



Influence of habitat utilization strategies on trace element signatures in egg contents of green turtles nesting on Xisha Islands, South China Sea

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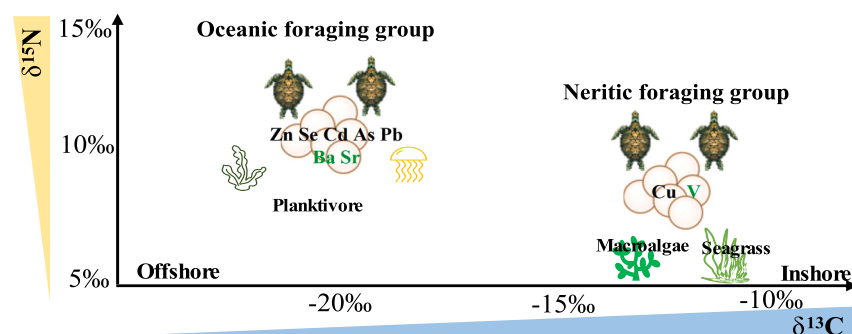
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HIGHLIGHTS

- 16 TEs and stable isotopes (C and N) values in *C. mydas* egg contents were reported.
- Nesting *C. mydas* were assumed to be divided into oceanic and neritic foraging groups.
- Eggs of *C. mydas* in two foraging groups accumulated different levels of TEs.
- Correlations between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and TEs provided new insights regarding the origin of TEs.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Julian Blasco

Keywords:

Green turtle
Trace elements
Characteristic elements
Stable isotopes
Foraging habitat

ABSTRACT

Habitat utilization significantly influences the accumulation of chemical pollutants, including trace elements (TEs), in the tissues of large marine organisms. Previous research has demonstrated that sea turtles nesting in the same location may employ distinct foraging strategies. This study investigated the influence of habitat use strategies on the concentrations of 16 TEs in the eggs of green turtles (*Chelonia mydas*) nesting on the Xisha Islands. The analysis incorporated stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes, as well as characteristic elements. Additionally, inter-relationships between TEs were examined. The nesting female green turtles were categorized into two foraging groups based on isotopic signatures, namely oceanic ($\delta^{13}\text{C}$ values: -21.5 to -17.0 ‰; $\delta^{15}\text{N}$ values: 7.10 to 12.5 ‰) and neritic ($\delta^{13}\text{C}$ values: -14.4 to -9.95 ‰ and $\delta^{15}\text{N}$ values: 5.10 to 10.0 ‰). Different TE patterns were observed in the egg contents of these two groups. The neritic group exhibited elevated levels of V and Cu, which positively correlated with $\delta^{13}\text{C}$ values. Conversely, the oceanic group displayed higher levels of Zn, Cd, Se, Sn, As and Hg, which positively associated with $\delta^{15}\text{N}$ values. This distribution pattern is attributed to variations in background TE concentrations in the respective foraging habitats. Additionally, prey items and trophic levels of green turtles may contribute to the observed inter-group differences in TE concentrations (e.g. Zn, As, Se, Sn) found in their eggs, warranting further research. This study provides valuable information about habitat utilization patterns and TE distribution in green turtles nesting on the Xisha Islands. The

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<https://doi.org/10.1016/j.scitotenv.2024.177149>

Received 5 February 2024; Received in revised form 18 October 2024; Accepted 20 October 2024

Available online 21 October 2024

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findings enhance our understanding of TE accumulation mechanisms in turtle tissues and eggs, which is significant for the conservation of this endangered species, the green sea turtle.

1. Introduction

Trace elements (TEs), including metals and metalloids, are ubiquitous pollutants in the marine ecosystems, originating from both natural and anthropogenic sources. These elements are characterized by their non-degradability, extended half-lives, and potential toxicity. Certain TEs, notably mercury (Hg) and cadmium (Cd), have been demonstrated to exert deleterious effects on the health and reproductive capabilities of marine organisms, including sea turtles (Day et al., 2007; Perrault et al., 2013). Thus, revealing the mechanisms driving TE accumulation in sea turtle tissues is of paramount importance for the conservation efforts directed towards these endangered species.

Foraging habits, dietary preferences, and individual foraging strategies have been identified as key factors influencing pollutant accumulation patterns in marine organisms, including sea turtles and whales (O'Connell et al., 2010; Bezerra et al., 2015; Remili et al., 2021). While numerous studies have revealed interspecific variations in TE accumulation among sea turtle species, fewer investigations have focused on intraspecific variations in pollutant levels (Sakai et al., 2000; Filippou et al., 2021). However, mounting evidence suggests that variations in dietary sources are prevalent among sea turtle populations. Research has demonstrated that green turtles nesting on the same beaches may utilize distinct foraging habitats. For instance, green turtles nesting on the Ogasawara Islands (Japan) (Hatase et al., 2006), Galápagos Islands (Ecuador) (Seminoff et al., 2008), and beaches in the eastern Mediterranean (Özdilek et al., 2023) are known to utilize both neritic and oceanic habitats. Therefore, sea turtles nesting in the same location but foraging in distinct habitats are expected to accumulate different levels of pollutants in their tissues. In recent years, various ecological parameters (e.g., food sources, prey items, trophic level) have been effectively assessed using stable isotope signatures (e.g., $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), providing valuable information for identifying chemical pollutant bioaccumulation pathways in marine food webs (Bezerra et al., 2015; Le Croizier et al., 2022; Remili et al., 2021). Several studies have found that the concentrations of chemical pollutants (e.g., some phthalate esters [PAEs], polychlorinated biphenyl [PCB] 138, Hg) in sea turtle tissues are related to dietary sources and trophic ecology, as inferred through stable isotope analysis. These studies have also revealed intraspecific variations in pollutant levels within green turtle tissues (Bezerra et al., 2015; Filippou et al., 2021; Blasi et al., 2022). Furthermore, specific elements have been employed as environmental markers of sea turtle foraging resources (Talavera-Saenz et al., 2007; du Preez et al., 2018). du Preez et al. (2018) discovered that strontium (Sr), which is abundant in marine environments, drives the division of TEs in the eggshells of the oceanic foraging leatherback turtle (*Dermochelys coriacea*) and the neritic foraging loggerhead turtle (*Caretta caretta*).

Avian and reptilian eggs are useful bio-indicators of environmental contaminants due to their relative ease of sampling compared to oviparous animal tissues. Previous research has demonstrated a correlation between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in unhatched sea turtle eggs and hatchlings with those of their maternal tissues (Frankel et al., 2012; Chabot et al., 2019). Furthermore, studies have established that both essential elements (e.g., zinc [Zn], selenium [Se], and strontium [Sr]) and non-essential elements (e.g., arsenic [As], mercury [Hg], cadmium [Cd], and lead [Pb]) can be transferred from maternal sea turtles to their eggs (Guirlet et al., 2008; Páez-Osuna et al., 2010; Van Dyke et al., 2014; Barraza et al., 2023). It is noteworthy that vitellogenesis occurs 8–10 months prior to the female sea turtle's arrival at the nesting beach (Rostal et al., 1996). Therefore, the statistical analysis of stable isotopes ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and trace elements in sea turtle eggs is expected to not only identify dietary sources of nesting female turtles but also reflect

information regarding their exposure to TEs in their foraging habitats.

The South China Sea hosts China's largest sea turtle population, with green turtles comprising 90 % of this population (Wang, 1993). However, due to a drastic decline over the last century, green turtles have been designated as a national first-level protected species in China (Chan et al., 2007). Studies have discovered elevated levels of specific TEs, notably Pb and nickel (Ni), in both the marine environment of the South China Sea and the biological samples (tissues and eggs) obtained from green turtles nesting along the southern coast of China (Lam et al., 2006; Xu et al., 2016; Ng et al., 2018). The Qilianyu Cluster within the Xisha Islands hosts China's largest nesting population of green turtles (Jia et al., 2019). However, information regarding habitat use and baseline TE values for this population remains scarce. Assuming that $\delta^{13}\text{C}$ values are indicative of neritic or oceanic foraging habitats (Reich et al., 2007; Özdilek et al., 2023), and considering that neritic and oceanic habitats have distinct elemental characteristics, this study aims to address two primary research questions: (1) Do green turtles nesting on the Xisha Islands utilize a single foraging habitat? (2) Do different foraging populations exhibit varying TE profiles in their egg contents?

To investigate these questions, we conducted an analysis of stable carbon and nitrogen isotope ratios, along with 16 trace elements, including characteristic elements, in the egg contents of green turtles nesting on the Xisha Islands. This analysis was performed to differentiate between foraging groups within the nesting population. Furthermore, we explored the relationships among TE content, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, and characteristic element concentrations to enhance our understanding of how foraging habitat utilization influences the accumulation of TEs in green turtles.

2. Materials and methods

2.1. Sample collection

Field surveys were conducted on the beaches of the Qilianyu Cluster (9°35'–9°47' N, 114°14'–114°33' E) in the Xisha Islands, South China Sea. During the nesting seasons (June–September 2019–2023), nightly walking patrols were performed from 21:00 to 03:00 h, except during extreme weather conditions, to locate nesting green turtles. Individual female green turtles were identified by inserting an internal tag (ϕ 2.12 × 12 mm, EM4305, Juanhui intelligent technology Co., LTD, Guangzhou, China) into the left front flipper or by verifying existing tags. Each nest was subsequently numbered. Daytime patrols supplemented daily nest counts when nocturnal patrols were not feasible. Unhatched green turtle eggs were collected from 89 nests within 3–4 days of incubation, with three unhatched eggs sampled from each nest. Due to extreme weather conditions and limitations in walking patrol accuracy, only 36 nest samples from distinct females nesting on North Island were obtained, representing approximately one-third to one-half of the nests in the Qilianyu Cluster. Additionally, 10 non-peak breeding nest samples were collected from North Island (before June or after September). A further 43 nest samples were gathered from uninhabited islands (South Island, North Sand, Middle Sand, South Sand) of the Qilianyu Cluster, where nocturnal tagging of nesting females was not feasible. Although these samples were selected based on nesting dates and the observed 9–15 day interval between clutches of green turtles, the possibility of multiple samples originating from the same nesting individual cannot be entirely excluded.

After collection, the eggs were cleansed with ultrapure water to remove coral sand, and the egg contents were separated from the shells. The contents of three eggs from the same nest were combined to form a bulk sample representative of a single nesting female. Egg contents were

then frozen at -18 ± 2 °C until further analysis.

2.2. Chemical analysis

2.2.1. TE analysis

Egg content samples were subjected to freeze-drying for 96 h, subsequently ground into a homogeneous powder, and divided into two subsamples: one for TE analysis and the other for isotope analysis. The analytical procedure employed was comparable to that described by Lam et al. (2006). The resulting dry sample (0.25 ± 0.0005 g) was processed in an acid solution comprising 6 mL of 65 % HNO_3 and 2 mL of 30 % H_2O_2 . Initial sample digestion occurred at room temperature (25 °C) overnight, followed by further digestion using a microwave digestion instrument (MARS6, CEM Corp., USA). The digestion process followed a temperature gradient: 0–15 min to 120 °C, 15–30 min to 190 °C, and 30–60 min at 190 °C. The digestion solution was then evaporated at 95 °C to approximately 1 mL and subsequently diluted to 25 mL with ultrapure water for analysis.

TE concentrations of vanadium (V), Zn, iron (Fe), manganese (Mn), Se, chromium (Cr), cobalt (Co), copper (Cu), Pb, Ni, Cd, barium (Ba), tin (Sn), and Sr were determined using inductively coupled plasma–mass spectrometry (ICP-MS, X Series 2, Thermo Fisher Scientific, USA). Arsenic (As) and Hg contents were quantified using atomic fluorescence spectrometry (AFS-3000, Beijing Haiguang, China). The limits of detection for TEs in egg contents were as follows (in $\mu\text{g}\cdot\text{g}^{-1}$): V (0.003), Cr (0.005), Mn (0.005), Fe (0.05), Co (0.002), Ni (0.002), Cu (0.01), Zn (0.01), Se (0.01), Sr (0.05), Cd (0.001), Sn (0.005), Ba (0.003), Pb (0.002), As (0.02), and Hg (0.001). The precision and accuracy of the analytical method for TEs in egg contents were estimated using duplicate samples, with three replicates for every 12 samples, blank samples, and standard reference material (standard material for analysis of elements in yellow fish meal GBW10253). Relative recoveries ranged from 86.0 to 112 %, which were within 15 % of the certified values.

2.2.2. Analysis of stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$)

Lyophilized egg content subsamples (1.5 ± 0.05 mg) were weighed in tin capsules and analyzed at the Analytical & Testing Center of Hainan University to determine $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. The analysis was conducted using a CNS Element Analyzer (Vario Pyro Cube, Elementar, Germany) interfaced with a stable isotope ratio mass spectrometer (IRMS; isoprime precisION, Elementar, Germany). $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were expressed in parts per thousand (‰) relative to standard reference materials: Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}\text{C}$ and atmospheric N for $\delta^{15}\text{N}$. The following equation was used:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000 \text{ (in‰)} \quad (1)$$

where R is the ratio of heavy to light stable isotopes ($^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$) in the sample (R_{sample}) and the standard (R_{standard}). The precision and accuracy of the analytical method for stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were estimated using analytical blanks and urea analytical standards (B2174). Analytical control and reproducibility were assessed for every 12 samples using a certified isotopic reference material (EMA-P2: B2155; IRMS EMA P2: B2205). Triplicate analyses were performed for every 12 samples, yielding a precision of ± 0.1 ‰ for $\delta^{13}\text{C}$ and ± 0.15 ‰ for $\delta^{15}\text{N}$.

2.3. Analysis of potential diets of green sea turtles

A literature review was conducted using Web of Science search engines, covering publications from 2000 to 2024. According to the reported potential food sources and foraging habitats of green sea turtles (Bjorndal, 1997), the search keywords were set as “seagrass/ macroalgae/ plankton + stable isotope + South China Sea”. Full-text articles were examined, and those reporting $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values of potential diets were retained. From a total of 49 screened documents, 8 were

selected for further analysis. For each potential diet, the sampling sites and corresponding $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values were recorded. Detailed data and references are shown in Supplementary Table S1.

The trophic position (TP) of green turtles was calculated based on a single diet approach following Post (2002), using the equation:

$$\text{TP} = (\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{baseline}}) / \text{TDF}_{\text{consumer}} + \lambda \quad (2)$$

where $\delta^{15}\text{N}_{\text{consumer}}$ represents the $\delta^{15}\text{N}$ values of green turtles from two foraging groups, $\delta^{15}\text{N}_{\text{baseline}}$ represents the $\delta^{15}\text{N}$ values of reference organisms (seagrass, macroalgae, phytoplankton, zooplankton), $\text{TDF}_{\text{consumer}}$ (trophic discrimination factor) is set at 2.48 ± 0.35 ‰ for adult green turtles (Vander Zanden et al., 2012). λ denotes the trophic level of the reference organism, with primary producer assigned a value of 1 and zooplankton assigned a value of 2.

2.4. Statistical analysis

Normality assumptions were tested using Shapiro–Wilk tests. Density plots and histograms of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were generated for the total sample set using OriginPro 2022. The number of modes, which corresponds to distinct foraging habitats, was determined from the distribution density plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ using the “Piecewise Fit” function in OriginPro 2022. The density distributions of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were fitted using the “piecewise fit anonymous function” mode in the “Piecewise Fit” application. Spearman's correlation analysis was used to evaluate correlations among TEs in green turtle egg contents, as well as between TEs and stable isotope values. Analysis of similarities (ANOSIM) was utilized to compare TE concentrations in green turtle egg contents within and between foraging groups, implemented using the vegan package in R statistical language (version 4.1.0). The Kruskal–Wallis test was utilized to evaluate differences in TE concentrations and $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values in green turtles eggs between different foraging group sets, as the assumption of normality was not met ($p < 0.05$). Correlations between the egg content of TE \log_{10} -transformed concentrations, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were characterized using linear regression analysis. Principal component analysis (PCA) was employed to explore the relationships among TE concentrations, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in the egg contents of green turtles from different foraging habitats. The statistical significance of the correlations was evaluated using SPSS 25.0 and OriginPro 2022 statistical software for Windows.

3. Results

3.1. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in green turtle egg contents

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in green turtle egg contents ranged from -21.5 ‰ to -9.95 ‰ and 5.10 ‰ to 12.50 ‰, respectively (Fig. 1). The distribution of both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values demonstrated multimodality (Supplementary Fig. S1). For $\delta^{13}\text{C}$, two modes were identified -20.0 ‰ and -11.5 ‰, with estimated density values of 0.13 and 0.14, respectively. The goodness of fit was confirmed by an R^2 value of 0.49 and a residual sum of squares of 0.02. Similarly, $\delta^{15}\text{N}$ values exhibited two modes at 7.97 ‰ and 10.6 ‰, with estimated density values of 0.09 and 0.13, respectively. The fit quality was substantiated by an R^2 value of 0.72 and a residual sum of squares of 0.003. Based on these $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, two distinct groups of nesting green turtles were identified, with non-overlapping $\delta^{13}\text{C}$ values (Fig. 1). Group A ($n = 50$) exhibited relatively lower $\delta^{13}\text{C}$ values ranging from -21.5 to -17.0 ‰ (mean = -19.5 ‰ ± 1.44) and higher $\delta^{15}\text{N}$ values ranging from 7.10 to 12.5 ‰ (mean = 9.96 ‰ ± 1.61). In contrast, Group B ($n = 39$) presented relatively higher $\delta^{13}\text{C}$ values ranging from -14.4 to -9.95 ‰ (mean = -12.2 ‰ ± 1.33) and lower $\delta^{15}\text{N}$ values ranging from 5.10 to 10.0 ‰ (mean = 7.35 ‰ ± 1.27) (Fig. 1). Despite some overlap, the mean $\delta^{15}\text{N}$ values in Group A were significantly higher than those in Group B ($H = 38.5$, $p < 0.001$).

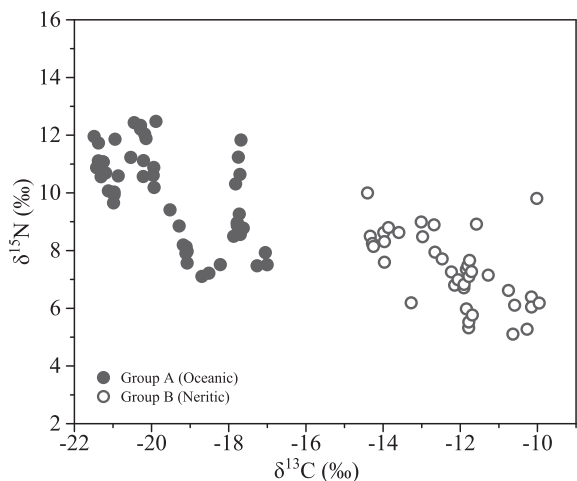


Fig. 1. Plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in egg contents of green turtles nesting in Xisha Islands, South China Sea.

The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values between green turtle egg contents and potential food items in the South China Sea were compared (Fig. 6A and Supplementary Table S1). Group A green turtles assumed $\delta^{13}\text{C}$ values similar to those found in plankton from coral reefs and bays in the South China Sea. In contrast, Group B green turtles exhibited $\delta^{13}\text{C}$ values consistent with those observed in seagrass and benthic macroalgae from the same region. The mean $\delta^{15}\text{N}$ values of seagrass and benthic macroalgae in neritic waters of Hainan Island, Xisha Island, and the northern Beibu Gulf ranged from 4.69 to 6.00 ‰. Additionally, the mean $\delta^{15}\text{N}$ values of plankton in coral reefs and bays in the South China Sea ranged from 6.38 to 8.97 ‰. Trophic position (TP) of the two green turtle groups were calculated based on baseline $\delta^{15}\text{N}$ values of single diets from potential foraging habitats (Fig. 6B and Supplementary Table S1). Results showed that neritic green turtles feeding on seagrass had a similar TP to oceanic green turtles consuming phytoplankton ($\text{TP}_{\text{mean}} \approx 2$). Furthermore, oceanic green turtles demonstrated a higher TP ($\text{TP}_{\text{mean}} = 3.3$) when consuming zooplankton in addition to phytoplankton.

3.2. TE concentrations in green turtle egg contents

The concentrations (median \pm MAD, $\mu\text{g}\cdot\text{g}^{-1}$, dry weight) of 16 TEs in the egg contents of green turtles nesting on Xisha Islands were quantified

and ranked in descending order: Sr (147 ± 36.3) > Fe (81.1 ± 13.9) > Zn (60.8 ± 11.1) > Ba (4.43 ± 1.27) > Sn (2.35 ± 1.26) > Cu (2.49 ± 0.527) > Se (1.75 ± 0.717) > Mn (1.13 ± 0.303) > Cr (0.553 ± 0.077) > Ni (0.190 ± 0.040) > V (0.103 ± 0.048) > As (0.071 ± 0.031) > Pb (0.035 ± 0.026) > Cd (0.030 ± 0.015) > Co (0.028 ± 0.012) > Hg (0.009 ± 0.004) (Fig. 2). Statistical analysis revealed a significant difference in the concentrations of the 16 TEs between the egg contents of the two green turtle groups (ANOSIM $r = 0.07$, $p = 0.007$). Group A exhibited lower concentrations of V ($H = 10.1$, $p < 0.01$) and Cu ($H = 6.53$, $p < 0.05$) compared to Group B. Conversely, Group A demonstrated higher concentrations of Zn ($H = 11.0$, $p < 0.01$), Cd ($H = 21.5$, $p < 0.001$), Sr ($H = 10.6$, $p < 0.01$), Mn ($H = 8.49$, $p < 0.05$), Se ($H = 25.7$, $p < 0.001$), Ba ($H = 19.8$, $p < 0.001$), Pb ($H = 27.0$, $p < 0.001$), and As ($H = 31.9$, $p < 0.001$) relative to Group B (Fig. 4). PCA was performed on 12 TEs (excluding Ni, Co, Pb, and Sn), resulting in two principal components that collectively explained 53.7% of the total variation (Fig. 3). The first principal component (PC1) accounted for 32.8% of the total variation and was characterized by high loadings (> 0.35) for Ba, Se, As,

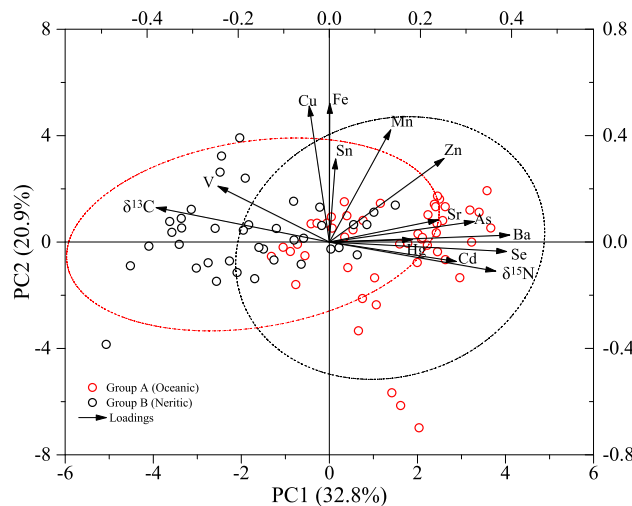


Fig. 3. The relative variation biplot of TEs in egg contents for oceanic and neritic foraging green turtles. Oceanic group = red circle; neritic group = black circle; PC1 explains most (32.8%) of the total variation; PC2 explain 20.9% of the total variation. Confidence Ellipse: red dashed line for Oceanic group, black dashed line for Neritic group.

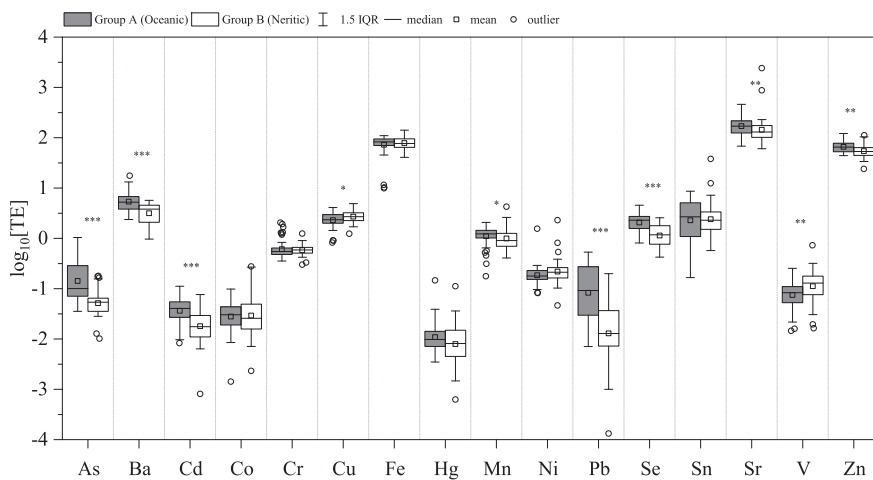


Fig. 2. TEs log 10-transformation concentrations in egg contents of green turtles from group A (gray box) and group B (white box) foraging habitats. Boxplots representing median (short line inside the box), mean (small square), 1.5 times interquartile range (box: 1st to 3rd quartile); Open circles are outliers. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Sr, Zn and $\delta^{15}\text{N}$, suggesting a strong influence of these elements on Group A. The second principal component (PC2) explained 20.9 % of the total variation and was associated with high loadings (> 0.35) for Fe, Cu, V and $\delta^{13}\text{C}$, indicating a significant influence of these elements on Group B.

3.3. Relationships between TE concentrations and stable isotope values

Analysis of all egg content samples revealed significant correlations between TE concentrations and stable isotope values. Positive correlations were observed between $\delta^{13}\text{C}$ values and \log_{10} -transformed concentrations of V ($r = 0.344$, $p < 0.01$), Ni ($r = 0.214$, $p < 0.05$), and Cu ($r = 0.293$, $p < 0.01$) (Fig. 4). Conversely, negative correlations were found between $\delta^{13}\text{C}$ values and \log_{10} -transformed concentrations of Zn ($r = -0.321$, $p < 0.01$), Se ($r = -0.684$, $p < 0.001$), Cd ($r = -0.504$, $p < 0.001$), Ba ($r = -0.545$, $p < 0.001$), As ($r = -0.549$, $p < 0.001$), and Hg ($r = -0.283$, $p < 0.01$). With respect to $\delta^{15}\text{N}$ values, positive correlations were observed with \log_{10} -transformed concentrations of Zn ($r = 0.382$, $p < 0.001$), Se ($r = 0.650$, $p < 0.001$), Cd ($r = 0.537$, $p < 0.001$), As ($r = 0.518$, $p < 0.001$), Sn ($r = 0.254$, $p < 0.05$), and Hg ($r = 0.265$, $p < 0.05$) across all green turtle egg content samples (Fig. 5). It is noteworthy that the concentrations of Cr, Co, Mn, and Fe in green turtle egg contents did not exhibit significant correlations with either $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values.

4. Discussion

4.1. Habitat utilization of green turtles nesting on Xisha Islands

Stable isotope analysis revealed two distinct modes in the distribution of $\delta^{13}\text{C}$ values and two clusters of $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ values, indicating that green turtles nesting on Xisha Islands employ two different foraging habitats. This polymorphic foraging pattern has been observed in green turtle populations across various regions, including the Pacific (Hatase et al., 2006), Indian Ocean (Richardson et al., 2013) and eastern Mediterranean (Özdilek et al., 2023). Stable carbon and nitrogen stable isotopes have been employed to distinguish between sea turtle groups foraging in oceanic and neritic habitats. Hatase et al. (2006) reported a wide range of $\delta^{13}\text{C}$ (-23.1 to -11.4 ‰) and $\delta^{15}\text{N}$ (6.6 to 14.2 ‰) values in egg yolk from green turtles collected from Ogasawara Islands in the Pacific. This variation was attributed to distinct habitat utilization: individuals with low $\delta^{13}\text{C}$ values in their egg yolk were associated with oceanic habitats and primarily consumed macroplankton, while those with higher $\delta^{13}\text{C}$ values were linked to neritic habitats and mainly foraged on macroalgae. Generally, benthic macrophytes, seagrass, and coastal phytoplankton exhibit higher $\delta^{13}\text{C}$ values than oceanic phytoplankton (Graham et al., 2010). For phytoplankton in coral reefs within the Pacific, mean $\delta^{13}\text{C}$ values ranged from -22 to -18 ‰ (Wyatt et al., 2013; Yñiguez et al., 2022; Yin et al., 2023). The elemental composition of sea turtle eggs can also be used to discriminate among individuals

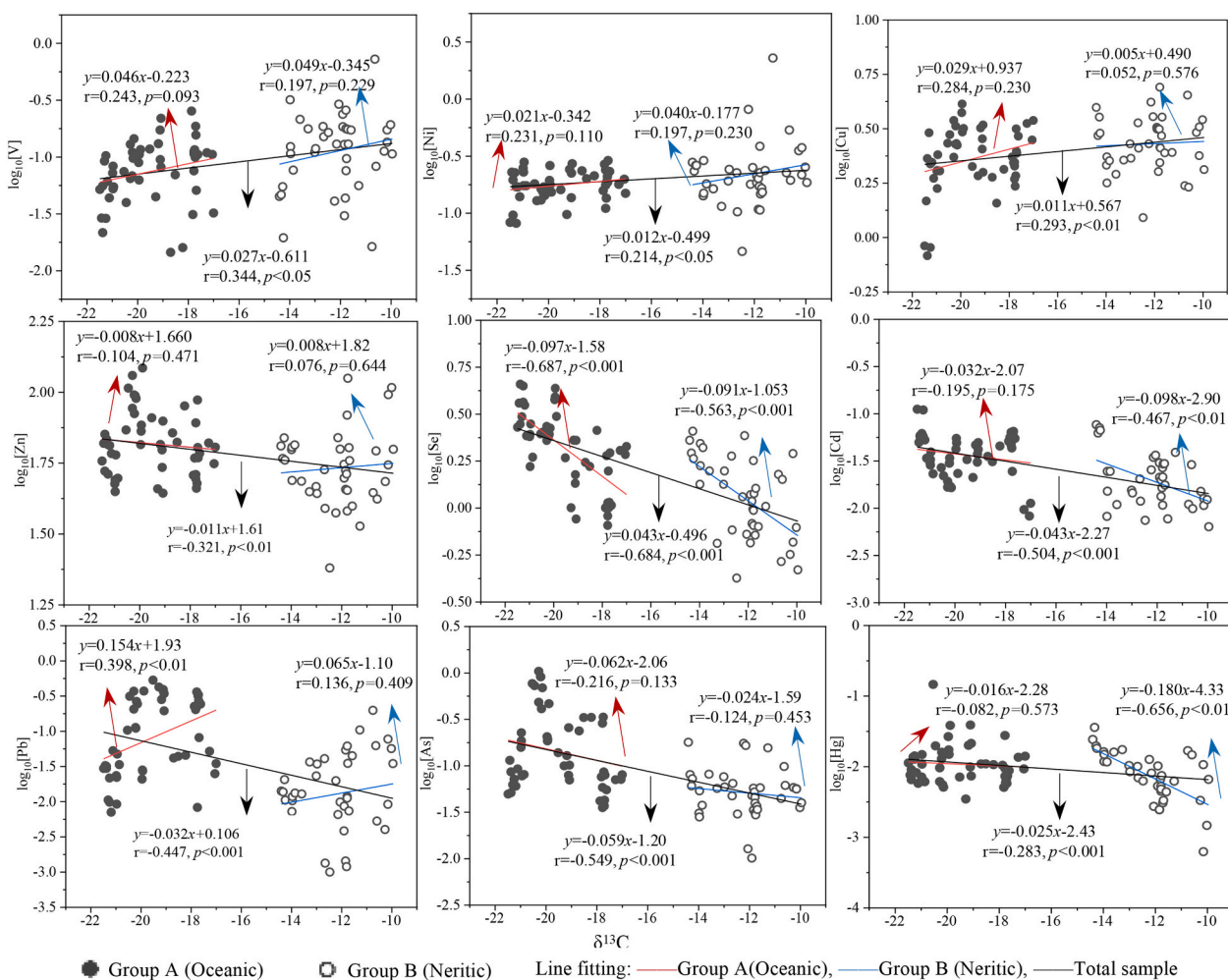


Fig. 4. Relationships between \log_{10} -transformed concentrations ($\mu\text{g/g}$, dw) of TEs and $\delta^{13}\text{C}$ in egg contents for neritic ($n = 39$) and oceanic ($n = 50$) foraging green turtles. The trendline is shown only for the TE that is significant related with $\delta^{13}\text{C}$ values at the total sample level. Blue arrow: the fitting result of neritic group; red arrow: the fitting result of oceanic group; black arrow: the fitting result of total sample.

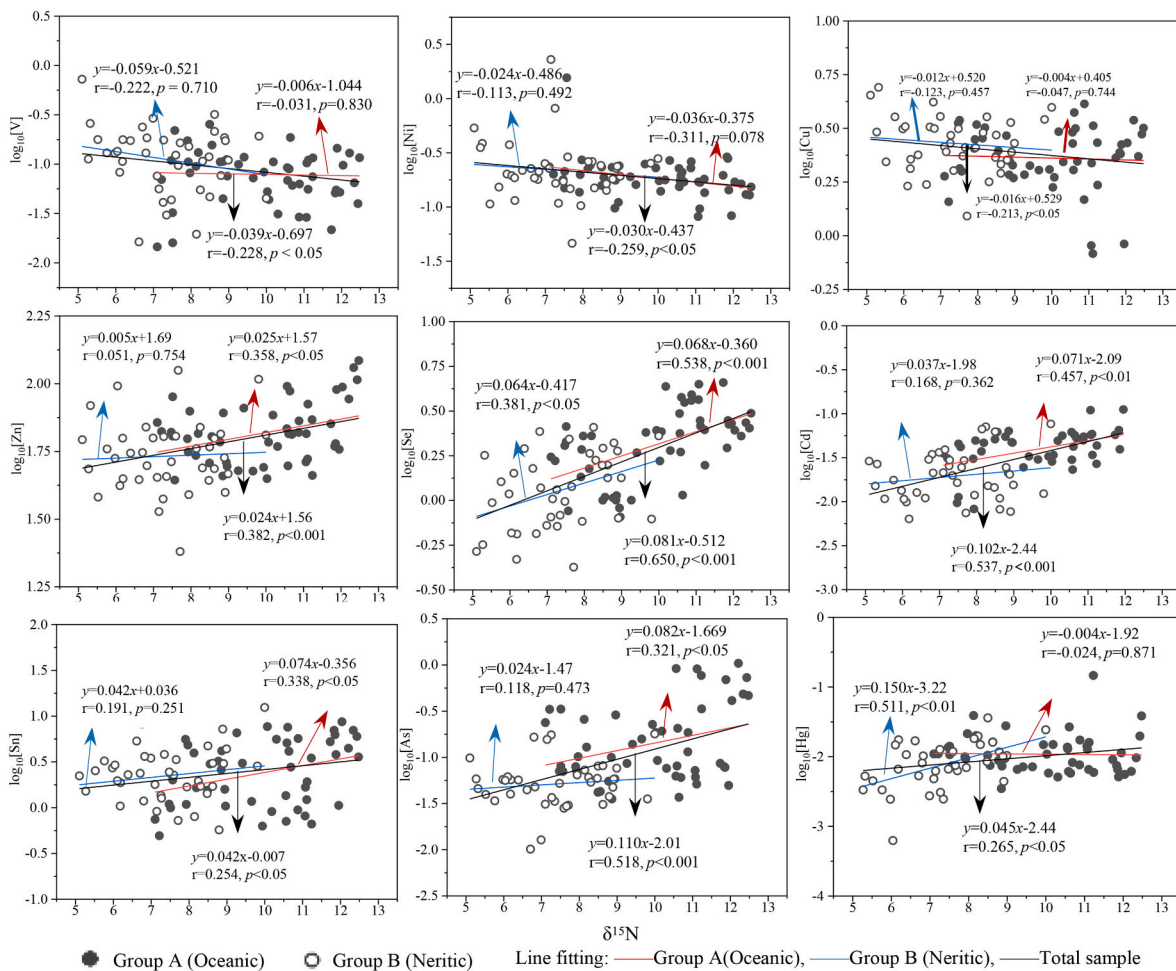


Fig. 5. Relationships between log10-transformed concentrations ($\mu\text{g/g}$, d.w.) of TEs and $\delta^{15}\text{N}$ in egg content for neritic ($n = 39$) and oceanic ($n = 50$) foraging green turtles. The trendline is shown only for the TE that is significant related with $\delta^{15}\text{N}$ values at the total sample level. Blue arrow: the fitting result of neritic group; red arrow: the fitting result of oceanic group; black arrow: the fitting result of total sample.

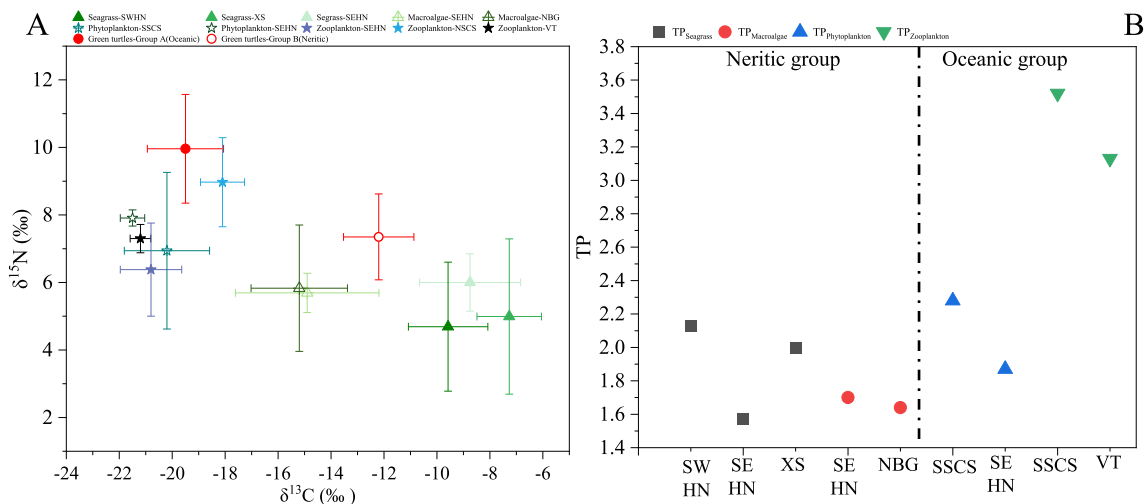


Fig. 6. A: The comparison of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in green turtles egg contents and their potential food items (in South China Sea). Values for prey items (large squares) are means \pm SD. The values are cited from literature (Supplementary Table S1). SWHN, Southwest of Hainan Island; SEHN, Southeast of Hainan Island; XS, Xisha Islands; NBG, Northern Beibu Gulf; SSCS, Southern South China Sea (Coral reef); NSCS, Northern South China Sea (Daya Bay); CVT, coastal waster of Vietnam. B: Trophic position (TP) of green turtles (assuming single primary producer-sourced diets). The calculation of $\text{TP}_{\text{Seagrass}}$, $\text{TP}_{\text{Macroalgae}}$, $\text{TP}_{\text{Phytoplankton}}$ and $\text{TP}_{\text{Zooplankton}}$ are based on the baseline values of seagrass, macroalgae, phytoplankton and zooplankton in different regions, respectively.

inhabiting different environments. For instance, Sr has been identified as a key element in differentiating between the eggshells of oceanic foraging leatherback turtles and neritic foraging loggerhead turtles (Talavera-Saenz et al., 2007; du Preez et al., 2018). In this study, discriminant analysis indicated significant differences in the characteristic elemental composition of V, Sr, and Ba in egg contents between the two green turtle groups (see section 3.2). V concentrations in coastal seawater are primarily affected by terrigenous inputs (Schuth et al., 2019), whereas Sr concentrations exhibit conservative relationship with salinity, showing a linear increase from freshwater to marine endmembers in coastal waters, with marine endmembers of Sr remaining stable (Surge and Lohmann, 2002; Nelson and Powers, 2020). Besides, Sr and Ba are influenced by marine carbonate-type sediment geochemical processes and are more affected by oceanic conditions (Chen et al., 2023). Analysis of the relationships between $\delta^{13}\text{C}$ values and Sr/V and Ba/V ratios revealed that $\delta^{13}\text{C}$ values of green turtle egg contents decreased with increases in $\log_{10}[\text{Sr}/\text{V}]$ ($r = -0.46, p < 0.01$) and $\log_{10}[\text{Ba}/\text{V}]$ ($r = -0.52, p < 0.01$) (Supplementary Fig. S2). Based on these findings, green turtles nesting on Xisha Islands can be classified into two distinct groups: an oceanic group (Group A, $\delta^{13}\text{C} < -14\text{‰}$) and a neritic group (Group B, $\delta^{13}\text{C} > -14\text{‰}$).

Moreover, the $\delta^{15}\text{N}$ values of egg contents from green turtles in the oceanic group were significantly higher than those in the neritic group. This observation aligns with findings by Arthur et al. (2014), who reported that green turtles captured from oceanic habitats (i.e., in the central North Pacific) exhibited higher $\delta^{15}\text{N}$ values in their tissues, indicating feeding at a higher trophic position compared to those in neritic habitats (Hawaii). Seminoff et al. (2021) noted substantial variability in green turtle tissue $\delta^{15}\text{N}$ values among neritic and oceanic sites, attributing this largely to spatial differences in baseline nitrogen isotopic compositions, and to a lesser extent, to trophic position differences among the green turtle foraging populations. As previously described (section 3.1), green turtles nesting on Xisha Islands were hypothesized to be divided into two foraging groups: oceanic planktivores and neritic herbivores. The neritic foragers consuming seagrass exhibited comparable TPs to oceanic foragers feeding on phytoplankton. However, oceanic foraging individuals that also consumed zooplankton demonstrated higher TPs. It is noteworthy that the calculated TPs ($\text{TP} < 2$) reported here were ecologically implausible, as the minimum trophic position for herbivores is $\text{TP} = 2$. This discrepancy may be attributed to the wide range of food sources available to green turtles, particularly considering the variability of $\delta^{15}\text{N}$ values in primary producers across different spatial and temporal scales. Furthermore, TDF values vary among green turtle tissues and foraging regions, highlighting the need for further investigation.

4.2. Effect of habitat utilization on TE accumulation in green turtle egg contents

This study revealed distinct patterns in TE concentrations between neritic and oceanic green turtle groups. Higher concentrations of V and Cu were observed in egg contents from the neritic group, exhibiting positive correlations with $\delta^{13}\text{C}$ values. Conversely, concentrations of Zn, Cd, Sr, Mn, Se, Ba, and Pb were elevated in egg contents from the oceanic group, presenting negative correlations with $\delta^{13}\text{C}$ values. These intra-species TE distribution patterns may be due to variations in feeding habitats (Le Croizier et al., 2021). Similar findings have been reported on the Great Barrier Reef, where green turtles foraging in coastal areas exhibited higher concentrations of Co, Mn, and Pb in their blood compared to those in offshore habitats. Additionally, seagrass in coastal bays contained higher concentrations of Cu, Pb, V, Mn and Co than that found in coral cays (Villa et al., 2017; Thomas et al., 2020). On the Brazilian coast, green turtles inhabiting coastal habitats displayed higher liver concentrations of Cu and V than those in oceanic habitats (Sulato et al., 2022). As previously described, V concentrations and Sr/Ba ratios represent characteristic indicators of neritic and oceanic

habitats, respectively. Analysis of the relationships between these characteristic element ratios (Sr/V and Ba/V) and TE concentrations revealed that the Cu concentrations in green turtle egg contents decreased with the increment of $\log_{10}[\text{Sr}/\text{V}]$ and $\log_{10}[\text{Ba}/\text{V}]$ ratios. Conversely, Se, Cd, As, and Zn concentrations increased with increasing Sr/V and Ba/V ratios (Supplementary Fig. S3). These findings suggest that the observed habitat-specific differences in Cu, Se, As, Cd, and Zn levels in eggs of *C. mydas* nesting on Xisha Islands may be influenced by background values or pollution levels specific to these distinct habitats.

In this study, $\delta^{15}\text{N}$ values in the egg contents of oceanic group green turtles were higher, and positive correlations were observed between $\delta^{15}\text{N}$ values and concentrations of Zn, Se, Cd, As, Sn, and Hg across all green turtle egg contents. In addition, significant positive relationships were identified between Zn, Se, Sn, Cd, As and $\delta^{15}\text{N}$ specifically within the oceanic foraging group, a correlation absent in the neritic foraging group, indicating trophic level transfer. While adult green turtles are traditionally considered obligate herbivores in neritic areas, increasing evidence indicates their utilization of oceanic areas as feeding habitats and consumption of animal matter in these environments (Arthur et al., 2014; Stubbs et al., 2022). As previously discussed (section 3.1), oceanic group green turtles that incorporate zooplankton in their diet occupy a higher trophic position. Briand et al. (2015) proved biomagnification of Zn, Se, and Hg across trophic levels in coral reef food webs. Besides, natural upwelling and coastal currents (carrying terrestrial-derived sewage) introduce elevated concentrations of nutrients (NO_x) and TEs (e.g., Cd, Zn, and As) to coastal coral reef areas (Cao et al., 2016; Jiang et al., 2017). Upwelling zones, widely distributed along the coasts of southeastern Hainan Island, Guangdong, and Vietnam, have been identified as green turtle feeding habitats (Chan et al., 2007; Liu et al., 2023). Therefore, the significant positive correlation between TEs and $\delta^{15}\text{N}$ values in the oceanic foraging group may be attributed to a common source for both TEs and nitrogen nutrients.

Hg concentrations in green turtle egg contents exhibited positive correlations with $\delta^{13}\text{C}$ ($r = -0.583, p < 0.001$) and $\delta^{15}\text{N}$ ($r = 0.495, p < 0.01$) values in the neritic foraging group. However, this relationship was not observed in the oceanic group, suggesting distinct sources and pathways of Hg bioaccumulation in green turtle eggs. A recent study by Yang et al. (2023) revealed that Hg in pelagic zone fish originates from atmospheric Hg deposition, while Hg in fish inhabiting the marine continental shelf is derived from sediments. These findings highlight the need for further research and advanced approaches to quantify TE sources in tissues of green turtles nesting on Xisha Islands.

5. Conclusion

The Qilianyu Cluster on Xisha Islands hosts China's largest nesting population of green turtles; however, information on the habitat utilization of this nesting group remains limited. While satellite telemetry is an effective method for assessing feeding ecology, its use is restricted in this region. The present study employed stable isotope and characteristic element analyses to investigate the habitat utilization of green turtles nesting on Xisha Islands. In particular, unhatched egg samples were utilized instead of female green turtle tissues for these analyses, providing a non-invasive method. The results demonstrated that green turtles nesting on Xisha Islands could be categorized into oceanic and neritic groups. This study established baseline values for 16 TEs in the egg contents of this nesting group and revealed that inter-group differences in TE concentrations were more pronounced than intra-group variations. Egg contents from neritic foraging green turtles exhibited higher concentrations of V and Cu, while those from oceanic foraging individuals contained higher concentrations of Zn, Cd, Se, Sr, Ba, Hg, Sn, and As. This distribution pattern may be attributed to variations in background TE concentrations between the two different foraging habitats. Furthermore, differences in prey items and trophic levels between the two groups of green turtles may contribute to the observed inter-group variations in TE concentrations (e.g. Zn, Se, As, Sn) within

the eggs. However, due to the lack of information regarding specific foraging site locations and dietary composition of this population, definitive conclusions cannot be drawn at this time. Further research is warranted to elucidate the precise foraging locations and dietary preferences of this nesting population, which would provide valuable insights into the factors influencing TE accumulation and distribution in green turtle eggs.

CRedit authorship contribution statement

Xiang Li: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Xiaobo Zheng:** Writing – review & editing. **Jingyue Peng:** Investigation. **Ting Zhang:** Investigation. **Liu Lin:** Investigation, Data curation. **Jichao Wang:** Writing – review & editing, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Acknowledgements

This work was supported by the National Natural Science Foundations (Grant No. 32160135, 42067040). The authors are grateful for assistance provided by the Sansha Ocean and Fisheries Administration and Qilianyu Administration Committee.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2024.177149>.

Data availability

No data was used for the research described in the article.

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