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Mother- Hatchling Isotopic Relationship in Green Turtle: Isotopic Niche-based Modelling

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The isotopic discrimination between the mother and the hatchlings has been modelled in various vertebrates, including sea turtles. In addition to the linear relation between $\delta^{13}C$ and $\delta^{15}N$ isotope values of the mother – hatchling couple, there is missing data on the combined effects of both isotopes, which represent ecological niches of a species. The stable $\delta^{13}C$ and $\delta^{15}N$ isotope signatures of live hatchlings and their mother's epidermis tissues were used in green turtles. The samples were taken from three main breeding beaches, Akyatan, Sugözü and Samandağ in Türkiye during the 2020 nesting season. It was found that both $\delta^{15}N$ and $\delta^{13}C$ values of hatchlings were not significantly different from those of mothers. Significant relationships were found between hatchlings and their mothers in terms of $\delta^{13}C$ value. Furthermore, when the hatchling isotopic niche size is known, the condition probability of estimating the mother isotopic niche size were 85.16% and 92.88% with the 0.95 and 0.99 alpha levels. In addition to showing a linear relationship between hatchlings and their mother's single isotopic composition, the current study offers a novel insight that proposes a niche overlap concept using two isotopes to comprehend the mother-hatchling relationship of green turtles living in the eastern Mediterranean.

Key words: *Chelonia mydas*, Stable isotopes, Hatchling isotope, Feeding ecology, Mediterranean

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BACKGROUND

Sea turtles are lecithotrophic reptiles, meaning that they get their nutrients from egg yolk. The yolk formation process is known as Vitellogenesis, and during this process the yolk precursor proteins (vitellogenin; VTG and very low-density lipoprotein; VLDL) are produced in the maternal liver and the yolk proteins represent the proteins found in female blood (Van Dyke and Griffith 2018). Mothers transport yolk precursors to the ovarian follicles, and after fertilization and ovulation, the developing embryo consumes yolk. Conversion of the yolk into follicles begins storing 7-8 months before the breeding migration in sea turtles (Wibbels et al. 1990), and the nutrients in the egg are derived from the food consumed by the female and stored at that time (Kwan 1994; Hamann et al. 2003). The yolk is expected to have particular $\delta^{13}C$ and $\delta^{15}N$ values, reflecting the diet of the female before migrating to the breeding beach. It is expected that the isotopic values of the mother and the hatchling will have a high correlation, due to the hatchling tissue ingredients being derived directly from the food sources consumed by the mother (Pilgrim 2007).

The isotopic discrimination factor between the mother and the hatchlings ($\Delta = \delta$mother – δhatcling) has been demonstrated previously in various vertebrates such as snakes (Pilgrim 2007), sharks (Vaudo et al. 2010), birds (Hahn et al. 2012). As well, there are a few articles on sea turtles in this issue in *Caretta caretta* (Zbinden et al. 2011; Frankel et al. 2012; Carpentier et al. 2015), *Dermochelys coriacea* (Caut et al. 2008) and *Chelonia mydas* (Chabot et al. 2019). Previous research used epidermis, keratin, or blood samples from mothers, together with egg yolk, dead or newly dead hatchlings from nests (Caut et al. 2008; Zbinden et al. 2011; Frankel et al. 2012; Carpentier et al. 2015; Chabot et al. 2019). Significant relationship between *C. mydas* maternal epidermis and unhatched eggs were recorded (Chabot et al. 2019). The association between the isotopic ratios of hatchling (yolk, unhatched, freshly dead, and dead) and mothers' tissue is described by allocating just a single isotopic ratio, most often $\delta^{13}C$ and $\delta^{15}N$ (Chabot et al. 2019). However, based on Hutchison's n-dimensional niche concept, the chemical structure of an animal is directly impacted by both bionomic and scenopoetic ($\delta^{15}N$ and $\delta^{13}C$) ecological information (Newsome et al 2007). In addition to a linear relationship between single isotope values of the mother and the hatchling, the combined effects of both isotopes ($\delta^{13}C$ and $\delta^{15}N$), which represent ecological niches of a species, may reflect the compatibility of niche spaces of the mother and hatchling. The niche region and niche overlap metric are used to find niche overlaps among ontogenetic or spatial subpopulations in the multidimensional ecological niches in various species (Swanson et al. 2015; Shipley et al. 2019; Gauffier et al. 2020). On the other hand, the formulation of niche overlaps between the mothers and their hatchlings may be another tool for understanding the isotopic discrimination between mothers and their offspring. It is expected that nesting females' isotopic niches may overlap the niches of hatchlings. This finding will be valuable in the fieldwork arrangement because, in contrast to the sampling of females, the sampling of hatchlings offers the potential for a larger sample size and a more straightforward fieldwork design. Thus, hatchlings may help better understand the feeding ecology of green turtles.

In order to study the feeding ecology of sea turtles, individuals must be sampled either in their feeding areas or on the nesting beach. The process of sampling of turtles can be both difficult and expensive, especially in feeding areas. In addition, on nesting beaches, it can sometimes be difficult to sample sufficiently without disturbing the individual in a short time. In this case, the isotope values of the hatchling can be used, assuming that they represent the mother. However, the isotopic composition relationship between the hatchling and the mother needs to be demonstrated with high-confidence tests. This has been demonstrated in *C. carreta* and *D. coriacea* species, but this is a lacking for the Mediterranean population of green sea turtles. The estimation of stable isotope conversion formulae between mothers and their hatchlings is a crucial concern in the field of conservation research. This is particularly relevant for understanding the trophic ecology of sea turtles using the analysis of their hatchlings (Kaufman et al. 2014; Carpentier et al. 2015).

In order to understand the feeding ecology of the Mediterranean population of green sea turtles, it is important to use particular techniques that are easy to sample, practical, and non-harmful. This study addressed this important issue and set two main objectives. One of the aims is to test the hypothesis that the isotope compositions of the mother and the hatchling will be similar in green turtles. Therefore, it will be possible to obtain green turtle specific conversion equations that can predict the isotopic value of the mother based on the regression between hatchling and mother tissue. The second aim of the study is to test the degree of niche overlap between the mother and the hatchling, assuming that the isotopic niche of hatchlings represents that of the mother's. If the dual-isotope values of the hatchling represent their mother's isotopic values, they represent the mother's isotopic niche.

MATERIALS AND METHODS

Study area and sample collection

This study was conducted at the three nesting beaches (Samandağ, Akyatan, Sugözü) located on the Eastern Mediterranean coast of Turkey during the 2020 nesting season (Fig. 1). Samandağ, Akyatan and Sugözü nesting beaches are the most prominent breeding areas of the entire Mediterranean (Casale et al. 2018). Therefore, a total of 19 nesting females from three beaches were used, assuming that they represented the Mediterranean (*n* = 8 for Samandağ, *n* = 8 for Akyatan, and *n* = 3 for Sugözü). Hatchling samples were gathered by randomly choosing 5 samples from each nest constructed by the mother turtles from whom the isotope sample was obtained.

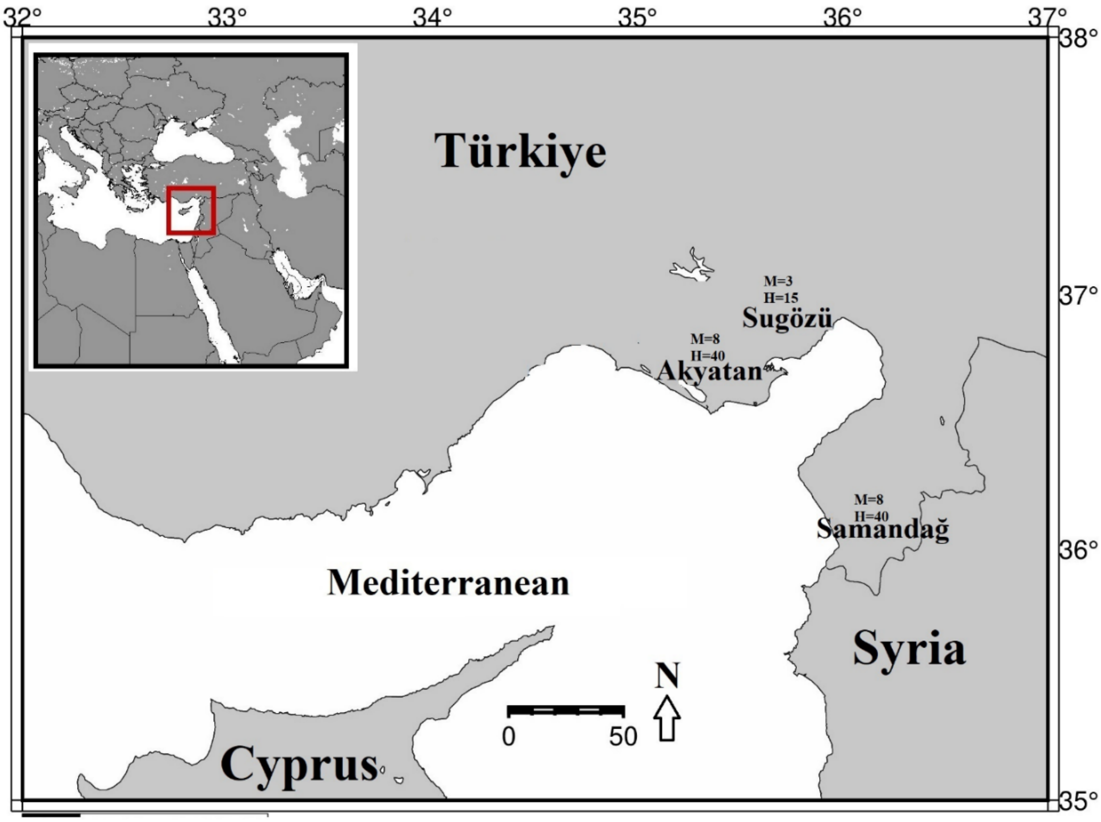

Fig. 1. The sampling beaches in the Eastern Mediterranean (M and H on the beaches where samples were taken indicate the number of mothers and hatchlings sampled respectively).

Before starting the sample collection, ethical approvals were obtained from the local ethical board for animal experiments (28/02/2018-1800028086). The green turtle sample collection on nesting beach was carried out during the night patrol in the 2020 nesting season. Five people patrolled the beach at night to observe female nesting turtles. The turtles were tagged and measured after they laid their eggs. The metal tags were used and placed on the trailing edge of the left forelimb flipper, as recommended by Balazs (1999). After nesting of the females, epidermal tissue samples were extracted by taking a piece of 4 - 6 mm wide and 3 mm deep from the trailing edge of a fore flipper using a 5 mm disposable biopsy punch (Vanderklift et al. 2020).

The nests of each female were marked with sticks and protected with a cage against the risk of predation. Following the hatching, using a 2 mm biopsy punch, tissues were sampled from 5 live hatchlings from the outermost projection of the marginal scute in each nest (Bjorndal et al. 2010; Türkozan et al. 2019). Hatchlings were examined in order to eliminate the decomposition effect of dead hatchlings, which was likely to significantly affect stable isotope values and lead to larger confidence intervals in discrimination factors (Frankel et al. 2012). Both adult females and hatchlings were released on the beach after the procedure was completed. All the tissues obtained from both adults and hatchlings were dried in an air circulating oven at 60°C for 24 hours, and these tissues were stored in vial tubes at -20°C in a deep freezer.

Stable isotope analysis

The tissue samples taken out of the deep freezer were kept in an oven (60°C) for one day, and then ground with a micro-dismemberator (1800 rpm/2 minutes) and turned into powder. The samples in powder form were weighed with 1 ± 0.005 mg microbalance (Sartorius) and packed in 9 x 9 mm tin cups. Later, they were analysed by a continuous-flow isotope-ratio mass spectrometer (Thermo-Electron Flash EA 2000 elemental analyser (EA) coupled via a Thermo Finnigan ConFlo IV Interface to a Delta V Thermo Finnigan Isotope Ratio Mass Spectrometer). In the mass spectrophotometer, CH-6 Sucrose (Vienna Pee Dee Belemnite sample for $^{13}C/^{12}C$ ($\delta 13C_{VPDB} - 10.45‰ \pm 0.04‰$) and USGS-34 Potassium Nitrate ($\delta 15N_{air} - 1.8‰ \pm 0.2‰$) standards were used for the carbon and nitrogen isotope ratios, respectively. The isotope ratios were calculated using the ratios in the tissue samples and the standard measured in the mass spectrophotometer as follows (Peterson and Fry 1987);

$$X = [(R_{Sample}/ R_{Standard})-1] \times 1000$$

where R_{Sample}: $^{15}N/^{14}N$ or $^{13}C/^{12}C$ ratio corresponding to the sample, $R_{Standard}$: atmospheric nitrogen for $^{15}N/^{14}N$ or Vienna Pee Dee Belemnite sample for $^{13}C/^{12}C$.

Data Analysis

In order to estimate the relationship between the isotopic composition of the mother (a dependent variable) and their hatchlings (an independent variable), linear regression was performed. In order to estimate the unknown parameters, a linear regression model was used. This method minimizes the sum of the squares in the difference between the observed and predicted values of the dependent variable configured as a straight line.

$$Y = \beta o + \beta X + \epsilon$$

In this formula, Y represents the mother's $\delta^{13}C$ and $\delta^{15}N$ values and X represents $\delta^{13}C$ and $\delta^{15}N$ values of the hatchlings. The average values of $\delta^{13}C$ and $\delta^{15}N$ for five hatchlings from each clutch, which are representative of the mothers, were considered. This model was used for all beaches combined.

We calculated the isotopic niche area and niche overlap metrics between mother and hatchlings in the R package *nicheROVER* v1.0 (Swanson et al. 2015). The *overlap ()* function with the use of $\delta^{13}C$ and $\delta^{15}N$ values. Ellipses were fitted to both the mother and the hatchlings, and the mean overlap was calculated using Bayesian 95 % credible intervals based on 10000 simulations. Because the overlap metric is directional, the mean overlap represents the probability that an individual from a hatchling will be found in the niche of its mother. This method does not assume a homogeneous distribution of individuals inside the niche region and is applicable to small and uneven sample sizes (Swanson et al. 2015).

RESULTS

Descriptive statistics of isotope values of epidermis tissue in nesting beaches according to hatchlings and their mothers are shown in Table 1. Although the hatchlings have a lower $\delta^{13}C$ value compared to their respective mothers, this difference is not statistically significant (Paired *t*-test = 0.30, *d.f.* = 18, $p > 0.05$). Also, the $\delta^{15}N$ value of the hatchlings was found to be not significantly higher than the $\delta^{15}N$ values of their mothers (Paired *t*-test = 2.09, *d.f.* = 18, $p = 0.05$).

Table 1. The descriptive statistics of isotope values of epidermis tissue in eastern Mediterranean green turtles nesting beaches according to hatchlings and nesting females and the conversion model equations between them (the isotope values of the hatchlings in each clutch were calculated based on the intra-clutch mean of the hatchling isotope values)

		n	Mean ± Sd (‰)	Min-max (‰)	Regression equation and "*p*" values
Hatchling	$\delta^{13}C$	95	-9.24 ± 1.93	-12.12 – -7.17	$\delta^{13}C_{mother} = -5.48 + 0.395*\delta^{13}C_{hatchling}$
Mother		19	-9.12 ± 1.42	-11.82 – -7.36	$R^2 = 0.29$ $p < 0.001$
Hatchling	$\delta^{15}N$	95	6.41 ± 0.69	5.03 – 7.59	$\delta^{15}N_{mother} = 3.62 + 0.342*\delta^{15}N_{hatchling}$
Mother		19	5.82 ± 1.17	4.34 – 8.12	$R^2 = 0.04$ $p > 0.05$

The models for δ^{13}C isotope value between hatchling and mother epidermis on overall showed significant relationships between them (Fig. 2). However, there is no statistically significant relationships between the δ^{15}N isotope value of hatchlings and that of the mother epidermis. The conversion model equation, which indicate a significant relationship between hatchling and female epidermis, are presented in table 1.

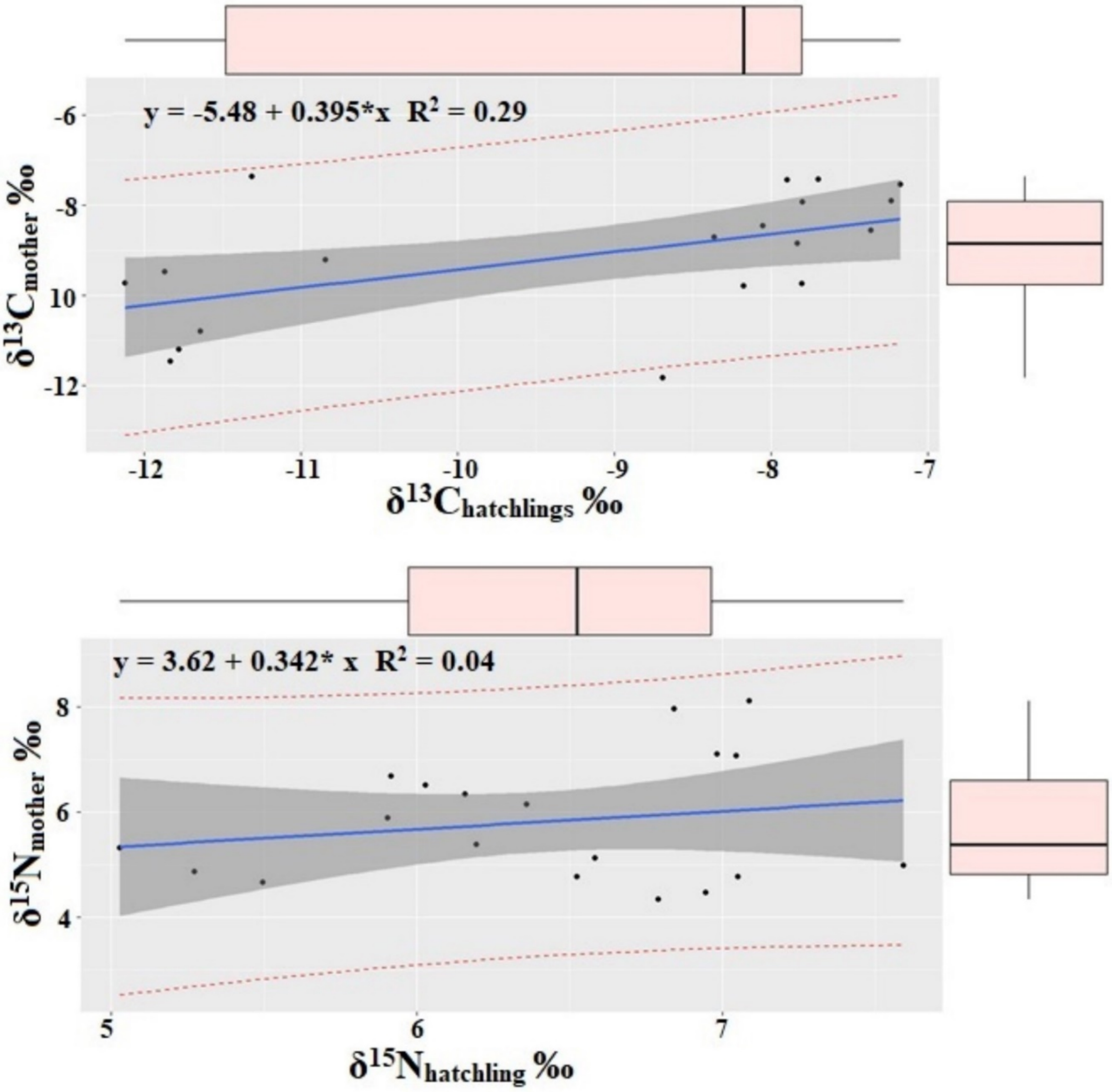

Fig. 2. The relationships of the mother and hatchling δ^{13}C and δ^{15}N epidermis values for the eastern Mediterranean with the 95% confidence (grey) and predicted interval (red), and conversion model equations (The red boxes indicate the mother and hatchling box plots. In the box plots, the medians of the mother and hatchling δ^{13}C values are -8.85 ‰ and -8.177 ‰, respectively, and the medians of the mother and hatchling δ^{15}N values are 5.38 ‰ and 6.52 ‰, respectively. The 25%-75% interquartile range of the distribution is -9.76 ‰ and -7.92 ‰ for the δ^{13}C values of the mother, and -11.48 ‰ and -7.8 ‰ for the hatchling. The 25%-75% interquartile range of the distribution for δ^{15}N values is 4.82 ‰ and 6.60 ‰ for the mother and 5.97 ‰ and 6.96 ‰ for the hatchling. The minimum and maximum values are given in Table 1).

The niche plots, density distributions, and raw data for each pairwise combination of isotope data for both maternal and hatchling (i.e., bivariate projections of 3-dimensional isotope data) were shown in figure 3. The niche sizes of mothers and hatchlings were estimated as 26.5‰ ± 5.96‰ and 20.3‰ ± 5.16‰, respectively. The hatchlings were found in the niche of mothers in the 85.2% and 92.8% in α = 95 and α = 99 levels, respectively.

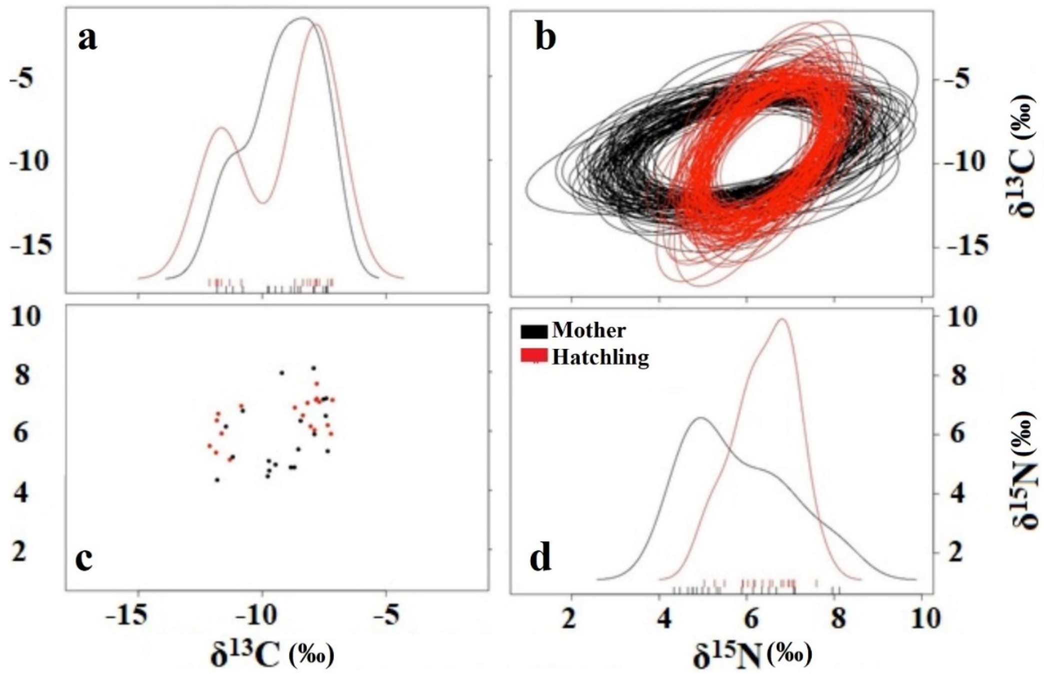

Fig. 3. Each pairwise combinations of isotope data for both maternal and hatchling on green turtle nesting beaches, a), Carbon density distributions of mother and hatchling, b) Niche plot graph of mother and hatchling, c) Dot plot of carbon and nitrogen values of mother and hatchling (the isotope values of the hatchlings in each clutch were calculated based on the intra-clutch mean of the hatchling isotope values), d) Nitrogen density distributions of mother and hatchling.

DISCUSSION

It was reported first time with this study that isotopic relationship between hatchling and mother, and first conversion equations in green turtles at eastern Mediterranean. Concerning the first objective of the present study, we determined that the $\delta^{15}N$ and $\delta^{13}C$ values of hatchlings did not exhibit any significant differences compared to those of mothers. Literature review shows that the $\delta^{13}C$ value of the unhatched egg was found to be depleted and the $\delta^{15}N$ value to be higher in green turtles (Chabot et al. 2019). Similarly, freshly dead hatchlings in loggerhead turtles have been reported to have higher $\delta^{15}N$ and depleted $\delta^{13}C$ values compared to their respective mothers (Frankel et al. 2012). Our results showed that $\delta^{15}N$ values were slightly enriched compared to their mothers, similar to previous studies. The higher values of $\delta^{15}N$ in hatchling is consistent with the idea that the hatchlings' trophic level is higher than their mothers'. Since hatchlings consume egg yolk produced by their mother, they are expected to have a higher trophic level (Partridge et al. 2001; Carpentier et al. 2015).

A positive relationship was found between the isotope values of mothers and their hatchlings. This suggests that stable isotope analysis of hatchlings is a viable method to assess foraging ecological questions in sea turtles. Therefore, the sampling from hatchlings can be used as a less invasive procedure to understand the diet of adult sea turtles instead of techniques such as gastric lavage or blood sampling during the nesting. Similar relationships have been found and proposed between various tissues of sea turtles and their hatchlings and/or eggs. However, model fit R^2 values in the present study are lower than in other similar studies between the mother and the hatchling in regression conversion equations. For instance, R^2 values in green turtles from the epidermis to unhatched egg conversion equations in the $\delta^{13}C$ and $\delta^{15}N$ models are $R^2 = 0.70$ and $R^2 = 0.64$ respectively (Chabot et al. 2019). Carpentier et al. (2015) reported $R^2 = 0.83$ and $R^2 = 0.86$ for $\delta^{13}C$ and $\delta^{15}N$ models, respectively, in loggerhead turtles. Similarly, Caut et al. (2008) obtained conversion equations between egg-yolk and plasma and red blood cells in leatherback sea turtles. They reported that R^2 values for $\delta^{13}C$ and $\delta^{15}N$ models were 0.84 and 0.89 respectively, between egg yolk and plasma, and 0.86 and 0.84 between egg yolk and red blood cell.

The lower explanatory power (R^2) of our study for the $\delta^{13}C$ and $\delta^{15}N$ models may be due to the possibility that the relationship between mother and their hatchling epidermis for isotope discrimination factors is weaker in green turtles than in other sea turtle species. A similar situation was also reported by Chabot et al. (2019) for lower explanatory power between unhatched egg contents and female epidermis in green turtles. Frankel et al. (2012) found that the r value for $\delta^{13}C$ ($r = 0.24$) lower than our study between fresh-dead hatchlings and their mothers in loggerhead turtles. Moreover, they noted that fresh-dead hatchlings showed less variation than the dead in nest, and fresh-dead hatchlings would provide a more acceptable estimation. Hatchling tissue collection in this study involved easy sampling for isotope analysis on nesting beaches. Sufficient samples can easily be obtained from a 2-mm biopsy punch, which does not require killing the hatchlings. Similarly, Frankel et al (2012) noted that hatchlings could be sampled using a 2 mm biopsy punch. This will not affect the growth rate and health status of hatchlings (Bjorndal et al. 2010). Based on the results of the study, hatchling tissues can indicate their mother's foraging ecology. However, since we investigated a single maternal tissue (epidermis), this may be limited to reflecting the mother, considering that the tissues had different incorporation rates and turnover rates. Therefore, it is important to reveal the relationships and differences between other tissues such as blood (plasma and red blood cell) of the mother and the tissue of the hatchling in future studies green sea turtles.

A positive relationship was found between the $\delta^{13}C$ isotope values of mothers and their hatchlings, but not between the $\delta^{15}N$. The limited correlation between the isotopic nitrogen content of hatchlings and their mothers may be due to a lack of differentiation between hatchlings and mothers, rather than the small sample size. The observed difference in the discriminant values of nitrogen and carbon between hatchlings and their mothers, as well as the findings published in the literature (Carpentier et al. 2015; Frankel et al. 2012), suggest that there may be variability in the transfer of carbon and nitrogen sources from the mother to hatchlings. Therefore, the use of dual isotopes presents a more viable approach for comprehending the ecological functions of sea turtles.

Isotopic niche analysis is a valuable tool for studying ecological niches due to the stable isotopes of sea turtles, which reflect both their prey and their environment. The generalist vs. specialist feeding strategy is particularly attributed to the wideness of isotopic niche space and the individual variation in resource use, which are taken into consideration to assess the feeding strategy of loggerhead turtles using stable isotopes (Vander Zanden et al. 2010). The large niche area of mothers is explained by the more generalist feeding strategy of green turtle populations. Even though the predictability of the mother's isotopic ratio from the hatchlings was remarkably high, the variation in the mother's epidermal samples still has to be taken into account. This indicates a possibility of the realization of food sources transferred by mothers to their hatchlings in the last few months with various foraging strategies. This finding is consistent with the polymorphic foraging strategy of each individual throughout their life span (Seminoff et al. 2007; Ferreira et al. 2021).

CONCLUSIONS

Stable isotope analysis provides valuable information about resource use in the trophic ecology of green turtles. The hatchlings reflect the isotopic composition of their mothers since they use the nutrients stored in the egg yolk during vitellogenesis. There was a significant relationship with a linear regression model between the mother and the hatchlings. In addition, there is a high probability that an individual hatchling was found in the niche area of respected mothers. However, the temporal and spatial intrapopulation variation in foraging strategies suggested that while assessing mother-hatchling isotopic compatibility, intra-population differences should also be considered.

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Authors' contributions: Şükran Yalçın Özdilek (SYO) conceived the ideas and designed methodology; Bektaş Sönmez (BS) and SYO collected the data; Burcu Mestav (BM) and SYO analysed the data; All authors writing of the manuscript and contributed critically to the drafts and gave final approval for publication.

Competing Interest: The authors have no relevant conflicts of interest to declare.

Availability of data and materials: The all data and analysis are transformed to R document and data that support the findings of this study are available from the authors, upon reasonable request.

Consent for publication: Not applicable

Ethics approval consent to participate: All applicable international guidelines for sampling, care, and experimental use of organisms for the study were followed and all necessary approvals were obtained.

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