this report, a total of 112 females and 34 male loggerhead turtles were tracked from Cyprus, Greece, Italy, Israel, Libya and Turkey. Also, 37 females and 1 male green turtle were tracked from Cyprus, Israel, Syria and Turkey. In addition to these numbers, 20 loggerhead turtles were tracked from Crete in another study (Patel et. al., 2015).

A reasonable number of loggerhead turtles were tracked in the Mediterranean up to date. However, available tracking data is concentrated in some major nesting areas. Further studies are required from other major nesting areas, especially from Turkey. Moreover, our knowledge on males and juveniles is limited and further studies should be prioritized to them. Available green turtle tracking data is limited in comparison with loggerhead tracking data in the Mediterranean. The main green turtle nesting areas in the Mediterranean are in Turkey but very few data is available on that subject. The green turtle individuals tracked in the Mediterranean were; two from Akyatan; one from Samandağ and eight from Yumurtalık. None were tracked from Kazanlı. Also, Syria might have important nesting sites so we recommend conducting some further studies there.

Tracking rehabilitated animals was questioned in previous studies whether they behave naturally or not after being released to the sea (Cardona et al., 2012). Nevertheless, tracking injured animals after treatment at rescue and rehabilitation centres is important to understand their behavior and identifying the foraging areas and migratory corridors of turtles.

Sea turtles can also be tracked with the use of temperature-depth data loggers, radio transmitters, and satellite transmitters in their foraging and nesting areas (Backof, 2013). Extracted information would provide insight and understanding habitat use in different habitats.

2.8. Strandings Information as Data Source

Usefulness of the stranding data is mentioned above in this report. Rescue and first aid centres are collecting data on stranded sea turtles. Ulmann and Stachowitsch (2015), listed available rescue and rehabilitation centres in the Mediterranean. There are 34 confirmed sea turtle rescue centres, 8 first aid stations and 7 informal rescue centres in the Mediterranean. On one hand, stranded animals are a cost-effective method to collect data and are used for identifying foraging areas, assessing the impact of anthropogenic activities on sea turtles, diseases, estimating growth rates and a good source for tissue sampling. On the other hand, there is no common database or networking among the rescue centres and first aid stations in the Mediterranean. A networking and common database for strandings are thought to provide valuable information about sea turtle populations and the biological traits in the Mediterranean. Sea turtles are a good indicator species to provide information about Good Environmental Status of marine areas and stranding data is a very good tool for that. Therefore, establishing regional stranding network and common database should be considered in the future.

2.9. In-Water Research

As mentioned above, it is necessary to obtain adequate information for assessment of a population status. Information about the spatial and temporal distributions of different life stages of sea turtles is required for assessing population estimates. Available data from previous monitoring studies in the Mediterranean was mainly based on reproductive data from nesting beaches and bycatch data. However, this data does not address the need for a whole population assessment. Therefore, monitoring populations of both juvenile and adult turtles in the water is required. There are very few in-water studies in the Mediterranean addressing other lifestages and sex (immature, male turtles or females in foraging areas) rather than nesting females. Most information on the distribution and status of sea turtle populations in the Mediterranean was derived from onboard observations, bycatch data and from interviews with local fishermen (Casale et.al, 2011; IÁ de Quevedo, et.al., 2010; IÁ de Quevedo, et.al., 2013).

In-water studies provide information for population modelling and also allow the acquiring of valuable data for genetic, survival rate and adult sex ratio studies. In-water studies may target different sea turtle species and life stages and in different habitats. These studies are usually conducted by Capture-Mark-Recapture (CMR) method. There are number of different capture methodologies and an appropriate one should be decided before starting an in-water study. Local environmental conditions and life stages of the target species should be taking into consideration while choosing the capture method. It should be also considered that in-water studies are time consuming and require long working hours in the field. In addition, it takes years to achieve sufficient information about a population by in-water studies and long-term planning is required.

The most commonly used visual method involved boat-based sightings. Sighting data are collected from a vessel by researchers who located sea turtles as they surfaced to breathe or who sighted sea turtles beneath the surface. The species and location were generally noted. Sightings may have been noted opportunistically while the boat was in transit or while researchers were conducting other activities. The preferred boat-based survey method, however, involved conducting standardized transects that used stationed observers. In this method for evaluating relative abundance, two observers were typically stationed atop a viewing platform located a couple of meters above the boat deck, with each observer scanning a different side of the boat for sea turtles. Another researcher will drive the boat in a transect which is recorded and used to measure effort. This effort is represented as a transect distance or, when an effective perpendicular sighting distance was measured, as transect area ([effective perpendicular sighting distance]*[transect length]). The species, exact location, and time of each turtle that surfaced were recorded in order to calculate a catch (or sightings) per unit effort (CPUE; e.g., turtles/km). Methods similar to those used in boat-based transects were employed for both aerial- and dive-transect surveys. In the aerial surveys adapted in one of the projects, the observers were stationed inside an airplane rather than on a boat platform.

Dive surveys (two projects) involve two divers being towed underwater behind a boat where they noted sightings of sea turtles (Makowski et al. 2005). In both of these methods, researchers calculated relative abundance as turtles/km of transect length or as turtles/km² of searched area. Capture methods varied greatly between projects and are based on the environmental conditions at the study area. We identified eight general techniques used to capture sea turtles, each with a different measure of effort. The specific parameters (e.g., net length, net depth, mesh size) for each of these techniques varied between projects. Due to the variations in the capture methods, CPUE units differed between them. This creates difficulties in the analyses using data pooled from all the projects.

3. POPULATION ESTIMATION

A few studies on modelling sea turtle populations used at least some demographic values of the Mediterranean loggerhead population. Cambell & Lagueux (2005) Mazaris & Matsinos (2006) and Mazaris et al. (2005) explored the effect of various reproductive patterns upon population dynamics. They suggested a relatively high importance of fecundity and of early juveniles' survival Mazaris et al. (2009) suggested the greater importance of hatching and emergence success over the nesting success for the overall hatchling production. Casale and Heppell (2016) derived a theoretical demographic structure of the Mediterranean population, assuming a stationary age distribution, and provided a likely order of magnitude of population abundance as a whole and in different life stages.

Another approach for population estimation is developed by Wade 1998 referred to as the Potential Biological Removal (PBR). This approach was developed mainly for marine mammals and can be used for population abundance estimates. In addition to this approach, simple deterministic age-structured models can also be used (Barlow et al. 1995, Caswell et al. 1998, Heppell et al. 2005, Monk et al. 2011). These models are used when the age distribution is stable and recruitment is constant when combined with an estimate of adult population. However, for this approach and model, lack of information on the juvenile stages will causes problem. The PBR approach can be used when anthropogenic mortality does not exceed 50% of the potential maximum productivity rate of the population. According to this approach, the studied population should recover if the anthropogenic mortality rate is lower than the PBR. However, differently from marine mammals, the size and demographic structure of sea turtle populations are unknown, and the numbers of nests or adult females are used as abundance indices which cause problems in application of PBR. Another approach was developed by Curtis & Moore (2013) for estimation of maximum bycatch which uses reproductive values depending on the number of individuals in a place.

To monitor the sea turtles and obtain the data, aerial or boat surveys, photo-identification, PIT tagging of flippers, Telemetry (satellite, GPS/GSM, radio telemetry) and loggers, capture-mark-recapture studies, Shipboard, aerial (including drone), or diver-based/video (potential),

Swimming/snorkelling surveys with photo-id and GPS in densely populated areas (e.g. certain breeding sites), CPUE (bycatch), Direct mortality rate, Post-release mortality rate, Nest counts, Photo-id of individuals, Time-Depth-Recorder tags and Beach strandings. These are the most commonly techniques used for estimating sea turtles populations.

3.1. Whole Population Abundance

In-water monitorings were usually done by visual counting via small boat and/or aerial surveys on coastal regions. The Bycatch data from fishermen were also used in open waters. Stranding information during beach monitoring were also recorded in most of the Countries and this also used to prove the distribution patterns based on satellite telemetry studies. Most researchers used tagging via CMR, metal-plastic tags and photo-identification methods.

Satellite tracking, GPS/GSM tracking, radio tracking and the use of loggers such as TimeDepthRecorders also provide detailed information about the movements of small number of individuals within a population.

Aerial surveys are being used to study the abundance of turtles at sea. New technologies such as drones are promising for future monitoring studies. Few studies adapting aerial surveys in the Mediterranean are available (Cardona et al. 2005; Gómez de Segura et al. 2006; Lauriano et al. 2011). Aerial surveys require information about the time spent at surface to produce absolute estimates of turtle abundance.

3.2. Population Size of Nesting Females

Although in-water studies are becoming more common, nesting females and their hatchlings remain the most accessible life stages. Findings in recent years show that it is essential to the conservation of sea turtles to know more about survival probabilities, breeding probabilities, and population size of nesting individuals (as well as population growth rates). In some cases adult females of a given nesting population are sufficiently philopatric that the population itself can be well defined. Nesting seasons are extensive, up to five months. Capture effort can take place throughout the season and occasionally during the night. Because sea turtles often lay more than one clutch, there is an opportunity to recapture a tagged female multiple times in a season. Because of females skipping nesting in that year, studies should be planned as an open population and be designed as primary and secondary seasons to overcome this issue.

Capture probabilities (p), recapture probabilities (c) and population size (N) can be estimated within primary sessions. The primary sessions are separated by longer time intervals (i.e. years). Also, the method has considered that the population is open, immigration, emigration, birth and death occurred between primary sessions. Thus, it also permits to the estimation of annual survival (Φ) as well as temporary emigration (γ). Under the Pollock's robust design, primary sessions contain secondary sessions that are separated by a short time interval and it is assumed that the population is effectively closed (i.e., no births, deaths, immigration, or emigration).

3.3. Computer Program MARK to estimate the population

MARK program can be used for population estimation if data about population Capture-Mark and Recapture are available. This program also used successfully for reptiles, such as sea turtles and amphibians.

This program can be used to estimate sea turtles survival probabilities, breeding probabilities, and population size of nesting individuals (as well as population growth rate). Nesting seasons are extensive, up to five months and the Capture effort can take place throughout the season, in some cases during the night. Because sea turtles often lay more than one clutch, there is an opportunity to recapture a given female multiple times in a season. Individuals are mark with metal tags after laying their eggs. In summary, sampling for a given year consists of multiple sampling periods, where each individual in the nesting population has a chance (assumed to be the same chance) of being captured in each sampling interval. With a couple of additional assumptions, this constitutes a robust design. Hereafter, a description of the closed robust design will be given. The design assumes that, for the duration of capture effort within a primary period, one of the following is true:

- (1) The population occupying the study area is completely closed to additions or deletions,
- (2) Individuals movement is completely random in and out of the study area,
- (3) All individuals were present in the first sampling occasion within a primary period, although marked and unmarked individuals could exit the study area (with the same probability) before the last sampling occasion for that season, or
- (4) Individuals could enter the study area between the first and last sampling occasion within a season, assuming all individuals are present by the last sampling occasion.

An additional assumption for conditions 3 and 4 is that capture probability within a primary period varies only by time (not trap response or individual heterogeneity).

In the case of nesting sea turtles, the above assumptions do not hold. In fact, turtles arrive to lay their first clutch in a staggered fashion, remain in the area to re-nest for variable periods of time, then complete nesting and return to foraging areas in a similar manner. In essence, there is an open population study going on within each nesting season. First arrival at the nesting beach is equivalent to birth, and departure for the foraging grounds after the last clutch is laid is equivalent to death in a modelling sense.

If each individual in the population could be relied upon to be on the nesting beach each year, then the data for the entire nesting season could be pooled into whether or not an individual was captured in year t . However, some individuals skip nesting in a given year, and therefore the nesting population and population of female breeders in a given year are not equivalent. If nesting were a completely random process (i.e., each adult female had the same probability of nesting), then a Comarck Jolly Seber analysis (CJS) from pooled data would

produce an unbiased estimation for survival, although breeding probability could not be estimated. With most species, however, breeding probability is more accurately characterized as a Markov process (i.e., the probability of breeding is dependent on whether or not an individual is currently a breeder), and for some species skipping at least one year after breeding is obligatory. In this case, if skipping is not accounted for, all estimations in the CJS model, including survival, will be biased.

On the other hands, the Pollock's (1982) robust design can be used to estimate population size and parameters for Dalyan population. It allows the estimating of capture probabilities (p), recapture probabilities (c) and population size (N) within primary sessions that are separated by longer time intervals (i.e. years). Also, it considers the population is open, so immigration, emigration, birth and death occurred between primary sessions. Thus, it also permits the estimation of annual survival (Φ) as well as temporary emigration (γ). Under the Pollock's robust design, primary session contains secondary sessions that are separated by a short time intervals and it is assumed that the population is effectively closed (i.e., no births, deaths, immigration, or emigration).

Models will be designed and constructed to test this hypothesis which represents an alternate biologically hypothesis, assuming that the population size $N(t)$ is year-specific and capture and recapture probabilities are equal in all eight models.

A few models are summarized as follows;

Model 1 and 2: Constant temporary emigration $\gamma(\cdot)$, constant and equal capture and recapture probabilities $p(\cdot)=c(\cdot)$. Survival rate is constant $\Phi(\cdot)$ (*Model 1*) or year-specific $\Phi(t)$ (*Model 2*). Our two models read as $\Phi(\cdot) \gamma(\cdot) p(\cdot)=c(\cdot) N(t)$ and $\Phi(t) \gamma(\cdot) p(\cdot)=c(\cdot) N(t)$ respectively.

Model 3 and 4: Temporary emigration was absent $\gamma(\cdot)=0$, constant and equal capture and recapture probabilities $p(\cdot)=c(\cdot)$ and survival rate is constant $\Phi(\cdot)$ (*Model 3*) or year-specific $\Phi(t)$ (*Model 4*).

Model 5 and 6: Temporary emigration was constant) $\gamma(\cdot)$, capture and recapture probabilities are year-specific and equal $p(t)=c(t)$ and survival rate is constant $\Phi(\cdot)$ (*Model 5*) or year-specific $\Phi(t)$ (*Model 6*).

Model 7 and 8: Temporary emigration is absent) $\gamma(\cdot)=0$, capture and recapture probabilities are year-specific and equal $p(t)=c(t)$ and survival rate is constant $\Phi(\cdot)$ (*Model 7*) or year-specific $\Phi(t)$ (*Model 8*).

These models are examples to estimate population size and related parameters and they can be re-built after data collection.

These models will be fit to the data and parameters will be estimated using the program MARK v. 4.3 (White and Burnham, 1999; Cooch and White, 2001). Model selection will be based on Akaike's Information Criterion corrected for small sample size (Burnham et al.

2002). To provide further information regarding model selection, the mean Akaike weights w will be calculated for each model across all years and the relative importance of each parameter will be assessed by summing mean Akaike weights across all models.

4. INTEGRATING DEMOGRAPHIC INFORMATION WITH ABUNDANCE ESTIMATES

The abundance estimates are critical for assessing sea-turtle populations, demographic or vital-rate parameters are critical for understanding and predicting trends in sea-turtle populations. Although there are some available data, the critical vital rates have not been adequately determined for the entire Mediterranean for both species. The Regional Activity Centre for Special Protected Areas (SPA/RAC) should prepare the most important procedures to improve coordination in data collection and availability. The archiving of tissue samples and their usage in population estimates should be established as Mediterranean based research projects. The most serious demographic data gaps are to be addressed including in-water abundance, hatchling production, survival of immature turtles and nesting females, age at sexual maturity, breeding rates, and clutch frequency. The available information compiled in this report and the research priorities for the gaps are suggested in the following chapter

5. RESEARCH PRIORITIES

Hamann et al (2010) reviewed the global research priorities for sea turtles: informing management and conservation stakeholders in the 21st century and identified 20 metaquestions classified under 5 categories: reproductive biology, biogeography, population ecology, threats and conservation strategies. More recently Rees et al. (2016) reviewed if researchers were working towards global research priorities for management and conservation of sea turtles. He found that there has been significant progress in all the key questions identified in 2010. During the 5th Mediterranean Conference on Sea turtles, 13 researchers have also identified the research priorities for the Mediterranean (Cardona et al. 2015). With the light of these three main studies, the main recommendations for the population studies in the Mediterranean are as following:

- Analysis of pressure/impact relationships for these sites and connectivity among the various sites in the Mediterranean.
- A manual to support the monitoring programme, which will provide more detailed information, tools, and advice on survey design, monitoring methodology and techniques that are most cost-effective and applicable to each of the sea turtle species. This will result in ensuring standardised monitoring procedures, comparable data sets, reliable estimates and trends. This will help to collect similar and comparable data among the Mediterranean countries.
- Databases and maps of known nesting, feeding, wintering habitats in each Contracting Party.

- Identification of the extent (area) baselines for each population/subpopulation and the habitats they dwell in.
- Identification of monitoring capacities and gaps in each Contracting Party.
- Identification of techniques to monitor and assess the impacts of climate change.
- Location of all breeding/nesting sites, all wintering, feeding, developmental sites of adult males, females and juveniles.
- Number of adults and juveniles frequenting wintering, feeding and developmental sites, along with how numbers vary across the season as individuals enter and leave different sites.
- Number of males and females frequenting all breeding/nesting sites each year (operational sex ratio), and the total number of individuals in the breeding populations.

Any minimal valid assessment of changes in species distribution (or distributional pattern) requires both spatially explicit reporting of animal abundances (coordinates of locations) and a measure of sampling effort. Locally, and when high quality data has compiled, it could be useful to try a density surface modelling approach.

5.1. Regional Molecular Projects

This should be established for both species. These projects should include the major nesting beaches and foraging grounds. A sample should be collected from every nest laid. Collected samples should be from 50 turtles of every species and from each major foraging ground. New generation sequencing markers should be used to 1) refine genetic structuring at nesting beaches and foraging grounds, 2) estimate remigration interval, 3) estimate clutch frequency through female fingerprinting and 4) resolve the contribution of nesting beaches to foraging grounds.

5.2. Beach Monitoring Projects

Beach monitoring projects should be established with the same methodology and collecting the information should be in the same format, i.e, the number unfertilized eggs, clutch information and hatching success in all countries.

5.3. Aerial Surveys and CMR Studies

This should be conducted in the main feeding and mating bays of every country.

5.4. Satellite Tracking Studies

Satellite tracking studies on the juvenile, adult male and female of both loggerhead and green turtles should be organised. Wild caught animals should be prioritized over rehabilitated ones.

5.5. A Regional Stable Isotope Project

Isotope analyses should be established for both species. A sample should be collected from every adult turtle that is satellite tracked and from every nest laid. Furthermore, samples should be collected from stranded and bycaught turtles at foraging grounds and also from potential prey.

5.6. National Stranding Networks

National stranding networks should be created in every Mediterranean country to collect data about turtle size and measurements. Samples (of at least skin) should be taken for stable isotopes and bone for skeletochronology. Stranding networks should be coordinated with rescue centres.

5.7. Regional Skeletochronology Project

This should be established for both species, with the aim to assess growth rate and age at first maturity. Analysis should be conducted independently for each foraging ground.

5.8. Regional Bycatch Projects

Bycatch projects should be established to update bycatch figures. Research on the post-release mortality of turtles' bycaught in bottom trawls and set nets should be included.

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