The following supplement accompanies the article

Satellite tracking and stable isotope analysis highlight differential recruitment among foraging areas in green turtles

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Supplement 1. Satellite tracking

Table S1. Data from 28 green turtles satellite tracked from northern Cyprus.

ID	PTT	Release date	Days	Foraging area	Transmitter	Calibration	SI sample 1	SI sample 2	SI sample 3	SI sample 4	Data source
G077	4150	27/07/1998	294	Libya (Gulf of Bomba)	Wildlife Computer	✓	2006*	2011*	<u>2014</u>		1,2,3
G078	4149	29/07/1998	222	Libya (Gulf of Bomba)	Wildlife Computer	✓	2006*	<u>2014</u>			1,2,3
G082	4148	01/08/1998	289	Turkey (Antalya Bay)	Wildlife Computer	✗					1,2,3
G125	6598	19/07/1999	243	West Libya (Gulf of Sirte)	Telonics ST18	✗					1,2,3
G059	4405	27/07/2002	403	Turkey (Antalya Bay)	Telonics ST6	✓	<u>2012</u>				2,3
G055	36638	12/07/2003	364	West Libya (Gulf of Sirte)	Sirtrack 2003	✓	<u>2006</u>	2010*	2015		2,3
G008	36639	24/06/2004	395	West Libya (Gulf of Sirte)	Sirtrack 2003	✓	<u>2012</u>				2,3
G076	49815	08/07/2004	61	Turkey (Antalya Bay)	Kiwisat 101	✗					2,3
G002	49816	23/07/2004	357	Libya (Gulf of Bomba)	Kiwisat 101	✓	<u>2010</u>	2014*			2,3
G044	49813	23/07/2004	311	West Libya (Gulf of Sirte)	Kiwisat 101	(C&N only)	<u>2007*</u>	2010*	2013*		2,3
Randall	95099	08/06/2009	81	Egypt (Lake Bardawil)	Kiwisat 101	✗					4
G015	95097	04/07/2009	486	West Libya (Gulf of Sirte)	Kiwisat 101	✓	2009	<u>2013</u>			3
G157	95101	05/07/2009	715	Libya (Gulf of Bomba)	Kiwisat 101	✓	<u>2009</u>				3
G166	95098	15/07/2009	116	Egypt (Gulf of Arab)	Kiwisat 101	✓	<u>2006</u>				3
G189	95102	24/07/2009	110	Egypt (Lake Bardawil)	Kiwisat 101	✓	2009	<u>2011</u>			3
G058	52820	16/06/2010	751	Libya (Gulf of Bomba)	Kiwisat 101	✓	2006*	2010	<u>2014</u>		3
G009	86898	26/06/2010	475	Libya (Gulf of Bomba)	Kiwisat 101	✓	2010*	<u>2014</u>			3
G163	52846	28/06/2010	348	West Libya	Kiwisat 101	✓	2005*	2007	2010*	<u>2013</u>	3
G080	52827	01/07/2010	407	West Libya (Tunisia border)	Kiwisat 101	✓	<u>2010</u>	2013*			3
G087	52949	07/07/2010	478	Libya (Gulf of Bomba)	Kiwisat 101	✓	2006*	2010*	<u>2014</u>		3
G006	86900	13/07/2010	412	S. Cyprus	Kiwisat 101	✓	2010*	<u>2015</u>			3

ID	PTT	Release date	Days	Foraging area	Transmitter	Calibration	SI sample 1	SI sample 2	SI sample 3	SI sample 4	Data source
G172	52888	21/07/2010	122	West Libya (Tunisia border)	Kiwisat 101	✓	2007*	2010	2013		3
Pepsi Kibris	52818	04/06/2011	134	S. Cyprus	Kiwisat 101	✓	2011				NA
G217	150429	30/06/2015	146	Egypt (Lake Bardawil)	Wildlife Computer	✓	2009*	2013	2015		NA
G252	150427	30/06/2015	68	Egypt (Lake Bardawil)	Wildlife Computer	✓	2011	2015			NA
G020	150430	01/07/2015	73	Egypt (Lake Bardawil)	Wildlife Computer	✓	2009	2011	2013	2015	NA
G201	150431	01/07/2015	58	Egypt (Lake Bardawil)	Wildlife Computer	✓	2008*	2011	2013*	2015	NA
G254	150428	01/07/2015	58	Egypt (Lake Bardawil)	Wildlife Computer	✓	2011	2015			NA

ID = turtle's identification number (names in bold = male green turtles), PTT = numeric code for the platform terminal transmitter, Days = number of days the turtle was tracked for, Foraging area = conclusive end point where turtle was deemed resident, Calibration = turtles used to calibrate the discriminant analysis, SI samples 1 - 4 = which year tissue samples were collected for stable isotope analysis. Sample years underlined were specifically used to calibrate the discriminant analysis, * = only analysed for $\delta^{13}C$ or $\delta^{15}N$. Data source legend, 1 = Godley et al. (2002), 2 = Broderick et al. (2007), 3 = Stokes et al. (2015), 4 = Wright et al. (2012) and NA = unpublished data.

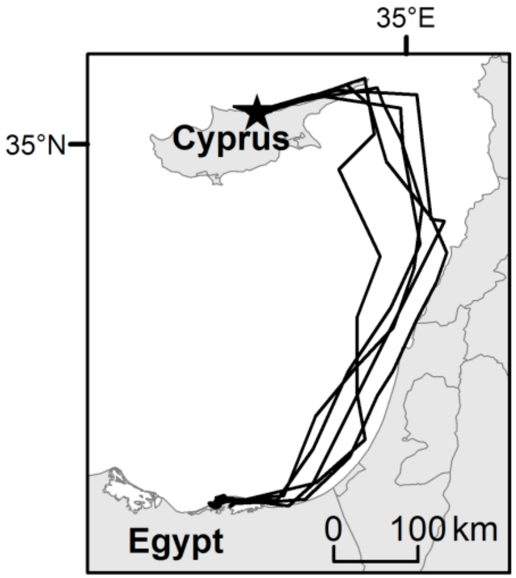

Fig. S1. Post nesting green turtle satellite tracks recorded in 2015 from Cyprus to Lake Bardawil, Egypt. These 5 turtles were specifically selected for PTT attachment based on their $\delta^{13}C$ and $\delta^{15}N$ values.

Supplement 2. Lipid extraction

Samples from 20 green turtles that nested among years (2009 – 2014) were used to determine whether lipid extraction was necessary by subdividing the sample so that half were not lipid-extracted whilst the other half were lipid-extracted using a 2:1 chloroform:methanol ratio in a Soxlet apparatus and heated for 1 hour.

The selected samples had a pre-extraction C:N ratio of 2.68 (±SD = 0.06). No significant differences were found between untreated and lipid-extracted tissue samples for $\delta^{15}N$ (paired t-test, $t_{19} = 1.70$, $p = 0.11$, S2a). Statistically significant differences were found for $\delta^{13}C$ (paired t-test, $t_{19} = - 4.0$, $p < 0.001$, S2b) with a mean difference of -0.18 (range =-0.27 – 0.09). However, the differences in $\delta^{13}C$ values between lipid extracted and untreated samples were not substantially different considering the mean difference in $\delta^{13}C$ among foraging areas (1.68 ‰) and lipid extraction was not considered necessary for the whole dataset.

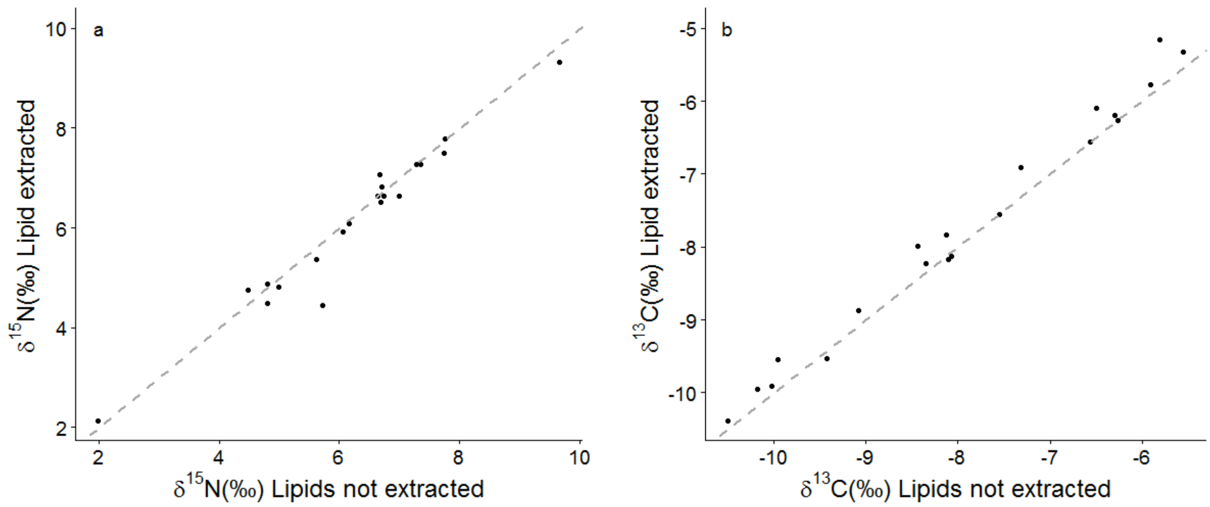

Fig. S2. Differences in stable isotope values between untreated and lipid extracted paired samples for a = $\delta^{15}N$ and b = $\delta^{13}C$ (n = 20). Grey dashed line = no difference (y = x).

Supplement 3. Storage concentration of ethanol

Paired epidermal tissue samples were collected simultaneously from 33 nesting females post-oviposition and stored in a 96% and 70% ethanol concentration for up to 5 months. The concentration of ethanol had no significant effect on δ^{15}N (paired t-test, $t_{32} = 0.673$, $p = 0.506$, S3a) or δ^{13}C values (paired t-test, $t_{32} = -0.129$, $p = 0.8981$, S3b) as no consistent enrichment or depletion of δ^{15}N or δ^{13}C values was found among samples.

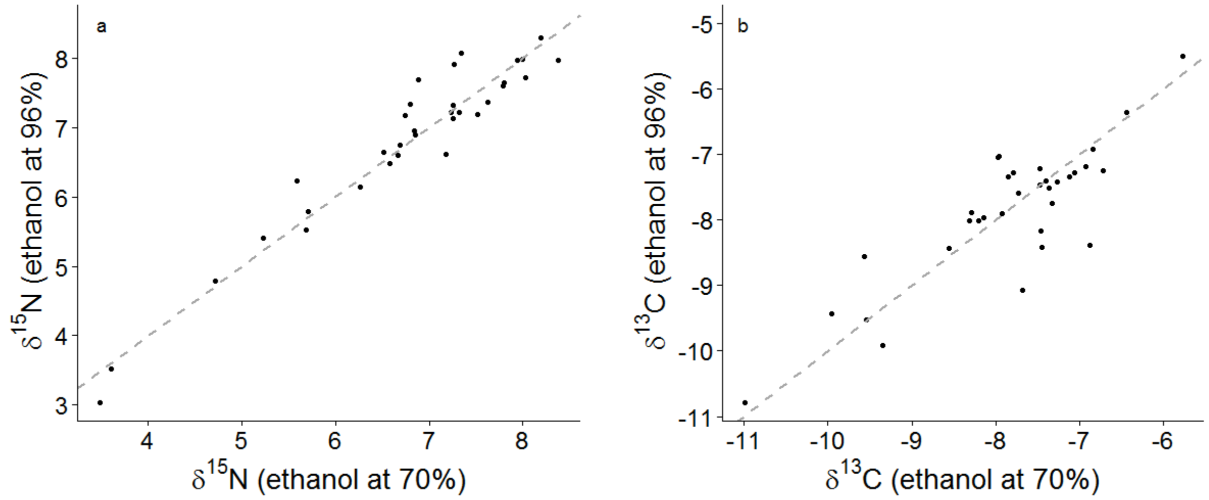

Fig. S3. Comparison of stable isotope values for paired green turtle epidermis samples (n = 33) stored in either 70% or 96% ethanol concentration, a = δ^{15}N values, b = δ^{13}C values. Grey dashed line = no difference (y = x).

Supplement 4. Predicting foraging area

This study adopted standard methods (Ceriani et al. 2012, Pajuelo et al. 2012, , Vander Zanden et al. 2015) to predict foraging areas using a discriminant function analysis evaluated by the leave-one-out cross validation method. Discrete differences in the combined isotopic values were assessed with a multivariate analysis of variance (MANOVA) with multiple pairwise comparisons conducted with Tukey's Honest Significant Difference to identify significant differences among foraging areas. Non-uniform priors were used based on the number of turtles tracked to each foraging area as they can improve the accuracy of assignment (Royle & Rubenstein 2004, Vander Zanden et al. 2015). We set a posterior probability of assignment at 80% or greater to maintain consistency among studies (Pajuelo et al. 2012, Seminoff et al. 2012, Vander Zanden et al. 2015) as this provides a 8 - 12 fold improvement in assignment over random odds considering 3 or 4 foraging areas, respectively (Wunder 2012, Vander Zanden et al. 2015).

Table S2. Sample sizes and the year that they were collected

	Preliminary DFA (conducted prior to 2015)				Post 2015 DFA + secondary classification			
Year sampled	N	Satellite tracked	Assigned	Unassigned	N	Satellite tracked	Assigned	Unassigned
2006	2	2			2	2		
2007	2	1	1					
2008	2		1		1		1	
2009	24	1 (1)	12	10	13	1	10	2
2010	14	4	10		7	2	3	2
2011	29	2	14	13	20	2	14	4
2012	15	2	13		6	2	1	3
2013	64	3 (1)	41	19	54	3	34	17
2014	51	4 (1)	32	14	43	5	25	13
2015					42	6	29	7

Total	203	19 (22)	124	56	188	23	117	48

Preliminary DFA was conducted using $\delta^{13}C$ and $\delta^{15}N$ prior to 2015, Post 2015 DFA + secondary classification was conducted using $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$. N = number of turtles sampled in each year, satellite tracked = number of turtles sampled that were satellite tracked to 1 of the 4 foraging areas, Assigned = number of turtles assigned to a foraging area, Unassigned = number of turtles that could not be assigned to a foraging area. Values in brackets under 'Satellite tracked' were the 3 turtles selected to characterise the unidentified foraging area and therefore their foraging area was not predicted

Supplement 5. Analysing foraging site fidelity

Foraging site fidelity was evaluated with a repeatability analysis employed in the R statistical package 'rptR' (Nakagawa & Schielzeth 2010) using a linear mixed-effects model based estimation for Gaussian data fitted with restricted maximum likelihood (REML). The identity of the turtle was the grouping factor and we controlled for the variance attributed to where a turtle forages by including this as a covariate, although 6 of the 45 turtles were unassigned to a foraging area. Confidence intervals (CI) were set at 95% and calculated through 1000 bootstrap statistics with asymptotic p-values calculated by 1000 permutations.

The differences in $\delta^{13}C$ and $\delta^{15}N$ values among serially collected samples were calculated using the first sample as a reference. The mean difference in $\delta^{15}N$ = 0.91‰ (upper & lower quantiles = 0.34 – 1.36‰, range = 0.02 – 2.50‰) and $\delta^{13}C$ = 0.61‰ (upper & lower quantiles = 0.27 – 0.81‰, range = 0.00 – 2.36‰, Fig.5 in the main text)

Supplement 6. Isotopic composition of the study population

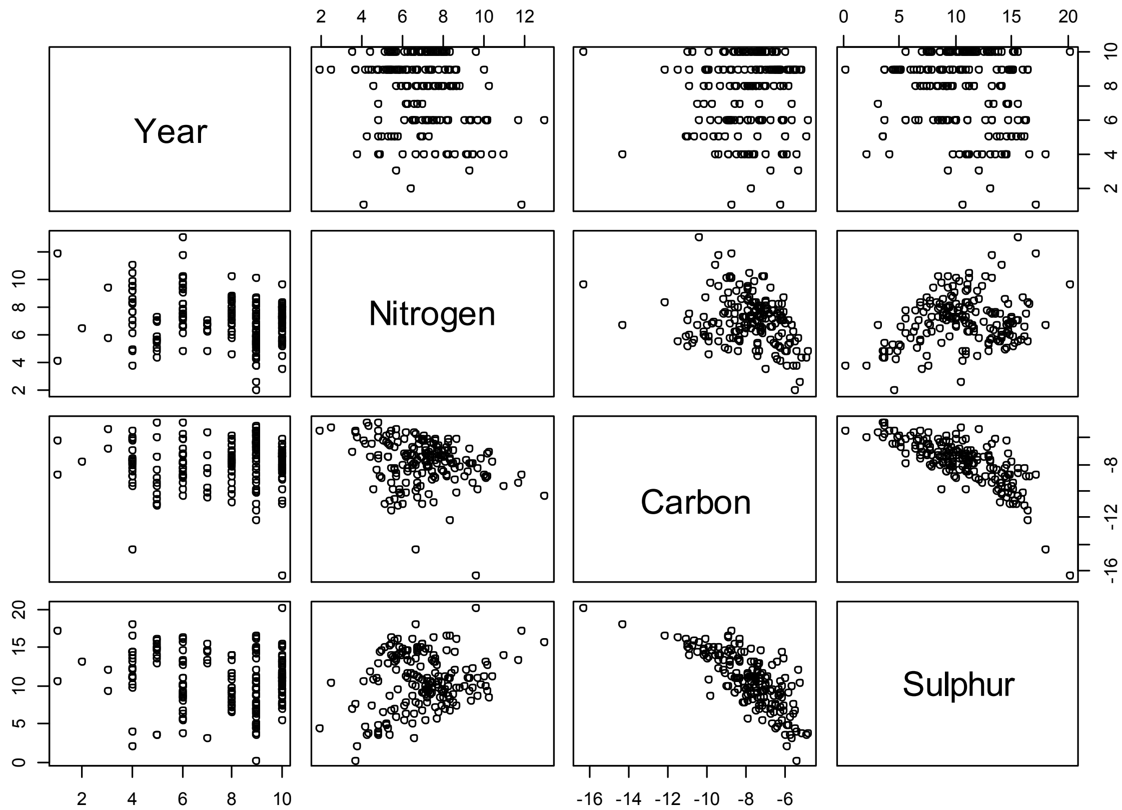

Fig. S4. Pairwise collinearity plot for the year the sample was collected and the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values of the turtle epidermis for individuals included within the second discriminant analysis conducted after the 2015 satellite tracking (n = 188). All pairwise comparisons for isotopic values were found to be significantly correlated (Pearson's product moment correlation coefficient, $p < 0.001$ in all cases $\delta^{13}C$ & $\delta^{15}N$, $r = -0.26$; $\delta^{15}N$ & $\delta^{34}S$, $r = 0.23$; $\delta^{13}C$ & $\delta^{34}S$, $r = -0.75$).

Supplement 7. Preliminary discriminant analysis to identify the origin of turtles from the foraging area not characterised through previous satellite tracking (1998 – 2011)

Stable isotope analyses conducted prior to satellite tracking can identify isotopic clusters to target foraging areas with specific isotopic profiles. When clusters are not evident, then groups of isotopic signatures can be selected to characterise the isotopic composition of the population. These groups can be used as pseudo-satellite tracked animals to calibrate a discriminant function analysis and obtain prior prediction probabilities for animals foraging in an area characterised by specific isotopic values.

For this study, we identified an area of isospace encompassing a large proportion of isotopic signatures which were not characterised by the pre-defined and calibrated foraging areas. To identify the origin of these isotopic values we selected 3 turtles from the 184 turtles of unknown origin that had temporal consistency in isotopic values over 2 breeding seasons and isotopically defined this region as the 'unidentified' foraging area. These turtles were used in addition to the 19 satellite tracked turtles to calibrate a discriminant analysis using $\delta^{15}N$ or $\delta^{13}C$ values. We predicted the putative foraging area for the remaining 181 turtles and produced a list of 48 turtles that were likely to forage at a greater than 80% probability in the 'unidentified' foraging area.

Supplement 8. Plots for final predictions of where turtles forage

The most discriminating isotopic criterion for turtles among foraging areas was visualised in a bivariate plot incorporating $\delta^{34}S$ and $\delta^{15}N$ (see main text Fig. 4). Here we present alternative plots incorporating the isotopic combination of $\delta^{13}C$ and $\delta^{15}N$ (S7a), $\delta^{13}C$ and $\delta^{34}S$ (S7b) and the full isotopic composition of the turtles ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) predicted to forage at each site (S7c).

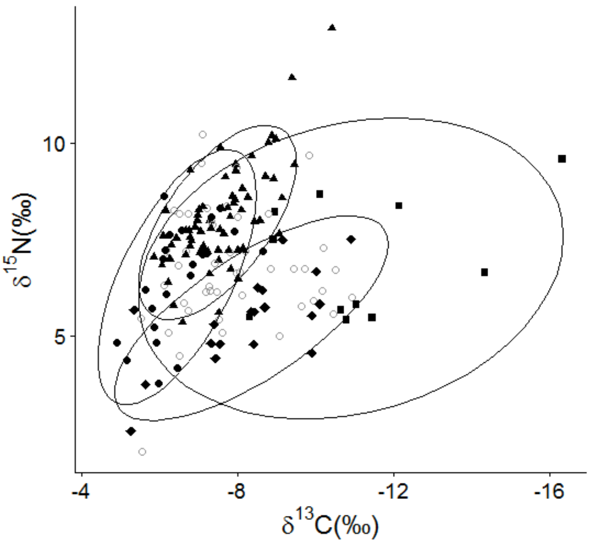

Fig. S5. $\delta^{13}C$ and $\delta^{15}N$ values for green turtles predicted to forage in: closed circles = Bomba (n = 22), triangles = Egypt (n = 65), squares = Turkey-Cyprus (n = 11), diamonds = West Libya (n = 19), open circles = unassigned (n = 48). Ellipses set at 95% CI, (total n = 165).

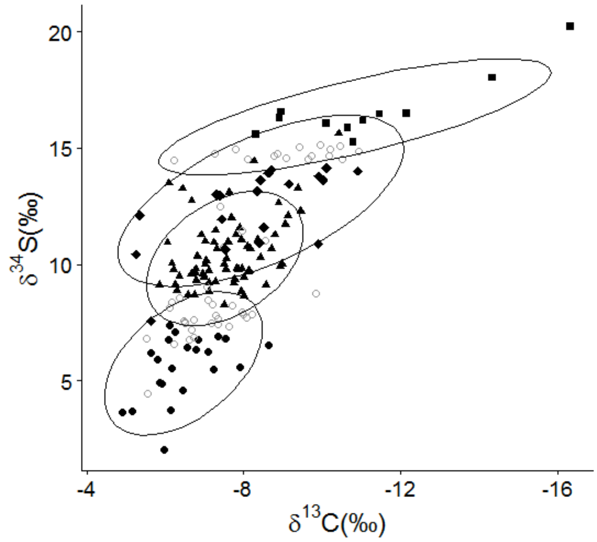

Fig. S6 $\delta^{13}C$ and $\delta^{34}S$ for green turtles predicted to foraging in: closed circles = Bomba (n = 22), triangles = Egypt (n = 65), squares = Turkey-Cyprus (n = 11), diamonds = West Libya (n = 19), open circles = unassigned (n = 48). Ellipses set at 95% CI, (total n = 165).

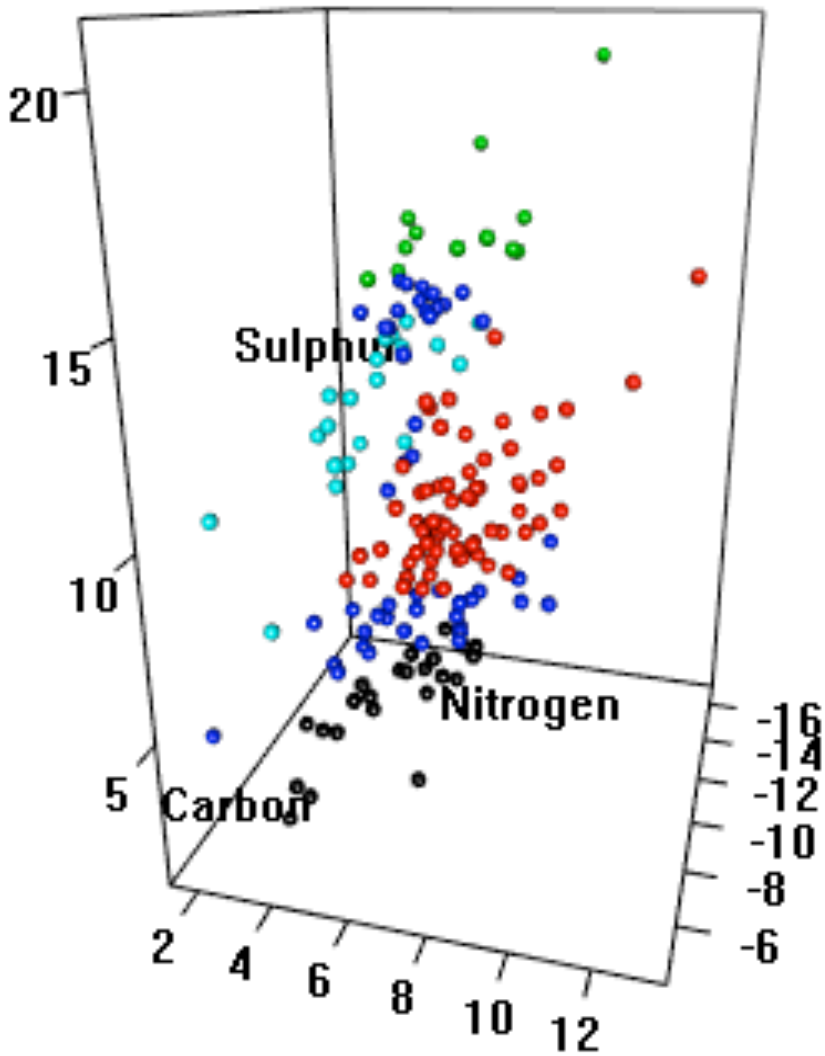

Fig. S7. $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ for green turtles predicted to forage in: black circles = Bomba (n = 22), red circles = Egypt (n = 65), green circles = Turkey-Cyprus (n = 11), cyan circles = West Libya (n = 19) and blue circles = unassigned (n = 48), (total n = 165).

Supplement 9. Evaluating foraging area specific contributions to the breeding cohort

We employed linear and non-linear mixed effects modelling to evaluate foraging area specific contributions to the breeding cohort. We evaluated autocorrelation through generalised least squares estimation models (GLS) within the R statistical package nlme (Pinheiro et al. 2016) as a general bi-annual pattern in foraging area contributions was observed. However, only the GLS model for turtles foraging in Egypt was significantly more accurate when accounting for autocorrelation based on AICc model selection (R statistical package MuMin for multi-model selection based on information criteria). Therefore, we did not account for autocorrelation within the full model incorporating all foraging areas. We employed a generalised linear model with a quasibinomial error structure to determine if the proportion of nesters from each foraging area to the breeding cohort significantly differed among years. The model was fitted with a proportional dependent variable based on the number of nesters from each site (number of nesters from x / total number of nesters – number of nesters from x) with year (also fitted as a quadratic variable) and foraging area as interacting fixed effects. A Tukey test of Honest Significant Differences (HSD) revealed that 3 out of 6 pairwise comparisons were significantly different (Fig. S8) with Egypt exhibiting a strong positive trend in the proportion of nesters contributed to the rookery whereas the other 3 sites showed a negative trend.

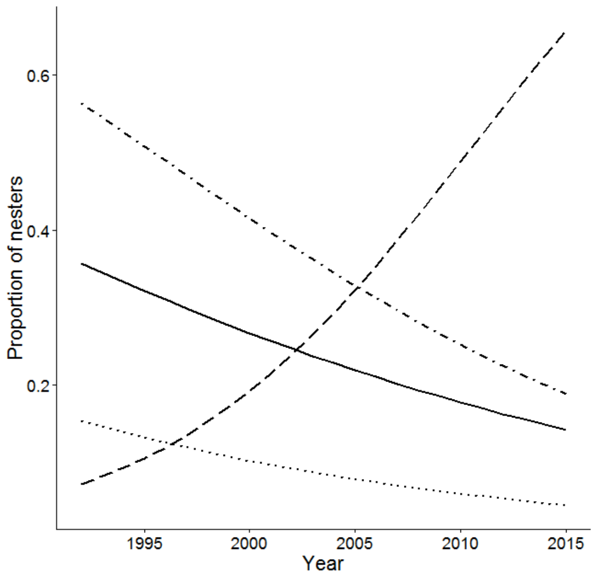

Fig. S8. Prediction from the GLM for the proportions of the nesting cohort contributed from each foraging area from 1992 – 2015. Dot-dash line = Bomba, dashed line = Egypt, dotted line = Turkey-Cyprus and solid line = West Libya.

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