Spatial ecology of loggerhead turtles: Insights from stable isotope markers and satellite telemetry

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Abstract

Aim: Using a combination of satellite telemetry and stable isotope analysis (SIA), our aim was to identify foraging grounds of loggerhead turtles (Caretta caretta) at important rookeries in the Mediterranean, examine foraging ground fidelity, and across 25 years determine the proportion of nesting females recruiting from each foraging region to a major rookery in Cyprus.

Location: Mediterranean Sea.

Methods: Between 1993 and 2018, we investigated the spatial ecology of loggerhead turtles from rookeries in Cyprus and Greece using satellite telemetry (\(n = 55\) adults) and SIA of three elements (\(n = 296\)).

Results: Satellite telemetry from both rookeries revealed the main foraging areas as the Adriatic region (Cyprus: 4\% of individuals, Greece: 55\%), Tunisian Plateau (Cyprus: 16\%, Greece: 40\%) and the eastern Mediterranean (Cyprus: 80\%, Greece: 5\%). Combining satellite telemetry and SIA allowed 64\% of all nesting females to be assigned to; the Adriatic region (Cyprus: 2\%, Greece: 38.5\%), Tunisian Plateau (Cyprus: 47\%, Greece: 38.5\%) and the eastern Mediterranean (Cyprus: 51\%, Greece: 23\%), which are markedly different to proportions obtained using satellite telemetry. The proportion of the Cyprus nesting cohort using each foraging region did not change significantly, with the exception that individuals foraging in the Adriatic region are only present in the Cyprus nesting population from 2012. Repeat satellite tracking (\(n = 3\)) and temporal consistency in isotope ratios (\(n = 36\)) of Cyprus females, strongly suggest foraging ground fidelity over multiple decades.

Main conclusions: This study demonstrates the advantages of combining satellite telemetry and SIA to investigate spatial ecology at a population level. The importance of the Tunisian Plateau for foraging is demonstrated. This study indicates that females generally show high fidelity to foraging grounds and shows a potential recent shift to foraging in the Adriatic region for Cyprus females, while the importance of other regions persists across decades, thus providing baselines to develop and assess conservation strategies.
1 | INTRODUCTION

Many marine species migrate over long distances, often travelling thousands of kilometres across remote areas, between critical habitats. Consequently, understanding their movements and identifying areas of habitat use can be challenging. Marine migrants are considered particularly vulnerable to overexploitation, habitat loss and climate change (Robinson et al., 2009), and a lack of knowledge of where and how populations move throughout their life cycle makes it difficult to identify potential risks to their survival. It is therefore necessary to understand the geographical range and migratory connectivity of a species for successful development of long-term conservation plans (Webster, Marra, Haig, Bensch, & Holmes, 2002).

Marine turtles often migrate across ocean basins between foraging and nesting grounds (e.g., Shillinger et al., 2008), and several geographically distinct foraging areas are typically used by individual nesting populations (e.g., Dujon, Schofield, Lester, Papafitsoros, & Hays, 2018; Hays, Hobson, Metcalfe, Righton, & Sims, 2006; Seminoff et al., 2008; Stokes et al., 2015). Traditionally, conservation, and conservation-driven research, of marine turtles has been focused on easily accessible nesting grounds (Bjorndal, 1999; Hamann et al., 2010), protecting nesting females and their eggs, thus potentially only protecting a small proportion of the life cycle of the species. The large geographical range over which marine turtles migrate and forage means that turtles are under high threat from fisheries so require a more diverse approach to conservation (Wallace et al., 2011). Bycatch is one of the key threats to marine turtles in the Mediterranean Sea resulting in high levels of mortality (conservatively 44,000 deaths per year, Casale, 2011; Casale et al., 2018). Consequently, working towards the conservation of critical marine regions, including foraging grounds and migratory routes, is considered a research priority in Mediterranean marine turtle ecology (Casale et al., 2018).

A common technique used in marine megavertebrate spatial ecology is satellite telemetry which enables migratory species to be tracked over long distances (e.g., Gillespie, 2001). This can provide fine-scale near real-time movement data on location and speed, but is an expensive technique, and this cost can often limit the sample size (Godley et al., 2008). In contrast, stable isotope analysis (SIA) is a powerful but relatively cheap forensic tool and has been used for several marine taxa (Bird et al., 2018; Newsome, Clementz, & Koch, 2010; Rubenstein & Hobson, 2004), including marine turtles (Figgener, Bernardo, & Plotkin, 2019a, 2019b; Haywood et al., 2019), to gain insights into the spatial and foraging ecology of marine species. Combining the locational data of satellite telemetry with stable isotope ratios allows scaling up and has been shown to enable inference of habitat use at a population level (e.g., Bradshaw et al., 2017; Ceriani et al., 2015; Ceriani, Weishampel, Ehrhart, Mansfield, & Wunder, 2017). This would enable conservation plans to be better informed, targeting foraging grounds that support the largest proportion of the nesting cohort.

Within low-metabolically active tissues of a consumer, the ratio of stable isotopes reflects the food that an individual has consumed and the location where it was ingested, therefore, acting as intrinsic habitat markers of migratory connectivity (DeNiro & Epstein, 1978). In marine research, the ratio of $^{13}$C:$^{12}$C (expressed as $\delta^{13}$C), $^{15}$N:$^{14}$N (expressed as $\delta^{15}$N) and $^{34}$S:$^{32}$S (expressed as $\delta^{34}$S) is most commonly used as geographical markers. Carbon isotope ratios reflect the primary producer at the base of the food chain in which feeding occurs (DeNiro & Epstein, 1978), with benthic and nearshore regions supported by algae and seagrass exhibiting high $\delta^{13}$C values in comparison with pelagic and oceanic regions supported by phytoplankton (DeNiro & Epstein, 1978; Graham, Koch, Newsome, McMahon, & Aurioles, 2010). Nitrogen isotope ratios of marine primary producers differ in relation to (a) nitrogen-based processes (e.g., nitrification, denitrification and $N_2$ fixation), and (b) nitrogen isotope ratios of their nutrient sources (e.g., $N_2$, ammonium and nitrate; Montoya, 2007). Sulphur isotope ratios in primary producers differ based on access to sulphides with inshore ecosystems supported by seagrass and microphytobenthos exhibiting low $\delta^{34}$S values when compared to offshore ecosystems supported by phytoplankton (e.g., Bradshaw et al., 2017). $\delta^{34}$S values are believed to be a true habitat marker as they are independent of fractionation from prey to predator, unlike $\delta^{13}$C and $\delta^{15}$N values (McCutchan, Lewis, Kendall, & McGrath, 2003).

Despite the benefits of analysing all three isotopes, only one previous study has used this methodology for loggerhead turtles (Tucker, MacDonald, & Seminoff, 2014). The oligotrophic Mediterranean Sea has regional heterogeneity in salinity, temperature and primary productivity, all of which influence nutrient cycling (Zotier, Bretagnolle, & Thibault, 1999). Therefore, the Mediterranean can support regions and food webs of differing isotopic compositions, and this variation can allow marine turtle foraging habitats to be inferred (e.g., Bradshaw et al., 2017; Cardona et al., 2014).

Fresh egg yolk and epidermis tissue sampled during the egg laying process are considered representative of the diet consumed in the foraging ground used several months prior to the tissue being sampled (Ceriani, Roth, Ehrhart, Quintana-Ascencio, Weishampel, 2014). The combination of satellite telemetry and SIA data allows the isotope ratios of specific foraging grounds to be determined. If isotope ratios of foraging grounds are distinct, this enables untracked females to be reliably assigned to putative foraging grounds from a single tissue sample, hence providing an understanding of the spatial ecology at a population level (e.g., Ceriani et al., 2015; Seminoff et al., 2012). In addition, the temporal consistency of isotope ratios has been used for confirming foraging ground fidelity in marine taxa (e.g., Newsome et al., 2010), including marine turtles (e.g., Bradshaw et al., 2017; Thomson et al., 2017).
If foraging ground fidelity occurs, then long-term studies enable the proportion of individuals in each annual nesting cohort using each foraging ground to be determined across multiple nesting seasons and therefore identifying potential shifts in population dynamics (e.g., Bradshaw et al., 2017; Ceriani et al., 2015, 2017). Temporal changes in the proportion of individuals using each foraging ground could be inferred as changes in the foraging ground dynamics, including changes in recruitment, survival of individuals or changes in foraging resources and environmental conditions. These could in turn be reflective of natural ecological or anthropological changes.

It is estimated that there are approximately 16,000 adult loggerhead turtles in the Mediterranean of which ~3,500 females nest annually (Casale & Heppell, 2016). The major foraging regions for these nesting females have been identified using flipper tag returns and satellite telemetry and include the northern Adriatic Sea, Aegean Sea, Turkey, Egypt and the Tunisian Plateau (Broderick, Coyne, Fuller, Glen, & Godley, 2007; Godley, Broderick, Glen, & Hays, 2003; Hays, Fossette, Katselidis, Mariani, & Schofield, 2010; Hays, Mazaris, & Schofield, 2014; Lazar, Margaritoulis, & Tvrtkovic, 2004; Margaritoulis & Rees, 2011; Patel et al., 2015; Schofield et al., 2013; Snape et al., 2016; Zbinden, Aebischer, Margaritoulis, & Arlettaz, 2008; Zbinden et al., 2011; see also reviews by Margaritoulis et al., 2003; Luschi & Casale, 2014; Casale et al., 2018). However, this information currently exists for only a small sample of these populations. Nest counts in Mediterranean rookeries are generally not increasing as rapidly as expected despite intensive conservation efforts on the nesting beaches (Casale et al., 2018). A more comprehensive picture of where Mediterranean loggerhead turtles are foraging would help target conservation strategies (Casale et al., 2018). Combining satellite telemetry and SIA, Bradshaw et al. (2017) described in detail the foraging grounds of a large proportion of nesting green turtles (Chelonia mydas) from an important rookery in North Cyprus. We aimed to replicate this study, and be the first to analyse green turtles (Chelonia mydas) from an important rookery in North Cyprus (Casale et al., 2018), combining satellite telemetry and SIA, to identify the foraging grounds used by loggerhead turtles from nesting populations in Greece and North Cyprus, examine the level of foraging ground fidelity and determine the proportion of the North Cyprus nesting cohort using each identified foraging ground during this multi-decadal study.

2 | METHODS

2.1 | Field data and sample collection

The beaches at Alagadi (35°20‘N, 33°29‘E) are major nesting grounds for loggerhead turtles in North Cyprus (Casale et al., 2018), where nest protection and monitoring has been implemented since 1992 (Broderick, Glen, Godley, & Hays, 2002, Figure 1). Nightly monitoring (for details see Stokes et al., 2014) took place between 20:30 and 05:00 during the nesting seasons between 1993 and 2018, from May to mid-August. For individual identification, after laying, females had flipper tags placed in the trailing edge of both fore-flippers and, since 1997, passive integrated transponders were injected into the shoulder muscle. Minimum curved carapace length (CCL, notch-to-notch, Bolten, 1999) was measured with a flexible measuring tape as an indicator of body size.

2.2 | Satellite telemetry

At Alagadi Beach, between 2001 and 2018, 32 Platform Terminal Transmitters (PTTs) were attached to 29 adult female loggerhead turtles after oviposition, with three of these individuals tracked on two occasions. In addition, eight PTTs were deployed from other beaches in North Cyprus: Akdeniz (35°20‘N, 32°56‘E), Iskele (35°16‘N, 33°55‘E) and Tatlisu (35°41‘N, 33°76‘E, Figure 1, see Appendix S1). The PTTs deployed between 2001 and 2012 from North Cyprus were previously published (Broderick et al., 2007; Godley et al., 2003; Snape et al., 2016), while 11 PTTs were attached in 2017–2018 on Alagadi (see Appendix S1). To further increase sample sizes, previously published satellite telemetry data for 18 individuals nesting at the Bay of Laganas on Zakynthos, Greece (37°72‘N, 20°86‘E, Zbinden et al., 2008, 2011) were included in our analysis (Figure 1, see Appendix S1). For details on the analysis of satellite telemetry data, see Appendix S1.

2.3 | Stable isotope analysis

Of 373 individual females that were recorded nesting at Alagadi between 2001 and 2018, epidermis tissue samples (<0.0025 m²) were collected using a scalpel from the trailing edge of the right fore-flipper (from the third membrane) or the shoulder (between the neck and fore-flipper) from 233 individuals (21 of which were satellite-tracked individuals). Until required for analysis, tissue samples were stored in either, >70% ethanol at room temperature (n = 421), >70% ethanol in a non-frost-freezer (n = 31), or frozen in sodium chloride solution (n = 28). Dermis tissue was separated from the skin samples in the laboratory, and only the epidermis tissue was used in the analysis. For details on the stable isotope analysis conducted, see Appendix S2.

Several individuals were sampled multiple times to determine the consistency of isotope ratios between left and right flipper samples (n = 38 females), between flipper and shoulder samples (n = 51 females), across successive clutches in the same season (sampled during first encounter and 10–16 days after the previous clutch, n = 30 females), and across nesting seasons (n = 36 females). For details on the methods of this analysis, see Appendix S2.

In addition, stable isotope ratios (δ¹³C and δ¹⁵N) were available for the present study from 12 satellite-tracked (see Appendix S1) and 51 untracked females from Zakynthos (previously published in Zbinden et al., 2011). Zbinden et al. (2011) collected yolk from unhatched eggs (during post-hatching clutch excavation) and fresh eggs (during laying). Yolk samples were frozen and subjected to lipid extraction. Therefore, to obtain comparable values to the present
study, we converted the isotope ratios of unhatched yolk to fresh yolk (by subtracting 0.49‰ from $\delta^{15}$N values, see Zbinden et al., 2011) and then to epidermis values using published tissue conversion equations for frozen fresh lipid extracted yolk to female loggerhead turtle epidermis values ($\delta^{13}$C$_{epi} = 0.90 \times \delta^{13}$C$_{yolk} - 0.95$, $\delta^{15}$N$_{epi} = 1.05 \times \delta^{15}$N$_{yolk} - 0.75$, Kaufman et al., 2014).

2.4 | Foraging ground assignment

Tissue samples were available for 21 Alagadi and 12 Zakynthos satellite-tracked females. Due to limited tissue quantity, six Alagadi individuals could only be run for $\delta^{13}$C and $\delta^{15}$N analysis, while 15 were analysed for all three stable isotope ratios (see Appendix S1). $\delta^{13}$C and $\delta^{15}$N analysis were prioritized for comparison to previous SIA research, as only four marine turtle studies to date have analysed $\delta^{34}$S values (see review by Haywood et al., 2019). From the previous study only $\delta^{13}$C and $\delta^{15}$N values were available from Zakynthos individuals. The PTTs of two Alagadi individuals ceased to function during migration; therefore, these were excluded from further analysis.

To enable assignment of untracked females to putative foraging grounds, statistically significant differences in stable isotope ratios among foraging grounds are required. To determine suitable geographical regions which are isotopically distinct, a principal component analysis was run, and an analysis of covariance was used to confirm if the isotope ratios of the identified regions were significantly different from each other. For details of this analysis, see Appendix S3. To assign untracked females to putative foraging grounds, the nominal assignment approach of linear discriminant function analysis (LDA) was used in the R-package “mass” (Venables & Ripley, 2002). Non-uniform priors based on the number of turtles tracked to each foraging region were used as recommended by Vander Zanden et al. (2015). As no Zakynthos individuals and not all Alagadi individuals had associated $\delta^{34}$S values, two LDAs were run. The first for $\delta^{13}$C and $\delta^{15}$N values while the second included all three isotopes. For the LDA using $\delta^{13}$C and $\delta^{15}$N values, the isotope ratios of 31 tracked females (Alagadi: $n = 19$; Zakynthos: $n = 12$) were used as the training dataset to develop the discriminant functions, while the remaining 265 untracked females (Alagadi: $n = 214$; Zakynthos: $n = 51$) were the test dataset for assignment to putative foraging grounds. For the LDA using $\delta^{13}$C, $\delta^{15}$N and $\delta^{34}$S values (using only Alagadi individuals), the training dataset consisted of 11 tracked females, while 160 untracked females were the test dataset for assignment. A jackknifed leave-one-out cross-validation method was used to assess the accuracy of the assignments. Assignments with posterior probabilities of ≥80% were considered successful.

2.5 | Foraging ground fidelity

Thirty-six individuals that had multi-year $\delta^{13}$C and $\delta^{15}$N values, and of those 23 individuals had multi-year $\delta^{34}$S values, allowed foraging ground fidelity to be examined for this population. Twenty-two individuals had isotope ratios for two nesting seasons, eight for three seasons, three for four seasons, and three for five nesting seasons. To test isotope temporal consistency, repeatability estimates using a linear mixed-effects model for Gaussian data fitted with restricted maximum likelihood were used in the R-package “rptR” (Stoffel, Nakagawa, & Schielzeth, 2017). Turtle ID was set as the grouping factor.

2.6 | Annual contributions to the Alagadi nesting cohort

Satellite-tracked individuals, with known foraging region, and individuals assigned to putative foraging regions (with posterior probabilities of ≥80%) were used to estimate the proportion of the annual cohort using each foraging ground through SIA. Tissue samples were only collected from 2001 onwards but some individuals, that were
identified from 1993 onwards, had samples collected in later seasons and therefore could be assigned to foraging grounds based on the assumption of foraging ground fidelity. To determine whether the proportion of nesters using each foraging ground differed among years (1993–2018), generalized additive models for binomial data were run for each foraging ground in the R-package “mgcv” (Wood, 2017), which took autocorrelation into account.

All analyses were performed with the software R 3.5.1 (R Core Team, 2018) and for statistical tests, the significance level was alpha = 0.05.

3 | RESULTS

3.1 | Satellite telemetry

From this study, a total of 40 PTTs were deployed on 37 females from four release sites in North Cyprus (three females were tracked twice from Alagadi, Figure 1). Locational data were transmitted for 6–2,007 days (mean: 371 days). Of these, 37 PTTs provided location data throughout the post-nesting migration to the foraging grounds. From Zakynthos, 18 females were satellite-tracked and all transmitted to confirmed foraging grounds and transmitted for 114–740 days (mean: 328 days). Satellite-tracked females from North Cyprus had mean CCL of 0.84 ± 0.04 m (range: 0.76–0.89 m), while turtles from Greece had mean CCL of 0.84 ± 0.04 m (range: 0.76–0.89 m). These CCL values are within the ranges recorded for nesting females at each representative site, showing satellite-tracked females represent the parent population well (Casale et al., 2018; Omeyer, Godley, & Broderick, 2017).

Post-nesting females from North Cyprus migrated via numerous migratory routes to the Aegean Sea, the Adriatic region (including the Adriatic Sea and the Gulf of Amvrakikos), and across a large extent of the eastern Mediterranean basin, to foraging grounds in Italy, Turkey, Cyprus, Syria, Lebanon, Israel, Egypt, Libya, Tunisia and the Tunisian Plateau (Figure 2a). Females nesting in Greece migrated to Croatia, Slovenia, Italy, Greece, Tunisia and the Tunisian Plateau (Figure 2b). Thirty-nine females remained in distinct foraging grounds all located on the continental shelf (within the 200 m isobath), fourteen females showed over-wintering behaviours moving to a second distinct area during the winter months, and two turtles (Turtle 21 and Turtle 27) conducted oceanic foraging throughout their deployments in waters >200 m (466 and 222 days, respectively, Figure 2a). These oceanic individuals were considered untracked for foraging ground assignment as they did not occupy a distinct foraging ground.

3.2 | Foraging ground assignment

The principal component analysis identified three isotopically distinct geographical regions (see Appendix S3a), the Adriatic region, the Tunisian Plateau and the rest of the eastern Mediterranean (Figure 2). The “Adriatic region” includes all individuals foraging in the Adriatic Sea and the North Ionian Sea (including the Gulf of Amvrakikos), the “Tunisian Plateau” includes all individuals foraging offshore the Tunisian coast (mean distance from coast: 68.5 km) on the Tunisian Plateau, and the “rest of the eastern Mediterranean” includes all individuals foraging in neritic regions in the eastern Mediterranean basin, including individuals foraging nearshore on the Tunisian Plateau (Figure 2). These regions had significantly different isotope ratios even when body size was taken into account (Analysis of Covariance, δ13C: F2,25 = 11.99, p < .001, δ15N: F2,25 = 14.62, p < .001, δ34S: F2,2 = 4.47, p = .05, see Appendix S3b). A post hoc Tukey’s Honest Significant Difference test revealed that significant differences occurred between all regions with the Adriatic region distinct based on high δ15N values, the Tunisian Plateau distinct based on high δ34S values, and the rest of the eastern Mediterranean distinct based on high δ13C values (see Appendix S3a).

Tissue samples were available from 265 untracked females (of which 51 were from Greece), which ranged in size between 0.59 to 0.94 m (mean: 0.722 m). Stable isotope ratios ranged from −18.18‰ for δ13C (mean: −15.47‰), 4.44–12.8‰ for δ15N (mean: 8.88‰), and 1.62–23.39‰ for δ34S (mean: 18.05‰). The LDA using δ13C and δ15N values correctly assigned 74% of satellite-tracked individuals to their foraging region (Alagadi: 69% and Zakynthos: 83%) as tested by the jackknifed leave-one-out cross-validation method. The LDA using δ13C, δ15N, and δ34S values correctly assigned 73% of the Alagadi satellite-tracked females (Zakynthos individuals did not have associated δ34S values so were not included). The resultant uncertainties in the LDA due to propagating the isotope analytical uncertainties are ±0.1‰ (for both cases, for details on this analysis see Appendix S5).

Untracked individuals included in both LDAs (n = 129) were assigned to the same foraging region, showing consistency in this method. For Alagadi, 70% of untracked females (n = 148) were successfully assigned to putative foraging grounds. Of those assigned, 2% were assigned to the Adriatic region, 47% to the Tunisian Plateau and 51% to the rest of the eastern Mediterranean. For Zakynthos, 25% of untracked females (n = 13) were successfully assigned to putative foraging grounds. Of those assigned, 38.5% were assigned to the Adriatic region, 38.5% to the Tunisian Plateau and 23% to the rest of the eastern Mediterranean. Due to posterior probabilities being ≤80%, 30% of untracked Alagadi females (n = 62) and 75% of untracked Zakynthos females (n = 38) remained unassigned (Figure 2, see Appendix S3a). Oceanic Turtle 27 was assigned to the Tunisian Plateau, while Turtle 21 was unassigned due to posterior probabilities being ≤80%. Isotope ratios of satellite-tracked and isotopically assigned females are shown in Table S3.2.

3.3 | Foraging ground fidelity

Three individuals (Turtles 1, 3 and 37) were tracked during two foraging seasons and showed strong foraging ground fidelity. The centroids of their foraging grounds were separated by 1.2 km (Turtle 1),
20.2 km (Turtle 3), and 0.2 km and 0.8 km (Turtle 37, Figure 3). Turtle 37, was tracked for 2,007 days across two foraging seasons, and shuttled repeatability between two foraging grounds 13 km apart but showed exceptionally high fidelity to both foraging grounds (Figure 3). For individuals sampled for SIA, the δ¹³C, δ¹⁵N and δ³⁴S values across multiple nesting seasons had highly significant repeatability estimates (Repeatability estimation (R)), δ¹³C: R ± Standard error = 0.92 ± 0.02, 95% Confidence Interval = 0.86–0.95, p < .001, n = 36, δ¹⁵N: R ± Standard error = 0.94 ± 0.02, 95% Confidence Interval = 0.89–0.96, p < .001, n = 36, δ³⁴S: R ± Standard error = 0.84 ± 0.06, 95% Confidence Interval = 0.68–0.92, p < .001, n = 23, Figure 4). The analysis was repeated 100 times while perturbing the isotope data using additive noise (with a noise distribution based on the analytical uncertainties). In all cases, the p-values remained <.05 (for details of this analysis see Appendix S5). Therefore, these results are considered to be insensitive to the isotope analytical uncertainties. Isotope ratios of the oceanic Turtle 21 did not differ between two nesting seasons despite not occupying a distinct
foraging ground (Figure 4, only one sample was available for the oceanic turtle Turtle 27).

3.4 Annual contributions to the Alagadi nesting cohort

Across the study period 70% (n = 148) of sampled Alagadi nesting females were successfully assigned to a putative foraging region. Of these, fewest females foraged in the Adriatic region (2%) whereas the remainder were approximately equally split between the Tunisian Plateau (47%) and the rest of the eastern Mediterranean (51%). By determining foraging ground use at a population level, this study shows the number of females utilizing each region is markedly different to the proportions obtained from using purely the satellite tracking data, which results in one Alagadi individual tracked to the Adriatic region (4%), four to the Tunisian Plateau (16%), and 20 to the rest of the eastern Mediterranean (80%, see Appendix S1). Of those assigned to the Adriatic region, half were remigrants (returning females), while 26% of Tunisian Plateau foragers and 29% of the foragers in the rest of the eastern Mediterranean were remigrants. The proportion of individuals assigned to all foraging regions did not differ among years (Generalized additive model, Adriatic region: t-value = 0.21, df = 25, p = .83, Tunisian Plateau: t-value = 0.97, df = 25, p = .34, rest of the eastern Mediterranean: t-value = 0.44, df = 25, p = .67, Figure 5, for the number of females assigned to each foraging ground see Table S4.3) but it should be noted that breeding individuals that use the Adriatic region were only recorded from 2012 onwards.

4 DISCUSSION

This study adds to the growing body of literature that demonstrates the benefits of combining the complementary methodological approaches of satellite telemetry and SIA in understanding the spatial ecology of animal populations (e.g., Ceriani, Roth, Evans, Weishampel, & Ehrhart, 2012; Seminoff et al., 2012; Reich et al., 2017; for a review and references therein see Haywood et al., 2019). This combined approach allowed us to understand the importance of foraging grounds for the broader population, which demonstrates a remarkable difference from results obtained from several individuals using satellite telemetry alone. When teamed with long-term individual-based monitoring programmes, these combined techniques can determine whether the importance of these foraging regions persist over decades and provide baselines to assess future conservation strategies (e.g., Bradshaw et al., 2017; Ceriani et al., 2015; Ceriani et al., 2017; Pajuelo et al., 2012; Vander Zanden et al., 2014).

Previous satellite telemetry has shown these North Cyprus and Greece nesting populations utilize a broad range of foraging grounds (Broderick et al., 2007; Godley et al., 2003; Hays et al., 2010, 2014; Schofield et al., 2013; Snape et al., 2016; Zbinden et al., 2008, 2011). The PTTs deployed in this study in 2017 and 2018 (see Appendix S1) continued to identify a wide range of migratory routes and new foraging grounds including the first use of the Aegean Sea, the Adriatic Sea, and the western Mediterranean basin, none of which had previously been observed for the North Cyprus nesting population (Figure 2a). Satellite telemetry results suggest the majority of the Alagadi nesting population forages around the eastern Mediterranean Basin (80%),
while few forage on the Tunisian Plateau (16%) and in the Adriatic region (4%). However, by combining telemetry results with the powerful forensic tool of SIA, this study identifies the importance of the Tunisian Plateau as a foraging region for this population. This region appears to support almost half of the Alagadi nesting population (47%), despite being a relatively small geographical region and is a considerable distance from the rookery (2,500 km). This result can be used to better inform conservation, suggesting this relatively small foraging region, which supports a large proportion of the nesting cohort, is targeted for future management.

Prior satellite tracking and SIA studies have also shown the Tunisian Plateau to be a major foraging ground for loggerhead turtle rookeries across the Mediterranean Sea (e.g., Cardona et al., 2014; Hays et al., 2010; Schofield et al., 2013; Snape et al., 2016; Zbinden et al., 2011) as well as for male (Casale, Freggi, Cina, & Rocco, 2013; Hays et al., 2014; Schofield et al., 2013) and juvenile loggerhead turtles (Casale et al., 2012). In addition, both our satellite telemetry and SIA results support previous work showing foraging ground fidelity occurs in this species (Figures 3 and 4, Broderick et al., 2007; Schofield et al., 2010; Thomson et al., 2012; Tucker et al., 2014). In comparison with satellite telemetry alone, using isotope ratios to investigate foraging site fidelity not only enhances the sample size but enables tracking over decades. This is the first loggerhead turtle study to use this method on multi-decadal data showing foraging site fidelity across five nesting seasons. Isotopically tracking individuals over decades provides a baseline to potentially investigate shifts in habitat use as well as to provide pre- and post-disaster information (e.g., Reich et al., 2017).

In the Mediterranean Sea, bycatch is one of the most important threats to marine turtles and the Tunisian Plateau has some of the highest rates (Casale, 2011; Casale et al., 2018; Casale, Cattarino, Freggi, Rocco, & Argano, 2007). In Alagadi and Zakynthos, nest counts are not increasing as rapidly as expected (Casale et al., 2018), suggesting that alternative conservation approaches are needed. This study supports the need to focus site-specific conservation strategies to key marine habitats, such as the Tunisian Plateau, which may dramatically increase the survival of individuals in this foraging ground and aid in the recovery of many loggerhead rookeries across

**FIGURE 4** (a), (c) and (e) show temporal consistency in δ^{13}C, δ^{15}N and δ^{34}S values of samples collected from female loggerhead turtles across multiple nesting seasons in Alagadi Beach, North Cyprus. The oceanic Turtle 21 is highlighted in red. (b), (d) and (f) show differences in isotope ratios between samples using the first nesting season as a reference.
the Mediterranean. Potential future conservation management approaches to reduce bycatch in important foraging areas, such as the Tunisian Plateau, have been reviewed in Casale et al. (2018). The review highlights the need of monitoring and reporting bycatch, the enforcement of changes to less detrimental fishing gears, as well as mitigation measures such as the use of turtle excluder devices by bottom trawlers or “circle hooks” by longliners (Casale et al., 2018 and references therein).

Both satellite telemetry and SIA show the Adriatic region is a more important foraging area for those nesting in Greece than nesting females in North Cyprus. The use of the Adriatic region by nesting populations in Greece (Cardona et al., 2014; Hays et al., 2014; Lazar et al., 2004; Schofield et al., 2013; Zbinden et al., 2011) and the limited use by eastern nesting populations, such as North Cyprus, have been previously reported (Margaritoulis & Rees, 2011; Snape et al., 2016). Hatchling dispersal studies suggest adult foraging grounds may be selected based on the passive dispersion of hatchlings by surface currents, with those originating from Greece dispersed to the Adriatic region (Casale et al., 2010; Casale & Mariani, 2014), while those from eastern nesting sites are restricted from entering this region (Casale & Mariani, 2014). Therefore, with the importance of foraging grounds likely to differ between nesting populations, this study should be replicated for all major nesting grounds to ensure all critical marine habitats for this species are considered in conservation plans.

Temporal differences in hatchling dispersal have been simulated and are thought to be due to fluctuations in surface currents (e.g., Hays et al., 2010). With shifts in ocean circulation likely with future climate scenarios (Hoegh-Guldberg & Bruno, 2010), shifts in hatchling dispersal and in turn adult foraging grounds may occur (Hays et al., 2010). Shifts in foraging grounds will not only determine the potential of fisheries interactions due to variable bycatch rates across the Mediterranean (Casale, 2011) but could also influence reproductive output, with individuals foraging in areas of high productivity, such as the Adriatic region, being larger with larger clutch sizes (e.g., Cardona et al., 2014).

Collecting long-term individual-based data at easily accessible nesting beaches can allow monitoring of shifts in the importance of foraging grounds (e.g., Bradshaw et al., 2017; Ceriani et al., 2015, 2017). Although loggerhead turtle foraging grounds have been identified, how each foraging ground contributes to a nesting cohort on a long-term scale has not been investigated in the Mediterranean and no marine turtle study has investigated this over multiple decades. This study shows that the proportion of the Alagadi cohort using each foraging region did not significantly differ across this multi-decadal study. This suggests little shift in the importance of these regions, with recruitment, survivorship, and conditions potentially remaining similar. In contrast, significant shifts in the relative contributions to foraging grounds have been reported in major loggerhead turtle rookeries in the Atlantic (Ceriani et al., 2017; Pajuelo et al., 2012; Vandersanden et al., 2014).

Over this 25 year study period, individuals foraging in the Adriatic region were only seen in the Alagadi cohort from 2012 onwards (Figure 5) and could suggest differences in recruitment and survivorship in some areas or a range shift possibly due to climatic variations in the environmental conditions or anthropological changes (Casale et al., 2018). We support the recommendation by Ceriani et al. (2017) that multi-decadal studies are required to detect long-term trends in population dynamics, providing a baseline to assess temporal shifts in foraging ground importance enabling conservation management to be adapted and targeted appropriately. It also provides baselines to develop and assess future conservation strategies.

This is the first instance of oceanic foraging behaviours reported for the North Cyprus nesting population (Figure 2a); however, this behaviour has occasionally been recorded previously for adult females in other regions of the Mediterranean Sea (e.g., Bentivegna, 2002; Schofield et al., 2010; Zbinden et al., 2008). Despite oceanic foraging, Turtle 21 showed temporal consistency in isotope ratios (Figure 4) suggesting they are consuming similar prey items from similar food chains across years. Oceanic foraging could reduce the accuracy of using SIA for foraging ground assignment as foraging ground fidelity is required; however, a small proportion of females are doing this. Both oceanic foragers were relatively small in comparison with the other satellite-tracked females. A size difference between foraging strategies has been reported in previous SIA studies investigating neritic versus oceanic foragers and was attributed to sparsely distributed planktonic prey in oceanic habitats leading to smaller individuals in comparison with those foraging on nutritional neritic prey (Cardona, Martins, Uterga, & Marco, 2017; Eder et al., 2012; Hatase et al., 2002).

Oceanic Turtle 21 spent 230 days in the Strait of Sicily before entering the western Mediterranean basin. This is the first report of a westerly migration for the North Cyprus nesting population. Although juvenile loggerhead turtles originating from the eastern Mediterranean have been previously reported to forage in the western basin (e.g., Margaritoulis et al., 2003), few adults have been
observed to migrate here (e.g., Margaritoulis et al., 2003; Schofield et al., 2013). The Strait of Sicily has strong south easterly currents year-round (Poulaín & Zambianchi, 2007), which may have restricted Turtle 21 from entering the western basin sooner. Strong surface currents may limit hatching dispersal to this region reducing the likelihood of adult foraging areas in the western basin (Hays et al., 2010; Casale & Mariani, 2014).

For SIA to successfully assign individuals to putative foraging grounds isotopically distinct regions must be used, and three were identified in this study, the Adriatic region, the Tunisian Plateau and the rest of the eastern Mediterranean (Figure 2, see Appendix S3a). Individuals in the Adriatic region have relatively low δ13C values and high δ15N values. This is because they are foraging on food chains strongly influenced by major river systems supplying terrestrial organic matter, which have lower δ13C values than marine organic matter (Degobbis & Gilmaritn, 1990; Vizzini, Savona, Do Chi, & Mazzola, 2005; Zbinden et al., 2011) and are likely to have a substantial amount of highly enriched 15N anthropogenic waste and agricultural run-off (e.g., Degobbis & Gilmaritn, 1990; Zbinden et al., 2011). This trend has been previously reported in Mediterranean loggerhead turtles (Cardona et al., 2014; Zbinden et al., 2011); notably, the eastern Mediterranean basin (including the Tunisian Plateau) has high levels of N2-fixation and therefore lower baseline δ15N values in comparison with the Adriatic region (Pantoja, Repeta, Sachs, & Sigman, 2002), explaining the low δ15N values reported for these foragers.

Individuals foraging on the Tunisian Plateau are foraging further offshore (mean: 68.5 km) than those in the rest of the eastern Mediterranean (mean: 11.0 km) or the Adriatic region (mean: 4.8 km). Although still on the continental shelf it is likely loggerhead turtles on the Tunisian Plateau are foraging on food chains with phytoplankton as the primary producer. Individuals foraging on the Tunisian Plateau have relatively low δ13C values and high δ34S values. This is expected as less productive pelagic and oceanic regions supported by phytoplankton have lower δ13C values and higher δ34S values in comparison with productive benthic and nearshore regions supported by algae and seagrass (DeNiro & Epstein, 1978; Graham et al., 2010). This trend has been previously reported in benthic communities (Pinnegar & Polunin, 2000) and for green turtles (Bradshaw et al., 2017; Cardona, Aguilar, & Pazos, 2009; Tucker et al., 2014).

In total, our study was unable to assign 30% of the Alagadi and 75% of the Zakynthos females sampled to one of three relatively broad geographical regions (Figure 2, see Appendix S3a). Samples from Zakynthos were run previously for only δ13C and δ15N analysis (by Zbinden et al., 2011), which likely contributed to the low assignment success observed. To better understand the spatial variation of loggerhead turtle isotopes in the Mediterranean Sea, we support the recommendation of previous studies (e.g., Bradshaw et al., 2017; Ceriani et al., 2012; Seminoff et al., 2012) in the use of these complementary tracking approaches and urge all future satellite telemetry studies to sample satellite-tracked individuals for SIA to understand the spatial ecology of marine vertebrates at a population level. In addition, the collaboration of researchers enabled the data for two major rookeries in the Mediterranean Sea to be combined to better understand the geographical differences in isotope ratios. This is the first study of this geographical scale in the Mediterranean. A basin-scale collaboration, combining data from foraging and nesting grounds across the Mediterranean, would enable species-specific isoscapes to be created (as recommended by Haywood et al., 2019), which would enhance our understanding on marine turtle ecology in this oceanographically complex region.

To date, only one loggerhead turtle study has analysed δ34S values to assign individuals to foraging grounds (Tucker et al., 2014). The present study is the first study to analyse all three isotopes for loggerhead turtles in the Mediterranean, a method that has been previously reported as vital for distinguishing green turtle foraging grounds in this region (Bradshaw et al., 2017). We strongly support the recommendation by Bradshaw et al. (2017) that sufficient tissue should be sampled to allow analysis of δ13C, δ15N and δ34S values. The Tunisian Plateau, for example, would not have been distinguishable from the rest of the eastern Mediterranean without δ34S analysis, and in turn the importance of this region would not have been highlighted. In addition, if sulphur had been analysed for the Zakynthos nesting population then it is likely a much larger proportion of nesting females would have been assigned to a foraging region. To better delineate isotopic profiles between multiple foraging grounds and help assign more individuals to putative foraging regions, analysis of additional intrinsic markers, for example trace elements (e.g., Ramirez et al., 2019) or additional analytical techniques such as amino acid compound specific stable isotope analysis (e.g., Seminoff et al., 2012; Vander Zanden et al., 2013) would also be beneficial. This could be especially important in regions of complex geography and oceanography with multiple foraging regions, such as the Mediterranean Sea (Bradshaw et al., 2017).

The large geographical range used by loggerhead turtles in the Mediterranean Sea makes protection challenging and requires a diverse approach to conservation (Wallace et al., 2011). Due to the collaboration of researchers, this is the first SIA study of this geographical scale in the Mediterranean, that combines satellite telemetry and SIA of three isotopes, enabling the critical marine regions of two major loggerhead turtle rookeries to be determined at a population level. This will enable conservation plans to be better informed, targeting foraging grounds that support the largest proportion of major nesting cohorts, where fisheries management can be directed. Continued monitoring of critical marine habitats is vital to detect changes in habitat use resulting from natural or anthropological changes, such as climate change. This would enable successful development of long-term conservation plans. By conducting the longest study of its kind, this research demonstrates the strength of stable isotope tracking to detect shifts in the importance of foraging regions across multiple decades and to direct management and conservation efforts to these critical habitats.

To summarize, to create a more comprehensive picture of where Mediterranean loggerhead turtles are foraging, we combined satellite telemetry and SIA to infer habitat use at a population level. This study confirms the importance of the Tunisian Plateau as a foraging
region and as a potential area for future conservation management. We demonstrate high foraging ground fidelity in this population and show that the importance of these foraging regions persists across this multi-decadal study, providing baselines to develop and assess conservation strategies. This work has greatly enhanced our understanding of the movements and habitat use of loggerhead turtles nesting in the regionally important rookery at Alagadi, North Cyprus and demonstrates the advantages of using complimentary tracking techniques to study the spatial ecology of elusive marine vertebrates. This study shows how this method could be a powerful tool in the designation of Marine Protected Areas designed to protect migratory species.

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DATA AVAILABILITY STATEMENT

Data can be made available by contacting the corresponding author directly.

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