REVISED CLUTCH FREQUENCY ESTIMATES FOR MASIRAH ISLAND LOGGERHEAD TURTLES (CARETTA CARETTA)

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Abstract.—The Sultanate of Oman hosts the largest Loggerhead Turtle (Caretta caretta) rookery in the Indian Ocean and was historically estimated as a third of the global stocks of the species. Early population estimates for the major rookery of Masirah Island in the Sultanate of Oman used track count surveys adjusted by an extrapolation of four nests per female as the average clutch frequency. We revisited the rookery to recalibrate a more rigorous estimate of clutch frequency by satellite tracking female loggerheads. We documented an estimated clutch frequency (ECF) for females of 5.5 nests in 2010, 5.2 nests in 2011, and 5.8 nests in 2012. Mean ECF of Loggerhead Turtles on Masirah Island = 5.4 nests ± (SD) 0.87 (range, 4–7 nests, n = 34). The revised ECF makes a -27% correction to earlier population estimates for the loggerhead management unit of the Northwest Indian Ocean. The revised ECF estimate can be combined with monitoring surveys to determine trends within this regional management unit and assess its conservation needs.

Key Words.—Indian Ocean; management unit; nest; population estimation; reproduction; satellite telemetry; sea turtle

INTRODUCTION

Surveys of remote sea turtle rookeries require accurate parameter estimates to generate population estimates that are less prone to biases (National Research Council 2010). The population estimates at turtle nesting beaches rely directly upon morning track count surveys or nocturnal tagging studies, with corrections for partial effort (Schroeder and Murphy 1999; Baldwin et al. 2003; Witherington et al. 2009; Pfaller et al. 2013). The realities of every sea turtle tagging or track count study include tag loss, few capture-recapture records in the first decade of study, variation in remigration schedules, variable female reproductive output, and unrecorded nesting events beyond the sampling area.

It can be straightforward to enumerate the sea turtle tracks on a beach and calculate a local abundance or density (Schroeder and Murphy 1999; State of the World’s Turtles [SWOT] 2011). However, it is understandably more difficult to acquire a valid estimate of individual female clutch frequency to convert track counts into a female abundance estimate (Tucker 2010; Richards et al. 2011; Stewart et al. 2014). In simple math, the annual nest numbers (numerator) divided by an accurate female clutch frequency (denominator) yields a population estimate for females that are reproductively active for that rookery in that season. Consequently, female population estimates for marine turtles are sensitive to the clutch frequency parameter (Richards et al. 2011), so innovative studies are required to define this key parameter accurately.

The Northwest Indian Ocean Management Unit of the Loggerhead Turtle (Caretta caretta) nests in the Sultanate of Oman (Baldwin et al. 2003; Wallace et al. 2010; Casale and Tucker 2015). The major loggerhead rookery on Masirah Island had historical track-count surveys conducted 1977–1979 and 1991 (Perran Ross, unpubl. reports). The historical track counts estimated 20,000 to 40,000 nesting females a year based upon a clutch frequency of four nests per female (Perran Ross, unpubl. reports). From a contemporary conservation standpoint, there was a valid concern that the historical
Tucker et al.—Masirah Island Loggerhead Turtles.

**Figure 1.** Beaches of Masirah Island, Sultanate of Oman, host the highest density of nesting loggerheads in the Northwest Indian Ocean at mean densities ranging from 1–23 nests km$^{-1}$ day$^{-1}$. The inset shows Masirah Island in the Arabian Sea off the southeastern facing coast of Oman. The arrow indicates the location of satellite tag deployments within the concentrated nesting zones.

clutch frequency estimates for Masirah were possibly inaccurate and should be rechecked by more modern methods such as satellite telemetry.

We reevaluated female clutch frequency during recent surveys of Masirah seasonal nesting abundance and trends. We tracked nesting females by satellite telemetry through three nesting seasons to determine an estimated clutch frequency (ECF). The study illustrates telemetry as a viable tool for demographic estimates at remote rookeries, provides a revised estimate of reproductive outputs by the major Indian Ocean loggerhead management unit, and calls attention to a future review of the regional population status.

### Materials and Methods

**Study area.**—The eastern beaches of Masirah Island, Oman (20.600 N, 58.906 E), are a main nesting aggregation of loggerheads in the Indian Ocean (Baldwin et al. 2003; Fig. 1). In general, loggerhead nesting occurs within temperate to subtropical zones. However, the tropical beaches of Oman are an exception (Pritchard 1979) because the country is exposed to the influence of a southwesterly monsoon exposing the rookery to more temperate air and sea temperatures during the nesting season. Nesting beaches of Oman are exposed beaches of coarse sand, backed by arid coastal plains or low rocky hills (Baldwin et al. 2003). Loggerhead nesting occurs upon at least 178 beaches along the mainland Oman coast in addition to the 84 km of beach on Masirah Island (Salm et al. 1993).

**Field methods.**—We visited the high density nesting beaches of Masirah Island as the nesting season began in mid to late April for three seasons (2010–2012) to account for potential inter-annual variability in nest production. We encountered turtles randomly and confirmed oviposition by visual inspection of eggs in the nest chamber. We recorded previous flipper tags or applied new tags (Stockbrands, Perth, Western Australia) to left and right flippers. For subsequent genetic studies, we took a skin biopsy from the trailing edge of the rear flippers, which we stored in 80% ethanol. We measured turtle length from notch to tip over the curved carapace length (CCL) to the nearest 1 cm with a stretched and calibrated soft tape measure.

We detached each turtle temporarily in a portable wooden box, cleaned the carapace of epibiota, and rinsed with fresh water and alcohol to ensure dryness. We attached GPS Argos transmitters (Fastloc MK10, Wildlife Computers, Redmond, Washington, USA; approximately 200–400 g in air) to the carapace using slow-curing construction adhesive smoothed into a hydrodynamic shape and coated by anti-fouling paint (Tucker 2010). The application process took 2 h to complete and we returned all turtles to the water before daybreak.

**Tracking and analysis.**—We organized, evaluated, and archived data in Satellite Telemetry Analysis Tool (STAT; Coyne and Godley 2005). A tracking path of movements connected latitude and longitude fixes of GPS and ARGOS Location Classes 3, 2, 1, 0, and A based on recommendations derived for marine turtles (Hays et al. 2001). We filtered location data in STAT to exclude unlikely data for swim speeds > 5.0 km hr$^{-1}$, or for angles < 15$^\circ$.

**Determination of clutch frequency.**—The observed clutch frequency (OCF) of a female based on field observations underestimates the actual number of nests when nests occur beyond the spatial or temporal coverage of observer patrols (Frazer and Richardson 1985; Tucker and Frazer 1991; Rivalan et al. 2006; Tucker 2010; Weber 2013). It was impractical on Masirah Island to empirically record observed clutch frequency because females reach densities up to 97 nesting attempts km$^{-1}$ per night across a 120 day nesting season, and there are 84 km of nesting beach (Perran Ross, unpubl. report). To overcome the coverage challenges, we used satellite telemetry to define terrestrial emergences and an estimated clutch frequency (ECF) as proposed and validated by other loggerhead tracking studies (Tucker 2010). We calculated ECF by including any undocumented nests that fit the expected interesting interval.
Some Masirah females were sedentary near shore in the internesting period, which added challenges to identify the terrestrial emergences from the satellite transmissions. We inferred terrestrial emergences in a tracking history by location fixes that corresponded to multiple criteria. The combinations of criteria (defined in Tucker 2009) included: (1) temporal - presumed haul-outs within the expected internesting intervals for loggerheads (9–15 d); (2) bathymetry - haul-out locations associated with depths of -0.5 to +0.5 m; (3) vector change - when the turtle movements were directed onshore followed by an immediate offshore vector; (4) classification difference - there was an improvement in location classes such as multiple LC 2 or 3 within a short time span; (5) signal shift - there was evidence of an increased surface interval in the transmitter data; (6) direct verification by nocturnal patrols if the female had site fidelity to the same beach as the patrols; or (7) genetic verification by genetic fingerprinting using molecular techniques. The Masirah study used only criteria 1–5 to identify an emergence. At the end of an internesting interval (temporal criterion), the improved LC with one or more locations in succession that coincided with coastlines and sea level indicated a difference between a false crawl (non-nesting emergence) and a nest. A characteristic departure vector directed to deeper water (bathymetry shift) was indicative of a nesting event after days of lingering nearshore in depths of -2 m to +2 m or of emergences spread over several nights before a successful nest.

Statistical analysis.—We studied the annual ECF determined by satellite telemetry for 3 y. We used a Kolmogorov-Smirnov one sample test with Poisson distributions to test differences from a hypothesized clutch frequency of four nests (setting $\lambda = 4$). We also evaluated a correlation of turtle size (CCL) against clutch frequency (ECF) for evidence if differently sized animals had capacity to lay more clutches. We used a factorial ANOVA evaluating year as a category against turtle CCL or clutch frequency as independent variables. We report the unadjusted $P$ values.

Results

Mean female CCL of tracked turtles was 98.8 cm ± (SD) 4.0 cm (range, 86–111 cm, n = 34). Female size was not significantly different across the study years ($F_{2,34} = 0.553, P = 0.579$). There was no predictive relationship of reproductive output determined solely by female body size ($P = 0.122$).

The mean ECF for 2010 turtles was 5.5 nests ± 1.3 nests (range, 4–7, n = 4), for 2011 turtles = 5.2 nests ± 0.9 nests (range, 4–7, n = 18), and for 2012 turtles was 5.8 nests ± 0.6 (range 5–7, n = 12; Fig. 2). The tracked turtles showed no significant difference of mean clutch frequency among years ($F_{2,34} = 0.549, P = 0.582$). A mean ECF of 5.4 nests ± 0.9 nests, n = 34 turtles) for our study was significantly greater than an ECF of 4.0 nests ± 0.9 ($Z = 8.4, \text{Bonferroni adjusted } P < 0.001, 95\% \text{ confidence interval} = 5.1–5.8$) from previous studies. A clutch frequency of 5.4 nests was 36% higher than the assumed reproductive values used by historical surveys (Perran Ross, unpubl. report) for a -27% correction to the historical population estimates.

Discussion

Assessments of marine turtle population trends based on track count or nest count data should be interpreted cautiously and re-evaluated whenever possible (National Research Council 2010). These are accepted concerns that apply to any population estimates of organisms with imperfect detection (Schwarz and Seber 1999). The ECF concept was introduced decades ago (Frazer and Richardson 1985), yet researchers have only recently relied on satellite telemetry as a more efficient mark-recapture tool to refine ECF estimates (Scott 2006; Rees et al. 2010; Tucker 2010; Weber et al. 2013; Esteban et al. 2017). It is unsurprising that ECF estimates improve as previously undocumented nesting events are added via telemetry. A conclusion in the Masirah case study is that estimates of this vital demographic parameter are impractical to derive by standard nocturnal tagging patrols.

The Masirah findings agree with earlier studies that clutch frequency of sea turtles is more accurately determined with the benefit of satellite telemetry. Some related examples included Georgia loggerheads (Scott 2006), Florida loggerheads (Tucker 2009; Tucker et al. 2010), Ascension Island Green Turtles (Chelonia mydas; Weber et al. 2013), and Chagos Island Green Turtles.
An earlier satellite telemetry study of Masirah loggerheads (Rees et al. 2010) suggested a minimum clutch frequency of 4.8 nests ÷ 1.2 nests (n = 8) for one season but admitted a negative bias in the ECF estimate by missing early nests. The earlier ECF value of four nests for Georgia loggerheads came from a long-term study based on saturation tagging patrols documenting 2.8–4.2 nests per female (Frazer and Richardson 1985). Notably, that same rookery later used satellite telemetry to recalibrate a higher ECF of 4.5 nests per season (Scott 2006), while a genetic fingerprinting mark-recapture study broadly covering the Southeastern U.S. region also found higher ECF because of nesting detected across many beaches (Shamblin et al. 2017). Early studies of clutch frequency without the benefits of satellite telemetry include Green Turtles by intensive patrols (Johnson and Ehrhart 1996; Alvarado-Diaz et al. 2003; Stokes et al. 2014) or radio telemetry (Weber et al. 2013), loggerheads by mark-recapture analysis (Frazer and Richardson 1985; Pfaller et al. 2013) or genetic fingerprinting (Shamblin et al. 2017), and leatherbacks by nocturnal patrols (Eckert et al. 1989; Tucker and Frazer 1991) or statistical estimation of stopover duration (Rivalan et al. 2006). All the foregoing studies should be recalibrated by satellite tracking to augment mark-recapture patrols and improve the accuracy of ECF estimates. We acknowledge that small sample sizes are inherent in most telemetry studies, and a caveat applies in the potential impact on results from making population-level inferences from a small sample size.

A methodological critique of any telemetry study regards the accuracy of signal inference. We did not duty cycle transmitters until several months after the season ended to not interfere with the multi-criteria approach of inferring terrestrial emergences. An impact of duty cycling is discussed by Witt et al. (2010) including: surfacing behavior, Argos receiver availability, and programmed duty cycling of Fastloc-GPS data acquisition. We used Fastloc-GPS tags to attempt the best accuracy and higher chances to collect data than the Argos system alone and rapid repetition rates to achieve the best signal output from the marine environment. However, there are still occasions where a missed signal by the GPS or Argos constellation or a lack of convergence of multiple criteria means a failure to detect a terrestrial emergence. The location errors with Argos signals (Montgomery et al. 2011; Boyd and Brightsmith 2013) in comparison with the higher frequency and accuracy of Fastloc-GPS location fixes (Hays et al. 2001; Costa et al. 2010; Witt et al. 2010) gave reasonable confidence that our approach would have sufficient precision to document terrestrial emergences (Jonsen et al. 2005; Hoenner et al. 2012).

Our study demonstrated a combination of judicious data filtering and multiple criteria matching can achieve reliable indicators of loggerhead haul-outs. However, discrimination between non-nesting or nesting emergence would not be certain until a track path veered offshore after a last location class of highest certainty near a nesting beach. Future studies that combine Fastloc transmitters and land-based receiving stations (also called Mote receivers) will enhance the interpretation of transmissions when turtles haul-out to nest (Jeanniard-du-Dot et al. 2017).

Masirah survey data from 2008–2016 documented a 72% nesting success (i.e., the percentage of emergences that yielded nest deposition; range, 67–77%; Five Oceans Environmental Services, unpubl. data) but do not report on the influence of nesting success on ECF. Field observations of arid coastal habitats of Masirah confirm that multiple nesting attempts in dry sand are common (Robert Baldwin and Andrew Willson, pers. comm.). In addition, nesting success was about 5–10% lower (i.e., more false crawls) on zones affected by beach driving (Five Oceans Environmental Services, unpubl. data). These caveats apply when studies focus upon the single parameter of clutch frequency and emphasize why track count surveys should be conducted across multiple seasons to account for inter-annual variability in individual clutch frequency.

Our study highlights the importance of accurate ECF for population estimates with a given management unit (Broderick et al. 2002; Mazaris et al. 2008; Richards et al. 2011). We found that clutch frequency was independent of female size, which agrees with other loggerhead studies (Broderick et al. 2003). However, it is best not to isolate on clutch frequency without regard to spatial variation in foraging habitat quality. Some regional stocks of loggerheads (e.g., Mediterranean rookeries) are relatively small-bodied and may in fact lay fewer clutches (Broderick et al. 2003; Schofield et al. 2013). An extended view of clutch frequency emerges in a context of growing, stable, or declining populations and the relative demographic compositions of younger and older individuals. Evidence suggests that younger females have lower ECF than demographically mature females (Tucker 2010). Our study cannot speculate if a recalibrated and higher ECF in the Masirah population represents one of these scenarios, but the question remains for future studies to address.

Furthermore, new insights on carry-over effects obtained by stable isotope studies suggest that foraging areas productivity can shape multiple facets of reproductive output including clutch frequency, reproductive size of females, clutch size, and remigration intervals (Cardona et al. 2014; Vander Zanden et al. 2014; Ceriani et al. 2015) even within the same ocean basin. Stable isotope studies have identified a nutrient-driven difference in reproductive outputs by ocean basins for loggerheads (Pajuelo et al. 2010) and for leatherbacks.
(Wallace et al. 2006). Extra complexity in provisioning a reproductive season is probable for turtles that transition seasonally between foraging habitats or that forage nomadically in ocean gyres. Much remains to be learned on energy reserves gained by marine turtles at foraging grounds and expended in migration and during the inter-nesting periods. The present study did not determine if turtles from different foraging grounds had different ECF, but that question might be pursued in a stable isotope study (Ceriani et al. 2015).

One may question whether ECF estimates were biased in any given year by applying satellite tags to early nesters of the season. Tagging early in the season reduces the likelihood of missing nesting events and captures the full extent of the nesting season for early nesters with low or high ECF. We assumed that late nesters with lower ECF would balance out with early nesters with low ECF. The consistency of ECF across three years affirms a primary point that females are nesting annually more than previously accounted for, regardless of whether individual females are differing by an onset of nesting or by nest frequency. A key message is that without satellite telemetry, researchers are simply underestimating female annual nest output when females nest beyond a study site. We therefore recommend that an ECF value generated by satellite tracking be used for future population estimates of Masirah loggerheads.

**Conservation implications.**—An ongoing inter-agency partnership is standardizing the nest surveys on Masirah Island during phase two of this project (Blair Witherington et al., unpubl. report). The surveys in progress document that current nesting abundances are lower than the historical counts conducted in the 1970s and 1980s (Five Oceans Environmental Services, unpubl. data). A renewal in the research and monitoring efforts at Masirah since 2006 should clarify the status of this population (Wallace et al. 2011; Casale 2015). The season-long nesting surveys with requisite data analysis are essential in monitoring the Masirah rookery, protected areas management planning, and a host of awareness raising activities within the local community.

A recalibrated ECF at Masirah affects any past or future estimates of the population abundance of Loggerhead Turtles in the Northwest Indian Ocean. Three factors that shape an IUCN Red List assessment are weighted scores on population abundance, trends, and threats. The global status for loggerheads was strongly shaped by two most abundant regions, i.e., the Northwest Atlantic (Florida) and Northwest Indian Ocean management units (Oman), which hosted 42.4% and 33.0% of the global population, respectively (Casale 2015). Female reproductive parameters have now been recalibrated in the two largest management stocks of Loggerhead Turtles by an ECF > 4, while Oman had an apparently declining nesting abundance (Blair Witherington et al., unpubl. report) and nest abundance in Florida increased in the last decade (Florida Fish and Wildlife Conservation Commission. 2017. Index Nesting Beach Survey Totals (1989-2017). Available from http://myfwc.com/research/wildlife/sea-turtles/nesting/beach-survey-totals/ [Accessed 22 January 2018]). Obviously, divergent trends at two major management units representing 75% of global loggerhead stocks reflect the underlying philosophy of a regional management unit framework (Wallace et al. 2010). Together, evidence of declining nest counts and a revised population estimate based upon accurate ECF are factors to carefully reassess in a future stocktaking of loggerhead stock in the Northwest Indian Ocean (Casale 2015). Similar demographic consequences of underestimating or overestimating clutch frequency would apply to other sea turtle stocks (National Research Council 2010). It is likely that findings from the satellite tracking project at Masirah will inform managers of potential threats to loggerheads near and away from Oman.

It is crucial to recognize that nonlinear responses are appropriate to consider when extrapolating sea turtle populations based on non-continuous monitoring across decades (Witherington et al. 2009; Van Houtan and Halley 2011; Arendt et al. 2013). Otherwise, for the Northwest Indian Ocean, there are only confusing statements of 37% of global stocks (Baldwin et al. 2003) and 33% of loggerhead global stocks (Casale and Tucker 2015), which may themselves be questionable. It is understood that inter-annual variation in sea turtle populations results from overlapping but non-synchronous cohorts with differing remigration intervals (National Research Council 2010), unequal detection (Pfläger et al. 2013), or influences of sea surface temperatures (Solow et al. 2002). Thus, researchers fully recognize the inadequacy of trends defined in extrapolations from 1980s surveys to recent surveys when there is a major gap of no surveys whatsoever. Nevertheless, the comparison of historical records for Masirah Island to a recent 2008–2014 dataset indicates a 70% decline over 20 y (Blair Witherington et al., unpubl. report). Survey results from 2015 and 2016 imply that nest counts may have declined even lower (Five Oceans Environmental Services, unpubl. data). Although efforts are underway to evaluate the potential influence of bycatch, recent events have exposed the rookery vulnerability to a host of escalating threats including marine debris from ship wrecks, beach driving, urban lighting, and development of beach habitat. The declines of this population management unit are reflected by an IUCN Red List status of Critically Endangered (Casale 2015). A documented decline and a revised population estimate based on recalibrated ECF
give some of the information needed by managers to identify critical threats and organize recovery efforts.

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