RESEARCH ARTICLE

WILEY

Trophic history of Hawaiian green turtles as revealed by stable isotope ratios (δ^{13}C, δ^{15}N and δ^{34}S) in the bones of museum specimens

Adriana Velasquez-Vacca [1,2] | Jeffrey A. Seminoff [3] | T. Todd Jones [4] |
George H. Balazs [5] | Luis Cardona [1]

[1]Department of Evolutionary Biology, Ecology and Environmental Science and IRBio, University of Barcelona, Barcelona, Spain

[2]Grupo de Ecología de la Universidad del Valle, Cali, Colombia

[3]NOAA Fisheries, Southwest Fisheries Science Center, La Jolla, California, USA

[4]NOAA Fisheries, Pacific Islands Fisheries Science Center, Honolulu, Hawai'i, USA

[5]Golden Honu Services of Oceania, Honolulu, Hawai'i, USA

Correspondence
Adriana Velasquez-Vacca, 9505 Hierba Road, Santa Clarita, CA 91390, USA.
Email: adrianavelasquezvacca@gmail.com

Funding information
Fundació Bosch i Gimpera, Grant/Award Number: 309765

Abstract

1. Understanding consumer trophic status and long-term dietary changes can yield information about impacts of altered habitats on their ecology. In Hawai'i, coastal ecosystems have been significantly modified by the introduction of invasive seaweeds and mangroves, high nutrient load and overfishing, but so far, much is still to be understood about how these changes have affected the green turtle (*Chelonia mydas*).

2. This study analyzed stable carbon, nitrogen, and sulfur isotope ratios in the bone tissue of modern and museum specimens of green turtles collected from 1901 to 2020 in Oahu and the North-western Hawaiian Islands to understand how their isotopic niche has changed through time, a crucial step towards restoring the ecological role of a formerly decimated species.

3. The standard ellipse size and the total area of the convex hull of the isotopic niche of green turtles in three periods (1901–1951, 1992–2008, and 2018–2020) were calculated.

4. The stable isotope values of ancient green turtles (1901–1951) suggest that they relied heavily on macroalgae even before the introduction of exotic species and eutrophication promoted the development of algal pastures.

5. However, a few ancient green turtles relied heavily on seagrasses, and others complemented their macroalgae-based diets with significant amounts of animal matter. Such diet specialists were missing from the sample of current green sea turtles, suggesting that these foraging strategies are less common or perhaps even absent in the current population. The results suggest that green turtles have converged on the use of the most abundant resource, red macroalgae, likely because of the homogenization and simplification of coastal habitats and food webs due to anthropogenic influences.

6. Restoring the population size of herbivorous fishes and a more diverse ecosystem structure may be necessary to recover the array of trophic strategies formerly present in the Hawaiian green turtle population.

1 | INTRODUCTION

Human activity dramatically reduced the abundance of many marine species worldwide during the 20th century (McCauley et al., 2015). Changes in population size were often associated with diet shifts (Cardona et al., 2020; Hanson et al., 2009; Zenteno et al., 2015) and hence altered the structure and dynamics of food webs, even when no species went extinct (Bas et al., 2019; Ólafsdóttir et al., 2021). Only recently have some populations started to increase as a result of protection (Duarte et al., 2020). Yet even with ongoing recovery of some species, restoration of ecological communities requires that former food web structure and species richness are achieved (Heithaus et al., 2014). The introduction of exotic species may make the restorations of food webs even more difficult, as these species may become major predators, competitors or prey for native species (Anton et al., 2020; Roemer et al., 2002). Thus, there is an urgent need to understand historical changes in the diets of consumers and the structure of marine food webs to provide adequate baselines for ecosystem restoration.

Green turtles *Chelonia mydas* are marine megaherbivores rebounding in warm-temperate and tropical marine ecosystems worldwide after several decades of conservation efforts (Casale et al., 2018; Kondo et al., 2017). Seagrasses are the staple food of green turtles in some locations, whereas in other places, macroalgae prevail in their diet (Esteban et al., 2020). Green turtle population increase has been particularly vigorous in the Hawaiian Archipelago (Kittinger et al., 2013), inhabited by a geographically and genetically discrete population (Seminoff et al., 2015). Nothing is known about the Hawaiian green turtle's population size in pre-human settlement times, but green turtle numbers are thought to have declined since human settlement in the archipelago (Van Houtan & Kittinger, 2014). The trend was reversed with the enactment of Reg 36 of the State of Hawai'i Department of Land and Natural Resources, banning all commercial harvest of green turtles in 1974, and the inclusion of green turtles in the US Endangered Species Act in 1978. Both were enforced by entirely shutting down commercial taking of green turtles in Hawai'i, and the Hawaiian green turtle population has been growing steadily since then (Balazs et al., 2015).

However, the recovery of Hawaiian green turtles is challenging because it takes place within a coastal setting highly modified by human activity, including the introduction of mangroves and several species of macroalgae (Allen, 1998; Russo, 1977), the decline of roving herbivorous fishes as a result of fishing (Helyer &

Samhouri, 2017) and coastal development and increased nutrient loading resulting from human population growth and fertilizer runoff from coastal farmland (Murray et al., 2019). During the second half of the 20th century, these processes operated synergistically to cause a reduction in the abundance of coral in favour of turf or macroalgae, which in turn might have been beneficial for the recovery of green turtles through providing more food for them. Diet analysis conducted since the 1970s revealed that Hawaiian green turtles rely largely on several species of exotic macroalgae, particularly at Kaneohe Bay on the island of Oahu (Russell & Balazs, 2015). However, the original diet of green turtles in the Hawaiian Archipelago remains unknown, and the potential contribution of the native seagrass *Halophila hawaiiana* is particularly intriguing, considering its former widespread occurrence and high productivity (Herbert, 1986) and the relevance of seagrasses in the diet of green turtle populations elsewhere (Esteban et al., 2020).

Identifying historic diet changes is challenging due to the absence of direct information from gut content analysis, but the stable isotope ratios in the tissues of museum specimens can provide some clues (Conrad et al., 2018; Saporiti et al., 2014). This is because the relative abundance of heavy and light isotopes of several chemical elements in the tissues of animals reflect those in their diets (Fry, 2006), and hence, stable isotope ratios offer a coarse proxy for trophic level and the contribution of major food sources (if isotopically distinct) to the diet of consumers (Swan et al., 2020). Furthermore, the stable isotope ratios in museum specimens are conserved through time, such that stable isotope analyses of 'old' bones can give insights from the diet when the bones were originally formed during somatic growth.

The stable isotope ratio informs about the relative abundance of the heavy and light isotopes of a chemical element in a sample compared to those in a reference material, and the stable isotope ratios in animal tissues are usually enriched in the heavy isotopes of any particular element compared to those of their diets, because of several physiological processes that are often not fully understood (Fry, 2006). For any given element, the difference in stable isotope ratios between an animal's tissue and diet is called the trophic discrimination factor (TDF). This value is tissue, diet and species specific (Hobson & Clark, 1992), and knowledge about TDF is an essential component of diet reconstructions using stable isotope mixing models (Turner Tomaszewicz et al., 2017). Major changes in the type of resources consumed (e.g., coastal vs. oceanic, benthic vs. pelagic and macroalgae vs. seagrasses) and the trophic position of the consumer can also be detected by means of stable isotope

analysis. Furthermore, a foraging population's trophic niche and diversity of individual foraging strategies can be depicted by metrics such as the convex hull total area (TA) and the standard ellipse area (SEA), which are both derived from isotope ratios of all individuals in the population (Quevedo et al., 2009). These metrics are informative of the diversity of food sources consumed by a population and the degree of individual dietary specialization, depending on the turnover rate of the tissue analysed (Drago et al., 2017). The latter is particularly relevant, because the degree of individual specialization may change in response to intraspecific and interspecific competition and resource availability (Pagani-Núñez et al., 2019).

In this study, stable isotope analysis was conducted on bone tissue from Hawaiian green turtle specimens housed at three museum collections from around the world to assess changes in isotopic niche over the last 120 years for this insular population. More precisely, the carbon, nitrogen and sulfur ratios ($\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$) of green turtle bones were measured, and changes through time in the isotopic niche breadth were assessed by SEA and TA. We tested the hypotheses that (1) reliance on macroalgae by green turtles has increased during this long-time span and (2) that trophic niche breadth has decreased since the 1970s due to increased green turtle population and macroalgae availability. Understanding how Hawaiian green turtle diet has shifted through decadal time scales could help pinpoint the potential environmental drivers for such change and provide insight about historic baselines and conservation measures necessary to restore the trophic ecology of Hawaiian green turtles.

2 | METHODS

2.1 | Prey sample collection

Previous gut content studies revealed that the diet of Hawaiian green turtles foraging in the coastal waters off Oahu from 1977 to 2012 was dominated by red macroalgae (*Acanthophora spicifera*, *Gracilaria salicornia* and *Hypnea musciformis*), with the seagrasses *Halophila decipiens* and *H. hawaiiana* supplying less than 15% of the ingested material and terrestrial vegetation having a very minor contribution (Russell & Balazs, 2015). In order to understand the variability of $\delta^{13}C$,

$\delta^{15}N$ and $\delta^{34}S$ values across those major diet sources, the following prey items were collected from Wailupe, Kāne'ohe flats, Kāne'ohe reef, Kāne'ohe sandbar and Wawamalu: the macroalgae *A. spicifera*, *G. salicornia* and *H. musciformis*; the seagrass *H. hawaiiana*; and red mangrove (*Rhizophora mangle*) leaves and turf algae. Turf algae are dense, multi-species assemblages of filamentous benthic algae, including small patches of macroalgae and cyanobacteria, typically less than 1 cm in height (Swierts & Vermeij, 2016). Macroalgae and turf samples were collected at the end of the dry (September 2018) and rainy seasons (February 2019), and the red mangrove leaves were collected in March 2021. Sample size was 10 for all the species.

2.2 | Turtle sample collection

A total of 39 samples were analyzed for this study. One set of samples was from museum collections of 25 bones from Hawaiian green turtles collected between 1901 and 2008 from Oahu ($n = 18$) and the North-western Hawaiian Islands ($n = 7$). These 25 bones had been preserved at the Smithsonian National History Museum ($n = 7$), the San Diego Natural History Museum ($n = 5$), and the Bishop Museum ($n = 13$). An additional set of samples was collected from 14 dead-stranded green turtles in Oahu between 2018 and 2020 (Figure 1).

Museum specimens were mostly skulls, but six had only ribs, and one had only a vertebra available. A previous study showed no significant differences in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of paired samples of squamosal bone and rib (Velasquez-Vacca et al., 2023), so any specimen represented by the squamosal bone (skull), rib or vertebra was sampled for analysis. The contemporary, dead-stranded turtles were frozen at $-20°C$ when found, and then necropsies were conducted by the Pacific Islands Fisheries Science Center (NOAA) staff in Oahu, Hawai'i. For each turtle, curved carapace length (CCL) was measured, and a piece of the skull (squamosal bone) or rib (when the skull was missing) was removed, washed, and air-dried. A cross-section of bone for all samples was then drilled using a Dremel™ tool with 0.1-in. drill bits.

CCL information was missing from most of the museum specimens, so the data from the 14 dead-stranded turtles collected

FIGURE 1 Origin of green turtles analyzed, according to the museum records or dead-stranding records. Dots indicate locations where green turtles were collected (blue dots denote turtles from museums, and green dots were dead-stranded turtles). Any given location includes more than one turtle. Maps modified from R tmap (Tennekes, 2018).
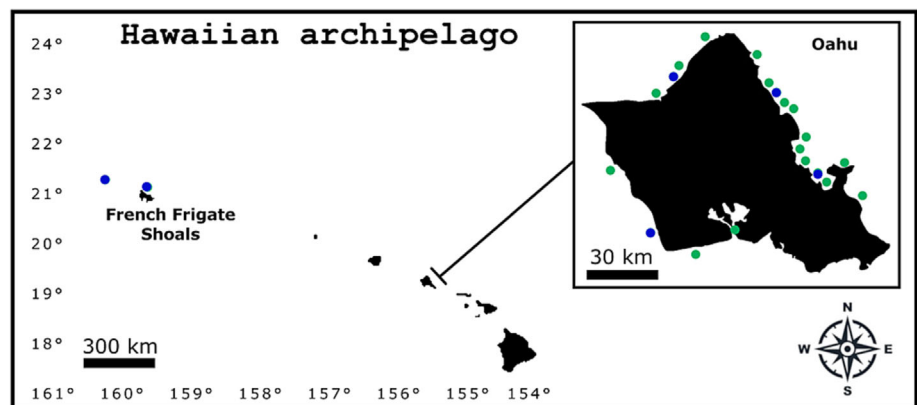
from 2018 to 2020 in Oahu were used to calculate the correlation between skull length and CCL (Equation 1). Later, the skull length of each museum specimen was measured in centimetre and used for Equation (1) to calculate the expected CCL.

$$CCL = 4.3257 \times skull\ length - 0.09 \qquad (1)$$

2.3 | Stable isotope analysis

Bone samples were dried at 50°C and processed for analysis of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$, without further treatment. Following Velasquez-Vacca et al. (2023), the $\delta^{13}C$ values of unprocessed samples were corrected for lipid content, based on their C:N ratio (Equation 2).

$$\delta^{13}C_{ale} = \delta^{13}C_{unprocessed} - 3.658 + \left(3.372 \times \ln(C:N)_{unprocessed}\right), \quad (2)$$

where $\delta^{13}C_{ale}$ (ale = acidification and lipid extraction) denotes the $\delta^{13}C$ value expected after acidification and lipid extraction, $\delta^{13}C_{unprocessed}$ denotes the $\delta^{13}C$ value in unprocessed samples and C:N$_{unprocessed}$ is the corresponding elemental ratio of unprocessed samples.

Samples of diet sources were cleaned of sand and debris, rinsed with distilled water, dried at 50°C for 24 h and ground to a fine powder.

After processing, all the samples were sent to the Stable Isotope Core Laboratory at Washington State University for stable isotope analysis (SIA). Only one sample was analyzed for each specimen, because of the small amount of bone powder available from the museum specimens. For carbon and nitrogen isotope analysis, 5 mg of bone powder or 15 mg of dietary sources was weighed and loaded into tin cups, combusted in an elemental analyzer (ECS 4010, Costech Analytical Valencia, CA), with resultant gases separated with a 3-m gas cromatograhy column and analyzed with a continuous-flow isotope ratio mass spectrometer (Delta Plus XP, Thermo Finnigan, Bremen) (Brenna et al., 1997). Isotopic reference materials were interspersed with samples for calibration. To correct the contribution of ^{17}O in CO_2, the Santrock correction was applied using the isotope ratio mass spectrometry (IRMS) software (Santrock et al., 1985). Carbon isotopic results are reported in parts per thousand relative to VPDB (Vienna Peedee Belemnite) using National Bureau of Standards 19 and lithium isotopes in lithium carbonate as anchor points. Nitrogen isotope ratios are reported in parts per thousand relative to N_2 in air.

Sulfur isotope analysis was conducted independently. Sample powder was weighed (10 mg in bone and 5 mg in diet items) and encapsulated, combusted with the same type of elemental analyser as for $\delta^{13}C$ and $\delta^{15}N$ analyses, and SO_2 gases were separated with a 0.8-m GC column (100°C) and analysed with a continuous-flow isotope ratio mass spectrometer (Delta Plus XP, Thermo Finnigan,

Bremen) (Qi & Coplen, 2003). The final determination of $\delta^{34}S$ was based on the collection of Ions 64 and 66 in SO_2. A dual-reactor configuration was used (Fry et al., 2002), with the second reactor full of quartz chips to buffer ^{18}O contribution to the SO_2. Approximately 5 mg of niobium pentoxide was amended to each sample to improve combustion. No correction for oxygen isotope contribution was made. Four isotopic reference materials were interspersed with samples for calibration (Costech Analytical BBOT, Alfa Aesar $BaSO_4$, Salt Lake Medals Ag_2S, Acros elemental S). A grizzly bear hair (*Ursus arctos horribilis*) sample from Pullman, WA, where the stable isotope laboratory is located, was also run with all samples as a blind quality control. Isotopic results are expressed in delta (δ) notation relative to VCDT (Vienna-Canyon Diablo Troilite) in parts per thousand.

2.4 | Statistical analysis

Turtles were grouped into three periods for analysis: 1901–1951 ($n = 12$), 1992–2008 ($n = 13$), and 2018–2020 ($n = 14$). Analysis of variance (ANOVA) was used to compare the CCL and stable isotope ratios of green turtles from three considered periods. Turtles from the first study period were sampled both at Oahu ($n = 5$) and at the North-western Hawaiian Islands ($n = 7$), whereas all the turtles from the other two study periods came from Oahu exclusively. Fisher F test and Student t-test were used to compare the variance and the average of the three stable isotope ratios of the turtles from the first study period collected in the North-western Hawaiian Islands and Oahu. As differences were not statistically significant (see Section 3), samples from the two locations were pooled for further analysis, and location was not considered as a factor in the following analyses.

General linear models (GLMs) were run in R version 4.1.1 (R-Team, 2021) to assess whether stable isotope ratios changed through time; CCL was included as a covariate. Location was not included as a factor because only during the first study period were some turtles collected at the North-western Hawaiian Islands, and hence, all the turtles from the two latter study periods were collected from Oahu. Furthermore, no statistically significant differences were observed in the variance and the mean of any stable isotope ratio of the turtles from Oahu and the North-western Hawaiian Islands from the first study period. To be able to compare these three periods, the change in the $\delta^{13}C$ baseline resulting from the burning of fossil fuels needs to be considered, because the emissions were greatly increased during the industrial era. To do this, the SuessR (Clark et al., 2022), an R package for applying mathematical corrections for the Suess and Laws effects with 1850 as the reference year, was used, because that is the onset of the industrial era and the beginning of exponential increases in anthropogenic CO_2 emissions into the atmosphere and ocean (Ruddiman, 2013). SuessR makes corrections at a regional scale (Clark et al., 2021), because changes in the $\delta^{13}C$ resulting from the burning of fossil fuels are dependent on surface water residence time, water temperature and biological production of CO_2 (Eide et al., 2017). The settings for this R function calculate the regional uptake constant

used to modify the global Suess effect curve to make it specific to a region. In this case, the Aleutian Islands values were used because they were not different from data for Hawai'i (Eide et al., 2017), and the SuessR package is still in development and adding more regions.

The δ^{13}C and δ^{15}N TDF values of green turtle cortical bone have been derived experimentally by Turner Tomaszewicz et al. (2017). Although they are not adequate to run mixing models on the basis of the stable isotope ratios of mixtures of cortical and trabecular bones (Velasquez-Vacca et al., 2023), they can be used as a benchmark. ANOVA was used to assess differences in the stable isotope ratios of diet items. Macroalgae and turf differed statistically from seagrasses in their δ^{13}C and δ^{15}N values (see Section 3), and *G. salicornia* was the macroalgae with the highest variability and the most extreme values, which were always higher than those of seagrass and encompassed those of other macroalgae and turf (range: 2.8‰–5.2‰; Table 1). According to the TDF of δ^{15}N reported for cortical bone ($+5.1 \pm 1.1$‰), the δ^{15}N values of the bone of green turtles consuming exclusively *G. salicornia* may range from 6.8‰ to 11.4‰, and hence, turtles with δ^{15}N above and below those thresholds are unlikely to rely solely on macroalgae or turf.

Stable Isotope Bayesian Ellipses in R (SIBER) (Jackson et al., 2011) was used to calculate the TA in the δ^{13}C–δ^{15}N, δ^{13}C–δ^{34}S, and δ^{15}N–δ^{34}S isospaces, the most frequent estimate of the area covered by the standard ellipses (SEA), the most frequent estimate of the area covered by the standard ellipse corrected for small samples size (SEAc) and the Bayesian estimate of the area covered by the standard ellipse (SEAb) in each period. The standard ellipse contains approximately 40% of the data (Batschelet, 1981), and the Bayesian approach allows for calculating their 95% credible intervals (Jackson et al., 2011). Estimating the credible intervals allows for assessing the statistical significance of differences in SEAb.

3 | RESULTS

The stable isotope ratios of vegetal food sources from Oahu are shown in Table 1. According to the ANOVA, they differed largely in their stable isotope ratios (δ^{13}C: ANOVA, $F_{5,54} = 76.50$, $p < 0.001$; δ^{15}N: ANOVA, $F_{5,54} = 64.68$, $p < 0.001$; and δ^{34}S: ANOVA, $F_{5,54} = 42.09$, $p < 0.001$; see Table S2 for the result of post hoc tests). Red mangrove leaves had the lowest δ^{13}C and δ^{34}S values and the highest δ^{15}N values of all the food sources considered (Table 1). The seagrass *H. hawaiiana* had the highest δ^{13}C values and the lowest

δ^{15}N values, and the three macroalgae species (*A. spicifera*, *G. salicornia* and *H. musciformis*) and turf had the highest δ^{34}S values (Table 1). Turf had δ^{15}N values similar to those of macroalgae, but the δ^{13}C of turf was lower than that of macroalgae.

The CCL of sampled turtles ranged 62–131 cm (mean $\pm$ SE = 80.2 $\pm$ 31.4 cm) in 1901–1951, 47–127 cm (mean $\pm$ SE = 86.3 $\pm$ 27.5 cm) in 1992–2008 and 46–103 cm (mean $\pm$ SE = 66.4 $\pm$ 18.9 cm) in 2018–2020. The individual values are shown in Table S1. There were no statistically significant differences in the average carapace length (CCL) of the turtles from the three study periods (ANOVA, $F_{2,36} = 22.062$, $p = 0.141$).

Turtles from the first study period from Oahu and the Northwestern Hawaiian Islands did not differ in their mean stable isotope ratios (t-test; δ^{13}C: $p = 0.974$, δ^{15}N: $p = 0.565$ and δ^{32}S: $p = 0.752$) and hence were pooled for further analysis. The average δ^{13}C, δ^{15}N and δ^{32}S values of the museum turtles from each period and the contemporary dead-stranded ones are shown in Figure 2 and the individual values in Table S1. Individual δ^{13}C values were corrected with SuessR for Suess and Laws effects. No statistically significant differences were found between the average of the three periods in any of the three stable isotope ratios (δ^{13}C: ANOVA, $F_{2,36} = 1.46$, $p = 0.246$; δ^{15}N: ANOVA, $F_{2,36} = 0.10$, $p = 0.897$; and δ^{34}S: ANOVA, $F_{2,36} = 0.98$, $p = 0.383$).

When using GLM to analyze the effect of CCL and year (Figures 3 and 4), neither independent variable had a significant effect on δ^{13}C (GLM, $F_{36,35} = -33.33$, $p = 0.110$), δ^{15}N (GLM, $F_{33,32} = -3.84$, $p = 0.840$) or δ^{34}S (GLM, $F_{37,36} = 0.81$, $p = 0.960$). Furthermore, the standard ellipses of the three periods overlapped broadly (Figures 5 and 6), although the areas of the standard ellipses of the most recent period (2018–2019) were significantly smaller than those of the two earlier periods (Table 2, 1901–1951 vs. 2018–2020: $p < 0.001$ and 1992–2008 vs. 2018–2020: $p < 0.001$). There was no difference between the size of the ellipses of the two first periods (1901–1951 vs. 1992–2008: $p = 0.645$).

4 | DISCUSSION

The results reported here revealed no significant change in the average δ^{13}C, δ^{15}N and δ^{34}S values in the bone tissue of Hawaiian green turtles from 1901 to 2020 but a significant and major reduction of the isotopic niche breadth in the most recent study period (2018–2020). Such reduction was particularly relevant in the δ^{13}C–δ^{15}N and

TABLE 1 Stable isotope ratios of the Hawaiian green turtle major diet sources in Oahu.

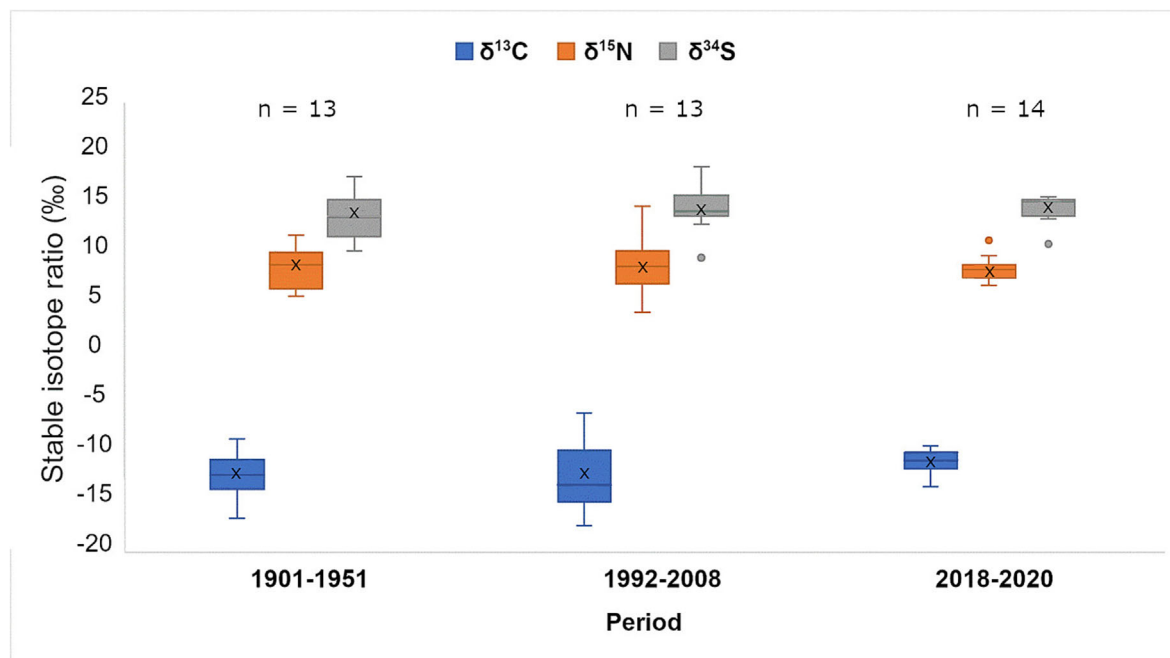| Diet source	δ^{13}C	δ^{15}N	δ^{34}S	*n*
Halophila hawaiiana (seagrass)	−8.1 ± 0.6	2.1 ± 0.3	18.5 ± 2.3	10
Acanthophora spicifera (macroalgae)	−14.4 ± 0.2	3.0 ± 0.2	22.2 ± 1.2	10
Gracilaria salicornia (macroalgae)	−16.3 ± 0.7	4.7 ± 1.2	21.5 ± 1.9	10
Hypnea musciformis (macroalgae)	−14.5 ± 0.8	3.7 ± 0.3	22.1 ± 1.7	10
Turf (short, filamentous algae)	−21.5 ± 6.6	4.9 ± 0.5	20.5 ± 2.2	10
Rhizophora mangle (red mangrove)	−30.3 ± 0.3	6.2 ± 0.3	12.6 ± 1.1	10

FIGURE 2 δ¹³C, δ¹⁵N and δ³⁴S in green turtle bone samples from Hawai'i from 1901 to 2020. Individual δ¹³C values were corrected with SuessR for Suess and Laws effects. The whiskers represent the minimum and maximum values, and the dots are the outliers. The mean is shown as an x, and the median as a horizontal line. The box represents the interquartile range (the bottom line of the box represents the median of the bottom half or first quartile, and the top line of the box represents the median of the top half or third quartile).
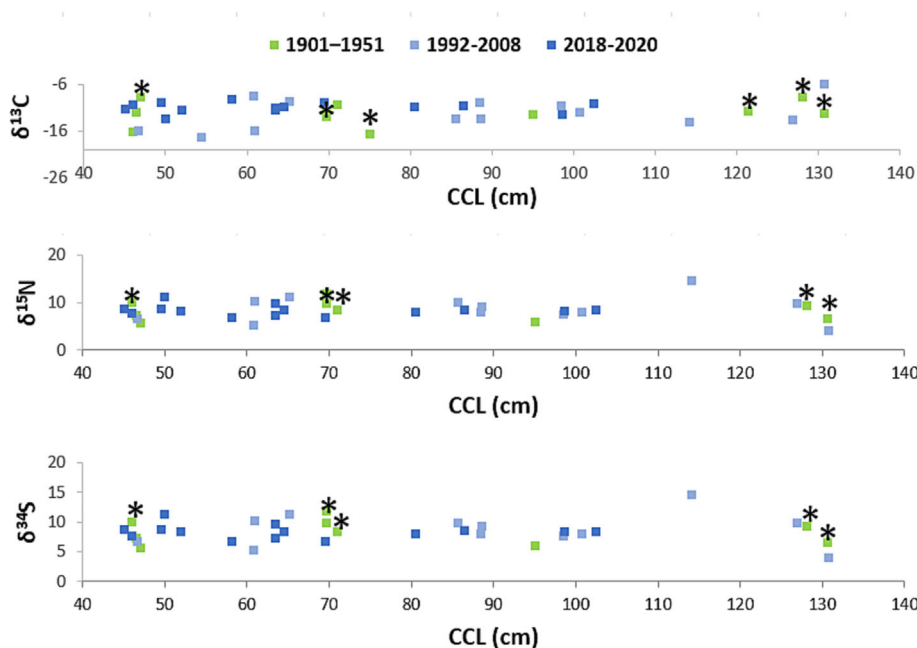
FIGURE 3 Curved carapace length (CCL) of green turtles from Hawai'i versus stable isotope ratios (δ¹³C, δ¹⁵N and δ³⁴S) in the bone tissue. There were no correlations between CCL and stable isotope ratios. δ¹³C values were corrected for the Suess and Laws effects (see Section 2). All turtles are from Oahu except for the ones marked with an asterisk, which are from the North-western Hawaiian Islands. Each period is denoted by a color: 1901–1951 in light green, 1998–2008 in light blue and 2018–2020 in dark blue.

δ¹³C–δ³⁴S isospaces, where current standard ellipses are less than 30% the size of standard ellipses from the two previous study periods. This suggests that, even though the average isotope values were statistically similar in the three considered periods, the scatter of the data was much smaller in the most recent period, despite a similar sample size. This is likely because of the absence of individuals with extreme δ¹³C, δ¹⁵N and δ³⁴S values in the most recent period relative to the two previous periods and likely indicative of lesser individual dietary specialization during the most recent sample period.

Extreme δ¹³C, δ¹⁵N and δ³⁴S values observed in some of the historical turtles analysed here are unlikely to be artefacts resulting from sampling biases and storage procedures, for three reasons. First, the average CCL of the turtles from the three study periods was not statistically different, and neither δ¹³C, δ¹⁵N nor δ³⁴S is currently
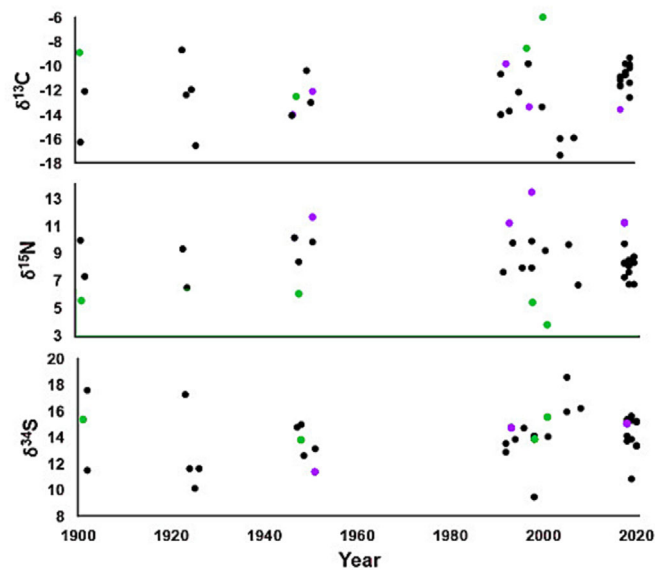
FIGURE 4 Collection or stranding year versus stable isotope ratios (δ^{13}C, δ^{15}N and δ^{34}S) in the bone tissue of green turtles from Hawai'i. δ^{13}C values were corrected for the Suess and Laws effects. Turtles in green had δ^{15}N values too low (<6.8‰), and turtles in purple had δ^{15}N values too high (>11.4‰) for diets based solely on macroalgae and turf, according to the trophic discrimination factor (TDF) of δ^{15}N reported for cortical bone (Turner Tomaszewicz et al., 2017) and the δ^{15}N values in macroalgae (this study).
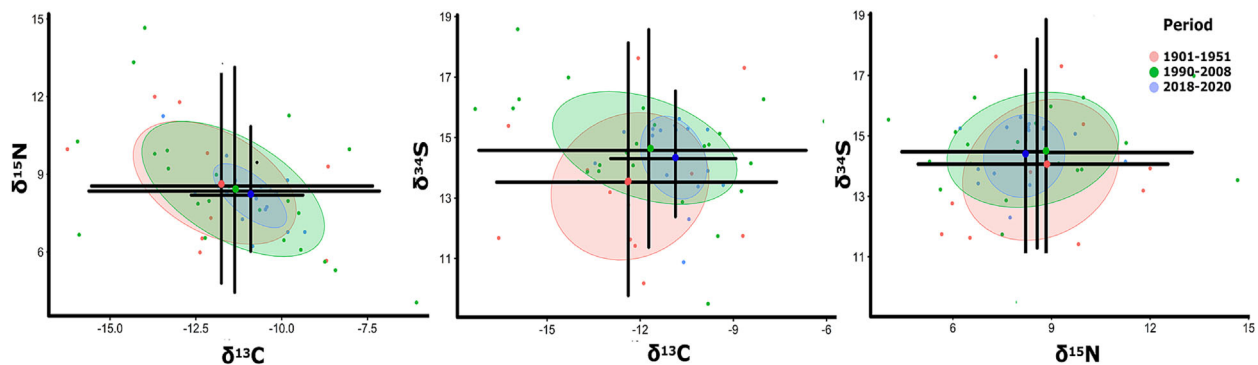

FIGURE 5 Values of individual green turtles in the isospace and standard ellipses (SEAc) of the population in the three periods: 1901–1951, 1992–2008 and 2018–2020. δ^{13}C values were corrected for the Suess and Laws effects with SuessR (see Section 2).

correlated with CCL in Oahu (Velasquez-Vacca et al., 2023), the origin of most of the samples analysed here. It should be noted, however, that nothing is known about the relationship between stable isotope ratios and CCL in turtles from the North-western Hawaiian Islands. Second, the inclusion of some green turtles from the North-western Hawaiian Islands in the first study period can also be ruled out as a reason for a broader isotopic niche, because this was also true for the second study period, when only turtles from Oahu were analysed. Furthermore, the results reported here revealed no statistically significant differences in the δ^{13}C, δ^{15}N and δ^{34}S values of the green turtles from Oahu and the North-western Hawaiian Islands collected during the first study period, although further research is needed to confirm the absence of geographic variability in stable isotope ratios of food sources. Third, the use of consolidants and preservatives is a matter of concern when

analysing bone samples from archaeological contexts (Groombridge & Luxmoore, 1989; Reynaga et al., 2023), but they are not used for the preservation of bone samples prepared from fresh specimens. Hence, the narrower isotopic niche observed in the most recent study is probably real and indicative of a lower variability in individual stable isotope ratios in green turtles. If so, the stable isotope ratios reported here suggest no major changes in the average diet of green turtles in the study locations during the past 120 years, although their current isotopic niche is much narrower than during the two previous study periods. The pattern might be different in other parts of the Hawaiian Archipelago, as green turtle diet varies across the archipelago (Arthur & Balazs, 2008; Velasquez-Vacca et al., 2023).

The absence of significant temporal changes in the average δ^{15}N values of the population is noteworthy, because sewage and farm
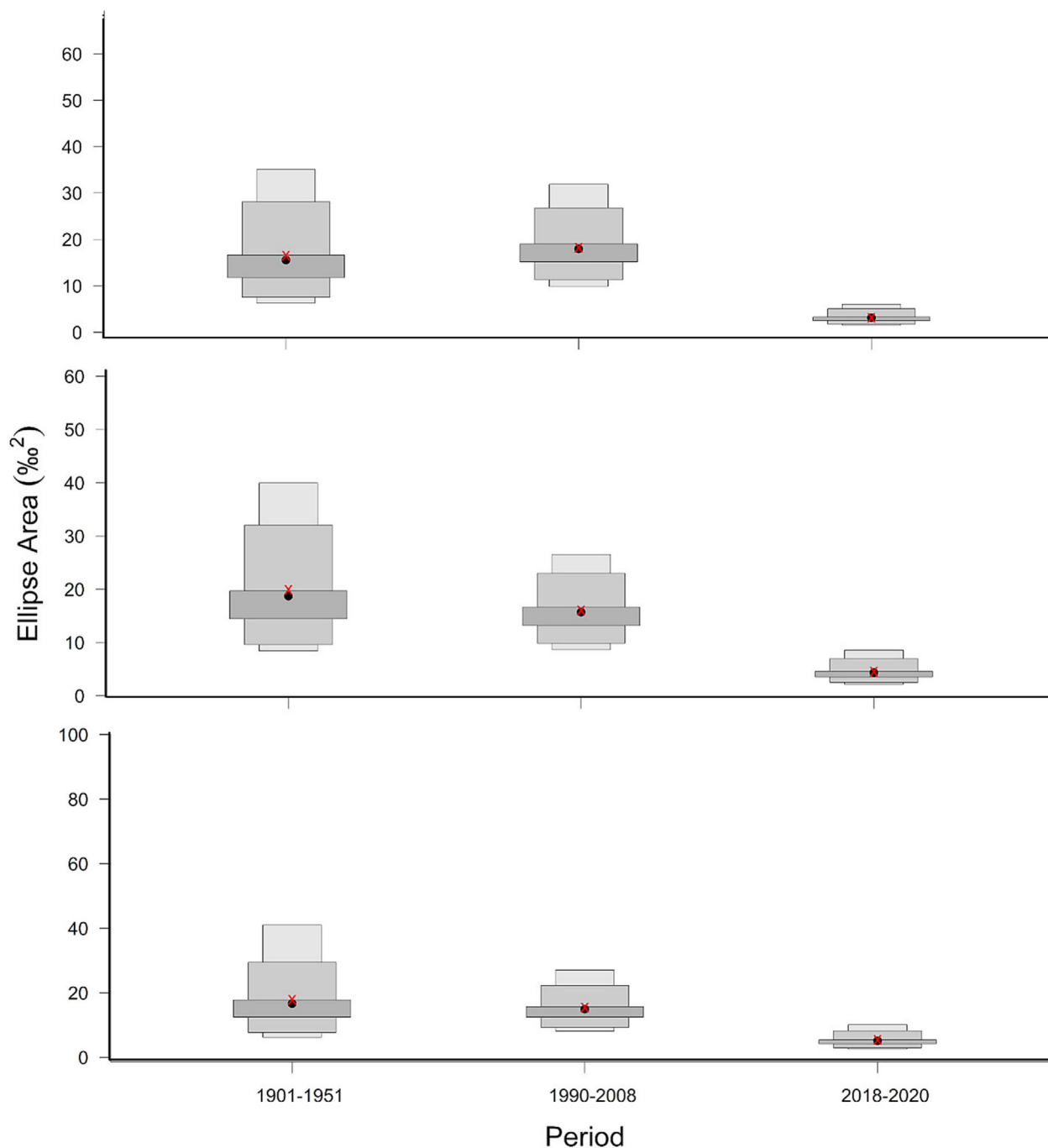
FIGURE 6 Bayesian estimates of the area of the standard ellipses (SEAb) in the $\delta^{13}C/\delta^{15}N$ (top), $\delta^{13}C/\delta^{34}S$ (middle) and $\delta^{15}N/\delta^{34}S$ isospaces (bottom) by period. Black dots represent the mode, the red x represents the mean and the shaded boxes represent the 40%, 95% and 99% credible intervals from dark to light grey. Periods on the horizontal axes are the same for the three figures and shown only for the lower panel.

runoffs are two major anthropogenic drivers of the $\delta^{15}N$ baseline in coastal areas of the Hawai'i Islands (Murray et al., 2019). Kaneohe Bay has a long history of nitrogen loading (Conklin & Stimson, 2004; Jouffray et al., 2015), and other populations of green turtles inhabiting similar habitats with a high N loading exhibit a consistent increase in their $\delta^{15}N$ values (Seminoff et al., 2021). Eutrophication resulting from high N loads may also modify the S baseline, because the higher organic matter load of the sediment caused by increased primary

production results in a lower oxygen content in the sediment and a lower redox potential, which enhances the product S^{-2} and hence the depletion in ^{34}S of the seagrasses rooted there (Connolly et al., 2004; Fry, 2006). In this scenario, the absence of any consistent change in the $\delta^{15}N$ and $\delta^{34}S$ values of green turtles during the study period indicates that any change in the $\delta^{15}N$ and $\delta^{34}S$ baselines that might have happened during the 1970s and 1980s had been reverted by 1992.

TABLE 2 Ellipse sizes for periods 1901–1951, 1992–2008 and 2018–2020 calculated with SIBER in R (Jackson et al., 2011).

	1901–1951	1992–2008	2018–2020
$\delta^{13}C$–$\delta^{15}N$			
°TA	29.6	58.6	9.0
°SEA	14.7	17.4	2.9
°SEAc	16.5	18.3	3.1
$\delta^{13}C$–$\delta^{34}S$			
°TA	46.2	50.5	11.2
°SEA	18.1	15.3	4.2
°SEAc	19.9	16.0	4.5
$\delta^{15}N$–$\delta^{34}S$			
°TA	54.8	15.9	17.9
°SEA	26.1	14.7	15.5
°SEAc	12.9	5.1	5.4

Abbreviations: SEA, standard ellipse area; SEAc, standard ellipse area corrected for small sample size; TA, total area of the convex hull.

Adequate TDF values for the squamosal or rib bone of green turtles are missing (Velasquez-Vacca et al., 2023), which prevents the use of mixing models to reconstruct the diet of turtles on the basis of the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values of such bones. Nevertheless, the $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values in the squamosal and rib bone of modern Hawaiian green turtles are indicative of the isotopic composition of diet, as they vary consistently with diet across ontogenetic stages and localities (Velasquez-Vacca et al., 2023). Currently, green turtles from Oahu rely primarily on red macroalgae, with seagrasses as a secondary diet source and terrestrial vegetation or mangrove leaves as a third, minor source (Russell et al., 2003; Velasquez-Vacca et al., 2023). Accordingly, the combination of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values observed in the bone tissue of the most recent green turtles analysed here should be indicative of that mixed diet. On this grounds, the absence of significant temporal changes in the mean $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ values of green turtle bone during the past 120 years suggests that a mixed diet, dominated by macroalgae and including smaller amounts of seagrass and mangrove/terrestrial vegetation, was also consumed during the two previous periods considered here. However, the existence of individuals with extreme $\delta^{13}C$, $\delta^{15}N$ or $\delta^{34}S$ values in the populations of the first two study periods might suggest the existence of diet specialists in the past, currently missing from the modern population.

Stable isotope analysis of different sections of metabolically inert tissues (e.g., bone, keratin and feathers) offers a valid alternative to assess individual specialization in species that can be captured only once (Araújo et al., 2011), and the analysis of thin sections of green turtle carapace scutes has demonstrated that generalist adult populations may be composed of individual specialists foraging consistently on the same resources through time (Vander Zanden et al., 2013). Most of the green turtle specimens in the museum collections analysed here are skulls (e.g., https://vertnet.org), and stable isotope ratios in tissues with a low turnover rate, such as bone,

integrate dietary information over months to years, and hence, individual values, when compared across the whole sample group, may also reflect the degree of dietary specialization (Drago et al., 2017). If so, the reduced individual variability reported here for the most recent study period suggests the absence of specialists in the population and the convergence of most of the population members on a similar diet, dominated by macroalgae. Including some specimens from the North-western Hawaiian Islands in the first study period might have increased artificially the isotopic variability of the sample if isotopically distinct, but their variance and average did not differ from those from Oahu from the same study period. Furthermore, individual variability was also high during the second study period, when only individuals from Oahu were analysed, and hence, the recent loss of diet specialist is a robust conclusion.

The most obvious group of specialists absent in the most recent study period is seagrass consumers, characterized by high $\delta^{13}C$ and low $\delta^{15}N$ values, represented by a few specimens in both 1901–1951 and 1992–2008 (Figure 4). Another absent group is the one characterized by low $\delta^{13}C$ and high $\delta^{15}N$ and $\delta^{34}S$ values, probably consuming significant amounts of animal prey and equally absent from the most recent study period in Oahu. The absence of such diet specialists explains the narrower isotopic niche of the modern population and might be caused by homogenization and simplification of coastal habitats and food webs, although sample size is small and further research is necessary to confirm that conclusion. Furthermore, identification of such specialists relies on the assumption that the variability of the stable isotope ratios of prey items was similar in the past, which remains unknown.

Optimal foraging theory predicts that the breadth of the isotopic niche results from the interplay between resource availability and interspecific and intraspecific competition (Araújo et al., 2011). The population of the Hawaiian green turtle has been growing steadily since they were legally protected in 1974 from all commercial hunting (Piacenza et al., 2016), and hence, intraspecific competition might have increased since then. However, the abundance of roving herbivorous fishes has declined simultaneously (Friedlander et al., 2003; Helyer & Samhouri, 2017), and exotic macroalgae have become dominant in the coastal ecosystems of Oahu (Allen, 1998; Russo, 1977), thus potentially increasing food availability for green turtles. Nothing is known about the extent of seagrass meadows in the past, to the best of our knowledge, but the former existence of some green turtles with stable isotope ratios consistent with seagrass-dominated diets can be explained only if more extensive seagrass meadows existed in the past (Campos & Cardona, 2020). In this scenario, the increasing homogenization and simplification of the coastal ecosystems in Oahu emerges as the leading reason for the current reduction of the isotopic niche of green turtles. If so, restoring the original diversity of trophic strategies in Hawaiian green turtles would require reverting the simplification of coastal ecosystems caused by macroalgal expansion and the decline of coral and, perhaps, seagrasses. Herbivorous fishes are major consumers of macroalgae, and the recovery of their populations is critical to restoring the structure of coral reef communities in the Hawaiian

Archipelago (Stimson et al., 2001). Increased competition with herbivorous fishes is expected to result in lower food availability for green turtles, which may trigger major changes in their foraging behaviour and diet (Velasquez-Vacca et al., 2023; Wabnitz et al., 2010). Hence, restoring the populations of herbivorous fishes can be considered a prerequisite to restoring the diversity of trophic strategies employed by Hawaiian green turtles, although such changes may adversely affect the body condition of green turtles. This is a reminder that restoring the population size of previously decimated species is often easier than restoring their ecological role in current, anthropogenically impacted ecosystems and that actions on the populations of competitors and prey should also be required to restore the ecological role of iconic species such as green turtles.

AUTHOR CONTRIBUTIONS

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST STATEMENT

None of the authors have conflicts of interest in this study. All activities performed to collect sea turtle samples were permitted by the National Oceanic and Atmospheric Administration (NOAA) and National Marine Fisheries Service (NMFS) permits 1581, 1581e01, 15685, 10027e05, 15661e01, 17022 and 21260; US Fish and Wildlife Service (USFWS) permits TE-70288A-3, 12533e12008 and 12533e13001; and State of Hawai'i permits 2013e32 and

2023-02. Sampling at the Kaloko-Honokōhau National Historical Park was conducted under special activity permit 2019-52 from the Department of Land and Natural Resources, Hawai'i, and permit KAHO-2018-SCI-00 for Scientific Research and Collecting Permit from the US National Park Service (NPS). All guidelines for sampling have been followed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Adriana Velasquez-Vacca https://orcid.org/0000-0002-4867-6371
Luis Cardona https://orcid.org/0000-0002-7892-1323

REFERENCES

Allen, J. (1998). Mangroves as alien species: the case of Hawaii. *Global Ecology and Biogeography Letters*, 7(1), 61–71. https://doi.org/10.1111/j.1466-8238.1998.00272.x

Anton, A., Geraldi, N.R., Ricciardi, A. & Dick, J.T. (2020). Global determinants of prey naiveté to exotic predators. *Proceedings of the Royal Society B*, 287(1928), 20192978. https://doi.org/10.1098/rspb.2019.2978

Araújo, M.S., Bolnick, D.I. & Layman, C.A. (2011). The ecological causes of individual specialisation. *Ecology Letters*, 14(9), 841–970. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Arthur, K.E. & Balazs, G.H. (2008). A comparison of immature green turtle (*Chelonia mydas*) diets among seven sites in the main Hawaiian Islands. *Pacific Science*, 62(2), 205–217. https://doi.org/10.2984/1534-6188(2008)62[205:ACOIGT]2.0.CO;2

Balazs, G.H., Van Houtan, K.S., Hargrove, S.A., Brunson, S.M. & Murakawa, S.K. (2015). A review of the demographic features of Hawaiian green turtles (*Chelonia mydas*). *Chelonian Conservation and Biology*, 14(2), 119–129. https://doi.org/10.2744/ccb-1172.1

Bas, M., Godino, I.V.i., Alvarez, M., Vales, D.G., Crespo, E.A. & Cardona, L. (2019). Back to the future? Late Holocene marine food web structure in a warm climatic phase as a predictor of trophodynamics in a warmer South-Western Atlantic Ocean. *Global Change Biology*, 25(2), 404–419. https://doi.org/10.1111/gcb.14523

Batschelet, E. (1981). *Circular statistics in biology*. New York: Academic Press.

Brenna, J.T., Corso, T.N., Tobias, H.J. & Caimi, R.J. (1997). High-precision continuous-flow isotope ratio mass spectrometry. *Mass Spectrometry Reviews*, 16(5), 227–258. https://doi.org/10.1002/(SICI)1098-2787(1997)16:5%3C227::AID-MAS1%3E3.0.CO;2-J

Campos, P. & Cardona, L. (2020). Trade-offs between nutritional quality and abundance determine diet selection in juvenile benthic green turtles. *Journal of Experimental Marine Biology and Ecology*, 527, 151373. https://doi.org/10.1016/j.jembe.2020.151373

Cardona, L., Campos, P. & Velásquez-Vacca, A. (2020). Contribution of green turtles *Chelonia mydas* to total herbivore biomass in shallow tropical reefs of oceanic islands. *PLoS ONE*, 15(1), e0228548. https://doi.org/10.1371/journal.pone.0228548

Casale, P., Broderick, A.C., Camiñas, J.A., Cardona, L., Carreras, C., Demetropoulos, A. et al. (2018). Mediterranean sea turtles: current knowledge and priorities for conservation and research. *Endangered Species Research*, 36, 229–267. https://doi.org/10.3354/esr00901

Clark, C., Cape, M., Shapley, M., Mueter, F., Finney, B., Misarti, N. et al. (2022). *Package 'SuessR'*. https://cran.r-project.org/web/packages/SuessR/SuessR.pdf

Clark, C.T., Cape, M.R., Shapley, M.D., Mueter, F.J., Finney, B.P. & Misarti, N. (2021). SuessR: regional corrections for the effects of anthropogenic CO_2 on $\delta^{13}C$ data from marine organisms. *Methods in Ecology and Evolution*, 12(8), 1508–1520. https://doi.org/10.1111/2041-210X.13622

Conklin, E.J. & Stimson, J. (2004). *An attempt to increase numbers of herbivorous fishes as a means of controlling populations of fleshy macroalgae on coral reefs in Kane'ohe Bay*. Hawai'i. *Pacific Science*, 58(2), 189–200. https://doi.org/10.1353/psc.2004.0015

Connolly, R.M., Guest, M.A., Melville, A.J. & Oakes, J.M. (2004). Sulfur stable isotopes separate producers in marine food-web analysis. *Oecologia*, 138(2), 161–167. https://doi.org/10.1007/s00442-003-1415-0

Conrad, C., Barceló, L.P., Seminoff, J.A., Tomaszewicz, C.T., Labonte, M., Kemp, B.M. et al. (2018). Ancient DNA analysis and stable isotope ecology of sea turtles (Cheloniidae) from the Gold Rush-era (1850s) eastern Pacific Ocean. *Open Quaternary*, 4(1), 3. https://doi.org/10.5334/oq.41

Drago, M., Cardona, L., Franco-Trecu, V., Crespo, E.A., Vales, D.G., Borella, F. et al. (2017). Isotopic niche partitioning between two apex predators over time. *Journal of Animal Ecology*, 86(4), 766–780. https://doi.org/10.1111/1365-2656.12666

Duarte, C.M., Agusti, S., Barbier, E., Britten, G.L., Castilla, J.C., Gattuso, J.-P. et al. (2020). Rebuilding marine life. *Nature*, 580(7801), 39–51. https://doi.org/10.1038/s41586-020-2146-7

Eide, M., Olsen, A., Ninnemann, U.S. & Johannessen, T. (2017). A global ocean climatology of preindustrial and modern ocean $\delta^{13}C$. *Global Biogeochemical Cycles*, 31(3). https://doi.org/10.1002/2016GB005473

Esteban, N., Mortimer, J.A., Stokes, H.J., Laloë, J.-O., Unsworth, R.K. & Hays, G.C. (2020). A global review of green turtle diet: sea surface temperature as a potential driver of omnivory levels. *Marine Biology*, 167(12), 183. https://doi.org/10.1007/s00227-020-03786-8

Friedlander, A., Brown, E., Jokiel, P., Smith, W. & Rodgers, K. (2003). Effects of habitat, wave exposure, and marine protected area status on coral reef fish assemblages in the Hawaiian archipelago. *Coral Reefs*, 22(3), 291–305. https://doi.org/10.1007/s00338-003-0317-2

Fry, B. (2006). *Stable isotope ecology*. New York, NY: Springer. https://doi.org/10.1007/0-387-33745-8

Fry, B., Silva, S.R., Kendall, C. & Anderson, R.K. (2002). Oxygen isotope corrections for online $\delta^{34}S$ analysis. *Rapid Communications in Mass Spectrometry*, 16(9), 854–858. https://doi.org/10.1002/rcm.651

Groombridge, B. & Luxmoore, R.A. (1989). *The green turtle and hawksbill (Reptilia: Cheloniidae): world status, exploitation and trade*. Cambridge, UK: IUCN Conservation Monitoring Centre.

Hanson, N.N., Wurster, C.M., Bird, M.I., Reid, K. & Boyd, I.L. (2009). Intrinsic and extrinsic forcing in life histories: patterns of growth and stable isotopes in male Antarctic fur seal teeth. *Marine Ecology Progress Series*, 388, 263–272. https://doi.org/10.3354/meps08158

Heithaus, M.R., Alcoverro, T., Arthur, R., Burkholder, D.A., Coates, K.A., Christianen, M.J. et al. (2014). Seagrasses in the age of sea turtle conservation and shark overfishing. *Frontiers in Marine Science*, 1, 28. https://doi.org/10.3389/fmars.2014.00028

Helyer, J. & Samhouri, J.F. (2017). Fishing and environmental influences on estimates of unfished herbivorous fish biomass across the Hawaiian Archipelago. *Marine Ecology Progress Series*, 575, 1–15. https://doi.org/10.3354/meps12235

Herbert, D.A. (1986). The growth dynamics of *Halophila hawaiiana*. *Aquatic Botany*, 23(4), 351–360. https://doi.org/10.1016/0304-3770(86)90083-5

Hobson, K.A. & Clark, R.G. (1992). Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. *The Condor*, 94(1), 189–197. https://doi.org/10.2307/1368808

Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER–Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, 80(3), 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Jouffray, J.-B., Nyström, M., Norström, A.V., Williams, I.D., Wedding, L.M., Kittinger, J.N. et al. (2015). Identifying multiple coral reef regimes and their drivers across the Hawaiian archipelago. *Philosophical Transactions of the Royal Society, B: Biological Sciences*, 370(1659), 20130268. https://doi.org/10.1098/rstb.2013.0268

Kittinger, J.N., Houtan, K.S.V., Mcclenachan, L.E. & Lawrence, A.L. (2013). Using historical data to assess the biogeography of population recovery. *Ecography*, 36(8), 868–872. https://doi.org/10.1111/j.1600-0587.2013.00245.x

Kondo, S., Morimoto, Y., Sato, T. & Suganuma, H. (2017). Factors affecting the long-term population dynamics of green turtles (*Chelonia mydas*) in Ogasawara, Japan: influence of natural and artificial production of hatchlings and harvest pressure. *Chelonian Conservation and Biology*, 16(1), 83–92. https://doi.org/10.2744/CCB-1222.1

Mccauley, D.J., Pinsky, M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H. & Warner, R.R. (2015). Marine defaunation: animal loss in the global ocean. *Science*, 347(6219), 1255641. https://doi.org/10.1126/science.1255641

Murray, J., Prouty, N.G., Peek, S. & Paytan, A. (2019). Coral skeleton $\delta^{15}N$ as a tracer of historic nutrient loading to a coral reef in Maui, Hawaii. *Scientific Reports*, 9(1). https://doi.org/10.1038/s41598-019-42013-3

Ólafsdóttir, G.Á., Edvardsson, R., Timsic, S., Harrison, R. & Patterson, W.P. (2021). A millennium of trophic stability in Atlantic cod (*Gadus morhua*): transition to a lower and converging trophic niche in modern times. *Scientific Reports*, 11(1), 12681. https://doi.org/10.1038/s41598-021-92243-7

Pagani-Núñez, E., Liang, D., He, C., Zhou, X., Luo, X., Liu, Y. et al. (2019). Niches in the Anthropocene: passerine assemblages show niche expansion from natural to urban habitats. *Ecography*, 42(8), 1353–1457. https://doi.org/10.1111/ecog.04203

Piacenza, S.E., Balazs, G.H., Hargrove, S.K., Richards, P.M. & Heppell, S.S. (2016). Trends and variability in demographic indicators of a recovering population of green sea turtles *Chelonia mydas*. *Endangered Species Research*, 31, 103–117. https://doi.org/10.3354/esr00753

Qi, H. & Coplen, T.B. (2003). Evaluation of the $^{34}S/^{32}S$ ratio of Soufre de Lacq elemental sulfur isotopic reference material by continuous flow isotope-ratio mass spectrometry. *Chemical Geology*, 199(1–2), 183–187. https://doi.org/10.1016/S0009-2541(03)00075-5

Quevedo, M., Svanbäck, R. & Eklöv, P. (2009). Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology*, 90(8), 2263–2274. https://doi.org/10.1890/07-1580.1

Reynaga, D.K.M., Munizzi, J.S., Mcmillan, R., Millaire, J.-F. & Longstaffe, F.J. (2023). Effects of consolidants and their removal by polar solvents on the stable isotope compositions of bone. *Quaternary International*, 660, 31–41. https://doi.org/10.1016/j.quaint.2022.12.004

Roemer, G.W., Donlan, C.J. & Courchamp, F. (2002). Golden eagles, feral pigs, and insular carnivores: how exotic species turn native predators into prey. *Proceedings of the National Academy of Sciences*, 99(2), 791–796. https://doi.org/10.1073/pnas.012422499

R-Team. (2021). *R: a language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org

Ruddiman, W.F. (2013). The Anthropocene. *Annual Review of Earth and Planetary Sciences*, 41, 45–68. https://doi.org/10.1146/annurev-earth-050212-123944

Russell, D.J. & Balazs, G.H. (2015). Increased use of non-native algae species in the diet of the green turtle (*Chelonia mydas*) in a primary pasture ecosystem in Hawaii. *Aquatic Ecosystem Health & Management*, 18(3). https://doi.org/10.1080/14634988.2015.1027140

Russell, D.J., Balazs, G.H., Phillips, R.C. & Kam, A.K. (2003). Discovery of the sea grass *Halophila decipiens* (Hydrocharitaceae) in the diet of the Hawaiian green turtle, *Chelonia mydas*. *Pacific Science*, 57(4), 393–397. https://doi.org/10.1353/psc.2003.0034

Russo, A.R. (1977). Water flow and the distribution and abundance of echinoids (genus *Echinometra*) on an Hawaiian reef. *Marine and Freshwater Research*, 28(6), 693–702. https://doi.org/10.1071/MF9770693

Santrock, J., Studley, S.A. & Hayes, J. (1985). Isotopic analyses based on the mass spectra of carbon dioxide. *Analytical Chemistry*, 57(7), 1444–1448. https://doi.org/10.1021/ac00284a060

Saporiti, F., Bearhop, S., Silva, L., Vales, D.G., Zenteno, L., Crespo, E.A. et al. (2014). Longer and less overlapping food webs in anthropogenically disturbed marine ecosystems: confirmations from the past. *PLoS ONE*, 9(7), e103132. https://doi.org/10.1371/journal.pone.0103132

Seminoff, J.A., Allen, C.D., Balazs, G.H., Dutton, P.H., Eguchi, T., Haas, H. et al. (2015). *Status review of the green turtle (Chelonia mydas) under the Endangered Species Act*. US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service. NOAA Technical Memorandum. Available at: https://repository.library.noaa.gov/view/noaa/4922 [Accessed March 2015].

Seminoff, J.A., Komoroske, L.M., Amorocho, D., Arauz, R., Chacón-Chaverrí, D., De Paz, N. et al. (2021). Large-scale patterns of green turtle trophic ecology in the eastern Pacific Ocean. *Ecosphere*, 12(6), e03479. https://doi.org/10.1002/ecs2.3479

Stimson, J., Larned, S. & Conklin, E. (2001). Effects of herbivory, nutrient levels, and introduced algae on the distribution and abundance of the invasive macroalga *Dictyosphaeria cavernosa* in Kāne'ohe Bay, Hawaii. *Coral Reefs*, 19(4), 343–357. https://doi.org/10.1007/s003380000123

Swan, G.J., Bearhop, S., Redpath, S.M., Silk, M.J., Goodwin, C.E., Inger, R. et al. (2020). Evaluating Bayesian stable isotope mixing models of wild animal diet and the effects of trophic discrimination factors and informative priors. *Methods in Ecology and Evolution*, 11(1), 139–149. https://doi.org/10.1111/2041-210X.13311

Swierts, T. & Vermeij, M.J. (2016). Competitive interactions between corals and turf algae depend on coral colony form. *PeerJ*, 4, e1984. https://doi.org/10.7717/peerj.1984

Tennekes, M. (2018). tmap: thematic maps in R. *Journal of Statistical Software*, 84, 1–39. https://doi.org/10.18637/jss.v084.i06

Turner Tomaszewicz, C.N., Seminoff, J.A., Price, M. & Kurle, C.M. (2017). Stable isotope discrimination factors and between-tissue isotope comparisons for bone and skin from captive and wild green sea turtles (*Chelonia mydas*). *Rapid Communications in Mass Spectrometry*, 31(22), 1903–1914. https://doi.org/10.1002/rcm.7974

Van Houtan, K.S. & Kittinger, J.N. (2014). Historical commercial exploitation and the current status of Hawaiian green turtles. *Biological Conservation*, 170, 20–27. https://doi.org/10.1016/j.biocon.2013.11.011

Vander Zanden, H.B., Arthur, K.E., Bolten, A.B., Popp, B.N., Lagueux, C.J., Harrison, E. et al. (2013). Trophic ecology of a green turtle breeding population. *Marine Ecology Progress Series*, 476, 237–249. https://doi.org/10.3354/meps10185

Velasquez-Vacca, A., Seminoff, J.A., Jones, T.T., Balazs, G.H. & Cardona, L. (2023). Isotopic ecology of Hawaiian green sea turtles (Chelonia mydas) and reliability of δ13C, δ15N, and δ34S analyses of unprocessed bone samples for dietary studies. *Marine Biology*, 170(7), 81. https://doi.org/10.1007/s00227-023-04232-1

Wabnitz, C.C., Balazs, G., Beavers, S., Bjorndal, K.A., Bolten, A.B., Christensen, V. et al. (2010). Ecosystem structure and processes at Kaloko Honokōhau, focusing on the role of herbivores, including the green sea turtle *Chelonia mydas*, in reef resilience. *Marine Ecology Progress Series*, 420, 27–44. https://doi.org/10.3354/meps08846

Zenteno, L., Borella, F., Otero, J.G., Piana, E., Belardi, J.B., Borrero, L.A. et al. (2015). Shifting niches of marine predators due to human exploitation: the diet of the South American sea lion (*Otaria flavescens*) since the late Holocene as a case study. *Paleobiology*, 41(3), 387–401. https://doi.org/10.1017/pab.2015.9

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.