Clutch Frequency for Loggerhead Turtles (Caretta caretta) Nesting in Kyparissia Bay, Greece

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Abstract.—Kyparissia Bay, Greece, currently hosts the largest Loggerhead Turtle (Caretta caretta) nesting aggregation in the Mediterranean Sea. We set out to determine the clutch frequency (number of clutches laid by a single female in one season) for turtles breeding in this rookery and to apply results in estimating breeding populations of the Mediterranean Management Unit. We used satellite tracking technology in 2018 and 2019 to follow 21 female turtles through their nesting seasons to reveal clutch frequency for each individual. The 2019 turtles deposited more clutches than those in 2018, likely because the delayed field work in 2018 missed early nesting. Average clutch frequency in 2019 was 3.8 nests/turtle and ranged from three to five nests. The 2019 clutch frequency value is higher than that of conspecifics in the region (2.2 in Cyprus), which we consider a reflection on methodological differences (satellite telemetry vs foot patrols) between locations in determining clutch frequency. Approximately 366 Loggerhead Turtles (range, 331–411, based on 95% confidence intervals of mean clutch frequency) may nest annually in southern Kyparissia Bay. The findings highlight that Regional Management Unit estimations may be overestimated by 73% and that conservation of adult females is likely more important than previously thought.

Key Words.—Mediterranean Sea; population estimation; reproduction; satellite telemetry; sea turtles

Introduction

All marine turtle species are of conservation concern and feature in the International Union for the Conservation of Nature for the Conservation of Nature (IUCN) Red List of Threatened Species (IUCN 2019). Loggerhead Turtles (Caretta caretta) are globally listed as Vulnerable, but regional subpopulations vary in status from Critically Endangered to Least Concern (Casale and Tucker 2017). The Mediterranean sub-population is one classified as Least Concern, but this is dependent on the continuation of long-term conservation programs that have maintained or increased historic nesting levels (Casale 2015).

Annual nest counts are the most common method of determining sea turtle population trends (Schroeder and Murphy 1999); however, to fully understand the status of a sea turtle population, it is essential to know more than the number of clutches deposited annually. That metric refers to only the annual reproductive output of adult female turtles, which are only a portion of all adult females, adult males, and juveniles that comprise the population. Life-history models (e.g., Heppell 1998) exploring demography and fecundity are important to interpret the impact of threats relative to age class. Furthermore, sea turtles are iteroparous, depositing more than one clutch of eggs at approximately two-week internesting intervals over a breeding lifespan that may last decades (e.g., Ondich and Andrews 2013). Estimating annual numbers of breeding females requires knowing clutch frequency (CF) values; the number of clutches of eggs a single female will deposit in a nesting season. Erroneous CF data typically result in overestimating population size (Tucker 2010; Richards et al. 2011; Weber et al. 2013; Esteban et al. 2017) and hence underestimate urgency for conservation.

Historically, CF values for sea turtles were derived from repeated observation of nesting females and limited interpolation of these results (e.g., Frazer and Richardson 1985). This method requires intensive nocturnal field-surveys over beaches that may possibly be several to tens of kilometers in length, to encounter each nesting female. Clutch frequency estimates are complicated by low site fidelity as turtles may disperse nests over wide spatial scales, leading to missed observations of nesting events. Due to both temporally and spatially incomplete surveying, sea turtle population-level CF values have been underestimated, e.g., three clutches or fewer per season, when individual Loggerhead Turtles are known to deposit up to five clutches in the Mediterranean (Broderick et al. 2002) or seven or even eight clutches elsewhere (Frazer and Richardson 1986; Tucker 2009).

More recently, CF for several sea turtle populations has been revised upwards through incorporation of tracking technologies. Both Loggerhead and Green Turtle (Chelonia mydas) populations in the Atlantic and Indian oceans have been reassessed where tracking has facilitated direct observation of turtles on the beach (Weber et al. 2013) or indicated nesting events additional to those detected by direct observation (Tucker 2010; Weber et al. 2013; Esteban et al. 2017; Tucker et al. 2013).
An alternative strategy to develop CF values is the use of widespread genetic sampling from each clutch deposited in a region (Shamblin et al. 2017), which, though labor intensive, produces robust results with near population-level sample size.

Clutch frequency values for Loggerhead Turtles have been updated for several global sites. In the southeast USA, original CF values of approximately 3.5 clutches (Frazer and Richardson 1985, 1986) have been superseded by higher values of approximately 4.5 (Shamblin et al. 2017) or 5.4 (Tucker 2010). In Oman, a CF of 4.0 had previously been adopted to determine the annual number of nesting sea turtles, this was updated and increased by Rees et al. (2010) and more recent work (Tucker et al. 2018) has indicated the value to be 5.4, thus reducing population size by 27% from original estimates. Clutch frequency estimates for other, globally important, Loggerhead Turtle breeding areas such as Brazil and the Cape Verde are lacking (Marcovaldi and Chaloupka 2007; Marco et al. 2012).

Across the Mediterranean region, lack of reliable CF estimates for Loggerhead Turtles is acknowledged as an important data gap (Casale et al. 2018) with values only determined for turtles nesting on Cyprus (Broderick et al. 2002). For Greece, individual CF values of up to three or four nests have been published for turtles nesting at Zakynthos (Margaritoulis 1983; Zbinden et al. 2007), and up to three nests in Kyparissia Bay (Margaritoulis 1988). No average values have been determined, however, for the largest rookeries in the Mediterranean (Casale et al. 2018). This study addresses the lack of robustly determined average CF of Loggerhead Turtles breeding in Greece. We tracked individuals (Fig. 1) for their nesting seasons, over two consecutive years in Kyparissia Bay, to facilitate a more accurate estimation of the annually nesting population size.

**Materials and Methods**

**Study area.**—Kyparissia Bay (37.3399°N, 21.6952°E) on the west coast of the Peloponnese, Greece, features a 44 km nesting beach. The southernmost 9.5 km of this beach concentrates about 84% of Loggerhead nesting as the core nesting area of Kyparissia Bay (Margaritoulis and Rees 2001). In recent years, an average of about 1,400 clutches have been deposited annually at the core area (Casale et al. 2018). We encountered all our study turtles within the core nesting area (Fig. 2).

**Field methods.**—ARCHELON (the Sea Turtle Protection Society of Greece) field studies have continued in Kyparissia Bay since the 1980s (Margaritoulis and Rees 2001), undertaking sea turtle nest monitoring and protection and a mark-recapture program. We applied uniquely coded metal tags (National Band and Tag Co., Newport, Kentucky, USA) to both front flippers of untagged turtles and recorded any existing tags to ensure that individual identities were assigned. We recorded straight carapace length from nuchal notch to tip of the longest supracaudal scute (SCLn-t; Bolten 1999) of each turtle to the nearest 0.5 cm. Due to logistical constraints, not every turtle that nested on the patrolled beach was encountered and tagged.
For the telemetry study, we commenced fieldwork in June, over two nesting seasons (2018 and 2019). To locate turtles, we conducted patrols between 2300 and 0300. We selected turtles for study on completion of a nesting emergence, confirmed by observation of oviposition.

We attached Platform Transmitter Terminals (PTTs; Model SPOT-375; Wildlife Computers, Redmond, Washington, USA) to a subset of tagged Loggerheads to track them using the Argos system (www.argos-system.org). We deployed nine PTTs in 2018 and 12 in 2019. We maneuvered turtles into a large plastic box to retain them on the beach during PTT deployment (for about 2 h). We attached the PTT to the carapace of the turtle, centered over the second vertebral scute, using the attachment kit of Wildlife Computers and their recommended methods (www.wildlifecomputers.com).

The 2018 nesting season in Kyparissia Bay started on 12 May (ARCHELON, unpubl. data), and we deployed PTTs between 14 and 19 June. The time period between the start of the season and PTT deployment was 33–38 d. The 2019 nesting season started on 31 May (ARCHELON, unpubl. data) and we deployed PTTs between 7 and 12 June. The time period between the start of nesting and PTT deployment was 7–12 d.

**Tracking and analysis.** — We used the Wildlife Computers data portal (www.wildlifecomputers.com) to retrieve, archive, and map Argos location data. We used positions from Argos Location Classes 3, 2, 1, 0, A and B (www.argos-system.org) to generate movement tracks and infer breeding activity and status of each turtle.

**Determination of clutch frequency.** — We attempted to derive estimated clutch frequency (ECF) for each tracked turtle through a number of methods: (1) through direct re-observation of the turtle on the beach during subsequent nesting emergences, (2) through interrogation of track trajectories and other Argos system data (see Tucker 2010 for details), because a turtle will return close to the beach to nest again after an approximately two-week interval, and (3) through using the telemetry-derived departure date (turtles depart their nesting site soon after depositing final clutch; Schroeder et al. 2003) to give nesting period (NP), which is divided by a typical internesting period (13–15 d; Margaritoulis 1983, 1988) to generate the ECF. Methods 2 and 3 have the advantage that they are able to incorporate nesting events outside the patrolled nesting area and do not require intensive fieldwork at night (Tucker 2010).

**Statistical analysis.** — Because of small sample sizes, we used the non-parametric Wilcoxon rank sum test to investigate interannual differences in ECF and SCLn-t and Spearman Rank Correlation to investigate the relationship between ECF and SCLn-t (pooled across years). Statistical significance was accepted at \( P \leq 0.05 \).

**RESULTS**

We were able to derive observed clutch frequencies for 10 turtles using method 1. Restricted, mainly nearshore, movements during the nesting season made it difficult to determine clutch frequency using method 2. We were able to determine clutch frequencies for all 21 tracked individuals using method 3 (Table 1). However, ECF values from 2018 were considered minima as PTT deployment that year commenced after turtles may have previously nested up to twice that season. Mean nesting period in 2018 was 24.9 ± 10.9 (standard deviation) d (range, 16–45 d; \( n = 9 \)) compared to 39.5 ± 9.8 d (range, 29–61 d; \( n = 12 \)) in 2019 (Table 1).

Only six of the 10 ECF values derived by direct observation (method 1) matched those derived from telemetry (method 3). The remaining four values were lower, as telemetry indicated turtles nested again after the final observation on the beach. Estimated clutch frequency in 2018 was a median of two and a mean of 2.4 ± 0.7 clutches (range, 2–4 clutches; \( n = 9 \)) and in 2019 a median of four and a mean of 3.8 ± 0.7 clutches (range, 3–5 clutches; \( n = 12 \); Fig. 3; Table 1). Clutch frequency differed significantly between years (\( W = 16, P = 0.005 \)). Mean turtle size (SCLn-t) in 2018 was 72.7 ± 3.1 cm (range, 68–76 cm; \( n = 9 \)) and in 2019 was 77.0 ± 4.9 cm (range, 72–87 cm; \( n = 12 \)), which did not differ significantly (\( W = 28, P = 0.069 \)). There was a moderate, but significant, positive correlation between turtle size (SCLn-t) and ECF (\( r_s = 0.542, P = 0.011 \)).

**Figure 2.** Kyparissia Bay currently hosts the largest nesting aggregation of Loggerhead Turtles in the Mediterranean. Arrow shows Platform Transmitter Terminal deployment location at the core nesting area. Inset places Kyparissia Bay, Peloponnese, Greece, in the context of the eastern Mediterranean Sea.
Our results confirm the benefits of using tracking technologies to determine ECF for marine turtles through the ability to overcome missed nesting events caused by incomplete surveying. Clutch frequency estimates were possible for all 21 study turtles using telemetry but for only 10 (48%) using physical re-observation. Enhanced PTTs capable of relaying additional data such as more accurate GPS locations, accelerometry and haul-out data would facilitate identification of individual nesting events that generate more detailed results. Additionally, complete data should be collected from several nesting seasons to account for potential interannual variability. Further improvements in CF estimations could come from ultrasound examination of nesting females to identify if clutch deposition had occurred prior to their initial encounter on the beach (Rostal et al. 1996) and to estimate the number of clutches a turtle is yet to deposit within a season (Blanco et al. 2011). Using ultrasound examinations would probably have improved accuracy of results from 2018; however, the turtles from 2019 were sampled at the onset of the nesting season and results that year are assumed to be accurate.

A lack of correlation between ECF and body size has been shown elsewhere (Frazer and Richardson 1986). The opposite result in this study is likely driven by the combination of smaller turtles being tracked later in the 2018 nesting season, and hence may have already deposited at least one clutch before receiving the PTT, and not a real biological trait. Intra-seasonal total reproductive output of individual turtles results from a combination of clutch frequency and clutch size. Maximum clutch size is somewhat related to body size due to physical constraints, with a high degree of individual variation (Broderick et al. 2003), whereas clutch frequency is likely dependent on energy reserves built up in the year(s) between nesting seasons (Broderick et al. 2003; Ceriani et al. 2015). Both clutch size and body size are affected by long-term foraging ground selection (Ceriani et al. 2015) thus population level reproductive output can vary depending on proportions of turtles from different foraging areas contributing to a breeding cohort. Body size is less relevant to clutch frequency, as non-ovulated ova are relatively small compared to shelled, oviductal eggs (Miller 1997), meaning several hundred may exist from the start of the nesting season.

Despite widespread nesting of Loggerhead Turtles around the Mediterranean, the Kyparissia ECF of 3.8 is only the second published estimate for the region, besides Cyprus. An ECF of 2.2 from Cyprus was derived from saturation surveying (nocturnal patrols for flipper-tagging) of 2 km of nesting beach, where 96% of clutches deposited were assigned to individual nesting turtles (Broderick et al. 2002). There may be a biological difference between the two populations as Cyprus turtles are smaller than those nesting in Greece (Margaritoulis et al. 2003). The lower number of CF in Cyprus, however, is likely driven by lack of nest site fidelity with the turtles encountered on the beach depositing clutches outside the surveyed area before first observation or after the final one (see Tucker 2010).

Applying our ECF (mean = 3.8 clutches per year, 95% confidence interval = 3.4–4.2) to the most recent published average number of nests (1,403 nests; Table S9 in Casale et al. 2018), we estimate that 366 adult female Loggerhead Turtles (95% confidence interval = 331–411) nest annually in the core area of Kyparissia Bay. This is the first estimate of nesting females for the Bay, based on topical data, and the first such estimate in Greece.

**Clutch frequency estimation methods.**—As previously identified (Tucker 2010), our results confirm that using telemetry to determine length of nesting period and subsequently estimate clutch frequency...
produces more accurate results than nocturnal ground patrols and for considerably less field effort. Exhaustive nocturnal ground patrols, however, have the benefit of obtaining a broader sample of clutch frequency records from individuals nesting at all stages of the breeding season, thus avoiding potential bias from selecting the earliest nesters (used in telemetry studies). While it is ideal to select turtles for tracking over a range of dates, it is important to intercept tracked females on their first nest, as highlighted by the difference in estimates between our full season tracking in 2019 and partial season tracking in 2018. Other studies (e.g., Esteban et al. 2017; Tucker et al. 2018) have successfully used telemetry data to pinpoint nesting dates and count clutches per season, but this method is less effective for populations such as the Kyparissia Bay Loggerheads, which exhibit restricted nearshore movements that prohibit identifying nearshore nesting-related behavior. Deriving clutch frequency from genetic analysis of a single egg from each clutch deposited (Shamblin et al. 2017) is less field-intensive and hence can cover a wider sampling area than nocturnal patrolling, but even spatially extensive egg sampling cannot match the potential coverage that tracking technologies can
offered. Furthermore, genetic analyses require a large investment in technical laboratory skills and data analysis to obtain improved clutch frequency estimates. Given that tracking turtles provides additional data, such as internesting habitat and migratory pathways, for a short amount of field time, it can be considered the prescribed manner to obtain clutch frequencies in many circumstances.

**Conservation implications.**—While more accurate, revised CF data do not alter long-term trends in nest numbers, they do highlight that regional population estimates based on the previously published CF of 2.2 from Cyprus (Broderick et al. 2002) are likely to be considerably overestimated, possibly by around 73%. These new data derived by satellite telemetry permit more realistic population models to be developed that combine all vital life stages. Given there are likely far fewer Loggerhead Turtles breeding in the Mediterranean than previously believed, increased effort for the conservation of these adult females may be warranted. The continued widespread discovery that CF values determined by telemetry are higher than previously estimated from foot patrols further emphasizes the importance of maintaining high rates of adult survivorship (Broderick et al. 2006), and why nesting trends can rise rapidly when protection is afforded to this demographic group (e.g., Balazs and Chaloupka 2004).

Richards et al. (2011), in their population estimates for western north Atlantic Loggerhead Turtles, highlight the need for research to better determine breeding intervals and clutch frequency. Progress has been made to this end. In the absence of telemetry, monitoring strategies to acquire these data have been assessed (Piccienza et al. 2019) and new models developed to determine internesting periods and clutch frequency from incomplete mark-recapture data (Hancock et al. 2019); thus tools are now in place to improve efficacy of future studies.

Within the Mediterranean region, population size estimates are generally compared based on nest counts (Casale et al. 2018), which provides an internally unified system. Using the estimated number of nesting turtles derived from nest counts and ECF values, however, may increase comparability of data from different global sea turtle populations. Data derived from this method can then be the adopted standard for global population assessments (Casale and Tucker 2017).

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**Literature Cited**


**Alan F. Rees** began his career in sea turtle research and conservation with ARCHELON in Kyparissia, Greece, in 1994 after graduating with an MSc in Biotechnology from the University of the West of England, Bristol, UK. His work with ARCHELON took him to Syria where he was the first to document a regionally important Green Turtle (Chelonia mydas) rookery. Since 2005, he extended his research further into the Middle East, undertaking several first-in-country sea turtle satellite tracking studies. He was awarded his Ph.D. in 2013 from the University of Exeter, Penryn, UK, which included analysis of his previous telemetry work together with results from an on-going in-water capture-mark-recapture study undertaken in collaboration with ARCHELON. He has published over 30 peer-reviewed articles and is on the editorial board of the Marine Turtle and Indian Ocean Turtle Newsletters. Alan’s dedication and skills as a sea turtle researcher have resulted in him serving a 5-year term on the Board of Directors of the International Sea Turtle Society and as Region Vice Co-chair of the Marine Turtle Specialist Group of the Species Survival Commission of the IUCN. Alan is currently an independent sea turtle researcher. (Photographer unknown).

**Panagiota Theodorou** completed her graduate and postgraduate law studies in Greece and Germany in 2009. After working as a lawyer for more than 3 y, since 2012 she has served ARCHELON as Peloponnese Projects Coordinator (2012–2015), as well as Conservation Coordinator (2015-present). She is specialized in environmental legislation, in shaping and implementing environmental policy with emphasis on coastal and marine ecosystems and the protection of sea turtles, in data collection and analysis, and in reporting. Panagiota has worked closely with local, regional, and national authorities, European and international institutions, research bodies, management agencies, and non-governmental organizations to promote the sustainable management of the natural environment, the implementation of legislation, and the resolution of conflicts. She has significant experience in cooperation, putting pressure, as well as in lobbying with authorities in managing local communities and other stakeholders in the direction of sustainable management of the natural environment. (Photographed by Hugo Baron).

**Dimitris Margaritoulis** discovered the nesting of Loggerhead Turtles in Greece (Zakynthos Island) in 1977, and he has worked since then on research, conservation, and management of sea turtles in Greece and in the Mediterranean Sea. He is a founding member (1983) of the Sea Turtle Protection Society of Greece (now ARCHELON), Athens, which through its pioneer sea turtle field projects, as well as its public awareness and environmental education activities, became a model non-governmental organization. Dimitris took active part in the elaboration of the Action Plan for the Conservation of Marine Turtles in the Mediterranean of the United Nations Environment Program/ Mediterranean Action Plan, and of the Global Strategy for the Conservation of Marine Turtles of the International Union for Conservation of Nature. He served for 10 y (1999–2009) as the Mediterranean Regional Chair for the Marine Turtle Specialist Group (MTSG) of the IUCN and was President of the International Sea Turtle Society (ISTS) 2005–2006. For his work on marine turtles, he has received several awards, among them the Athens Academy Award (1984) and the ISTS Life Achievement Award (2010). (Photographed by Anna Kremezi-Margaritoulis).