UNIVERSITAT DE BARCELONA

Tracking historical changes in the trophic ecology of the green turtle *Chelonia mydas* in the Hawaiian Islands

Adriana Velasquez Vacca

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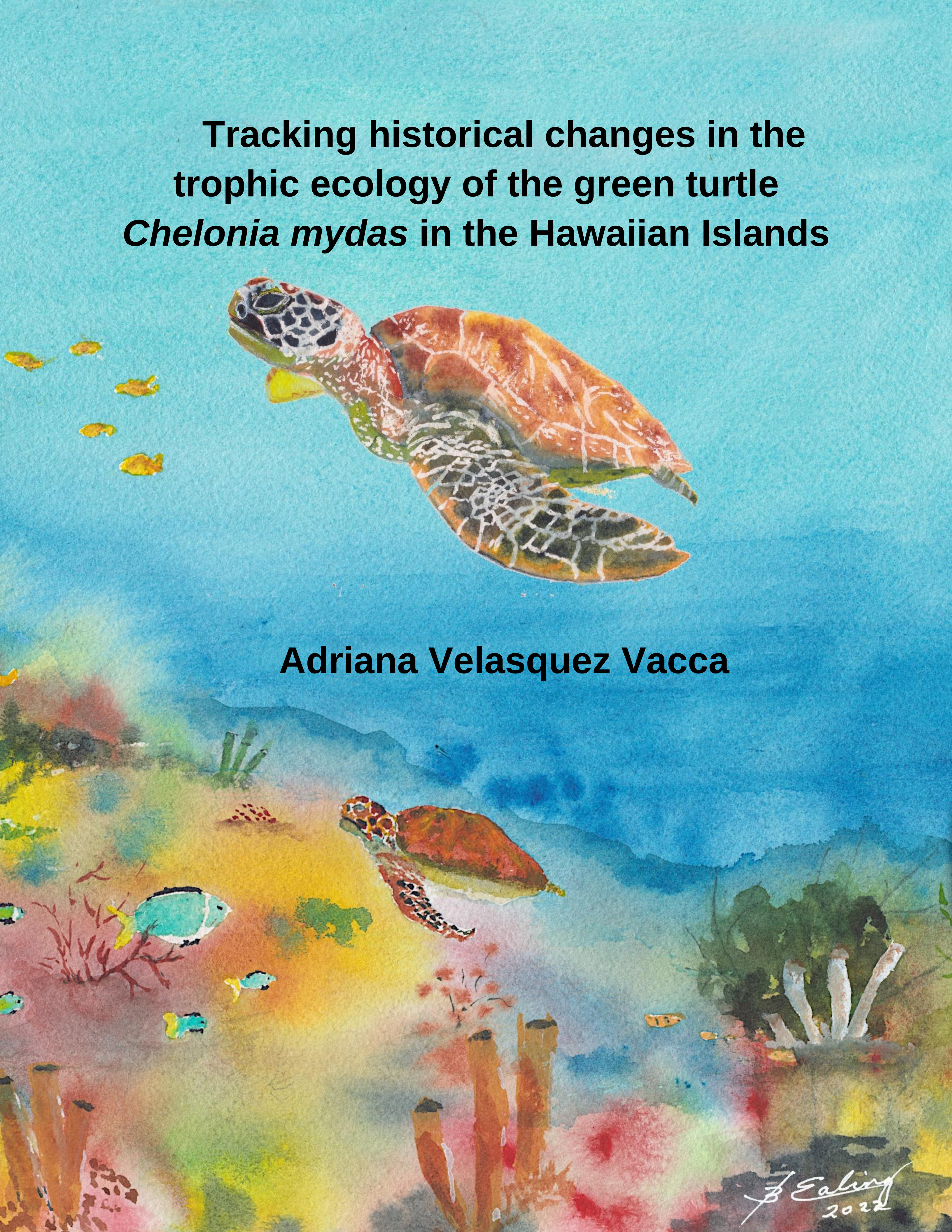

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UNIVERSITAT DE
BARCELONA

Departamento de Biología Evolutiva, Ecología y Ciencias ambientales

Programa de Doctorado en Biodiversidad (HDK04)

Reconstrucción de los cambios históricos en la ecología trófica de la tortuga verde (*Chelonia mydas*) en las islas Hawaii

Memoria presentada por Adriana Velasquez Vacca para optar al grado de doctora por la Universitat de Barcelona

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Certifica:

Que la presente memoria presentada para optar al título de Doctor, titulada "Tracking historical changes in the trophic Ecology of the green turtle *Chelonia mydas* in the Hawaiian Islands", ha sido realizada bajo mi dirección, y que todos los resultados presentados son fruto del trabajo experimental realizado por el mencionado doctorando.

Y para que así conste, expido y firmo el presente certificado.

Barcelona, 3 de diciembre de 2022

Lluis Cardona

Dr. Luis Cardona Pascual

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SUMMARY

Hawaiian green turtles *Chelonia mydas* were heavily exploited for their fat, meat and eggs by the Polynesians in the pre-contact era and continued to be commercially exploited until 1978 when they were officially protected. These conservation measures allowed its population to rebound, although it is still considered threatened by the Endangered Species Act. Hawaiian green turtles have been the focus of intense research, but surprisingly, little is known about its trophic ecology and how it might have changed due to the anthropogenic impacts in the coastal ecosystems of the archipelago. This thesis aims to better understand the current habitat use and diet of green turtles in the Hawaiian Islands and track possible historical changes in their ecological niche.

Underwater censuses in Oahu and the Kona coast revealed that green turtles had a strong preference for shallow, flat platforms covered with dense macroalgal pastures. Green turtle abundance was much lower in coral reefs, where they also had a modest contribution to the total biomass of herbivores, dominated by sea urchins and fishes. Not surprisingly, the stable isotope ratios of C, N and S in the epidermis of modern green turtles from east Oahu and the Kona coast confirmed a macroalgae-dominated diet, but seagrasses and mangroves had also relevant contributions to their diet in east Oahu, as well as fish in the Kona coast. Furthermore, the ontogenetic diet shift associated with the settlement of juvenile green turtles in neritic habitats is faster in eastern Oahu than in the Kona coast, perhaps because of the higher availability of macroalgae in the former.

The stable isotope ratios of C, N and S in the squamosal and the ribs of the same green turtle individuals revealed similar patterns of geographic and ontogenetic variability, hence confirming that unprocessed bone samples are informative of diet prior to death. This is the base for retrospective analysis using museum specimens. However, mixing models using the trophic discrimination factor (TDF) derived experimentally for cortical bone yielded unreliable results when used on unprocessed bone samples, suggesting that trabecular bone has a different TDF value. This is relevant, because the skulls and carapaces preserved at museums are made of skeletal elements with a thick core or trabecular bone.

Despite such limitation, the stable isotope ratios of C, N, and S in the skeletal elements of green turtles preserved in museums revealed minor changes in the isotopic niche of green turtles from east Oahu during the past 120 years. Nevertheless, the breadth of the isotopic niche decreased in the most recent years, indicating that ancient green turtles exhibited a broader diversity of individual foraging strategies, with a few individuals relying mostly on seagrasses and others consuming substantial amounts of animal matter. These trophic specialists are currently gone from eastern Oahu, where green turtles have converged on the use of the most abundant resource, red macroalgae, probably because of the homogenization and simplification of coastal habitats

Nevertheless, results confirm that macroalgae were the staple diet for the majority of the green turtle population before the introduction of exotic red macroalgae and hence it is a trait characteristic of the Hawaiian population. To understand the relationship between diet and the morphology of the skull and the mandible, geometric and traditional morphometrics were used, comparing skulls and mandibles of populations relying mostly on seagrasses and populations relying mostly on macroalgae. Results showed that macroalgae consumers have longer and narrower skulls than seagrass consumers, the former is more suitable for selective browsing and suction feeding and the latter is better adapted for grazing and stronger bite force. The skull morphology of Hawaiian green turtles fits that general pattern but is different from that of the green turtles inhabiting the Mexican Pacific, although both are genetically related.

Table of Contents

1. GENERAL INTRODUCTION…………………………………………………………...1

    Green turtle biology 2

    Overexploitation of the green turtle in Hawaii 13

    Reconstruction of past animal diets 16

2. OBJECTIVES……………………………………………………………………...19

3. CHAPTER 1: CONTRIBUTION OF GREEN TURTLES *Chelonia mydas* TO TOTAL HERBIVORE BIOMASS IN SHALLOW TROPICAL REEFS OF OCEANIC ISLANDS…………………………………………………………………………20

    Abstract 21

    Introduction.. 21

    Material and methods 23

    Study sites.. 23

    Underwater census.. 25

    Statistical analysis 26

    Results 27

    Discussion 29

    References 34

4. CHAPTER 2: RELATIONSHIP BETWEEN SKULL MORPHOLOGY AND DIET IN GREEN TURTLES…………………………………………………………………40

    Abstract 41

    Introduction.. 41

    Materials and methods 43

    Turtle sample collection... 43

    Statistical analysis 49

    Results 50

    Discussion 57

    References 59

5. CHAPTER 3: ISOTOPIC ECOLOGY OF HAWAIIAN GREEN TURTLES AND RELIABILITY OF STABLE ISOTOPES ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) IN BONE SAMPLES FOR DIETARY STUDIES……………………………………………………………63

Abstract 64

Introduction..64

Materials and methods 66

Turtle sample collection..66

Prey sample collection...68

Stable Isotope Analysis 69

Statistical analysis 71

Results 72

Discussion 82

References 87

6. CHAPTER 4: HISTORICAL CHANGES IN THE TROPHIC ECOLOGY OF HAWAIIAN GREEN TURTLES AS REVEALED BY STABLE ISOTOPE RATIOS (C, N, AND S) IN THE BONES OF MUSEUM SPECIMEN…………………………………...95

Abstract 96

Introduction..96

Materials and methods 99

Turtle sample collection..99

Prey sample collection...100

Stable Isotope Analysis 101

Statistical analysis 101

Results 102

Discussion 110

References 114

7. GENERAL DISCUSSION…………………………………………………………122

8. CONCLUSIONS………………………………………………………………………127

9. REFERENCES…………………………………………………………………………129

1. GENERAL INTRODUCTION

Green turtle biology

Sea turtles are classified in two taxonomic families, the Cheloniidae with six species, and the Dermochelyiidae with a single highly derived species, the leatherback turtle *Dermochelys coriacea*. The green turtle *Chelonia mydas,* known as honu by the Hawaiians, occurs in tropical and subtropical regions throughout the world's oceans (Seminoff et al. 2002a). It was first named by Linnaeus in (1758) as *Testudo mydas* and it was in 1812 when Schweigger first used the binomial *Chelonia mydas* that is used today (Schweigger 1812).

Among the seven species of sea turtles, the green turtle is the largest hard-shell species, and it grows to a maximum size of about 100 cm in curved carapace length (CCL) and a weight of 200 kg (Balazs 1980). It has a heart-shaped shell, small head, and single-clawed flippers (Seminoff et al. 2015). The carapace has five vertebral scutes, four pairs of costal scutes, and 12 pairs of marginal scutes. The head has a single pair of elongate prefrontal scales, four postorbital scales behind each eye, both of which are distinguishing characteristics that set it apart from other hard-shell sea turtles. They have a lower jaw edge that is coarsely serrated and strong grooves and ridges on the upper jaw that correspond with them (Carr 1952; Hirth 1997). This turtle has green subdermal fat (hence the name "green turtle") and externally it is light to dark brown, sometimes with olive and wavy markings of a darker color or with large blotches of dark brown (Carr 1952). This coloring could provide some camouflage while foraging on the bottom amongst corals, dark colored macroalgae and other substrate (Seminoff et al. 2015). The plastron is lighter and is usually yellowish. Hatchlings, on the other hand, are black dorsally and white ventrally (Seminoff et al. 2015). The Hawaiian green turtle population, as well as the Australian, has a well-developed "crop" in the esophagus that is not present in the Caribbean or eastern Pacific populations (Balazs 1980). In addition, juvenile green turtles in Hawaii have proportionally larger rear flippers than those in the western Caribbean (Balazs et al. 1998). These anatomical differences are believed to reflect adaptive variation to different environmental features in these regions (Balazs 1980).

The green turtle is circumglobally distributed and occurs throughout tropical, subtropical and temperate waters, even though is less common in the latter (Hirth 1997). In Hawaii, the most common sea turtle species is by far the Hawaiian green sea turtle, occurring in the foraging grounds of the 137 islands of the archipelago (Balazs and Chaloupka 2004b) and belonging to a distinct population characterized by six different mtDNA haplotypes reported to date in the nesting beaches at French Frigate Shoals (FFS) (Dutton et al. 2008). However, a few green turtles with haplotypes not found at FFS have been reported from foraging grounds in the archipelago, indicating that Hawaiian Islands might rarely be visited by green turtles from rookeries outside the Hawaiian Archipelago, both in the eastern and western Pacific.

The life cycle of Hawaiian green turtles is similar to that of green turtles elsewhere, although circumscribed to the Hawaiian archipelago. Each spring, in April and May, adults migrate to the Northwestern Hawaiian Islands to breed and nest (Figure **1**). This round-trip migration, beginning at foraging habitats in the main Hawaiian Islands, can extend over 1,200 miles. During the summer, in July and August, they return to nearshore environments in the main Hawaiian Islands to feed and bask (Rice and Balazs 2008).

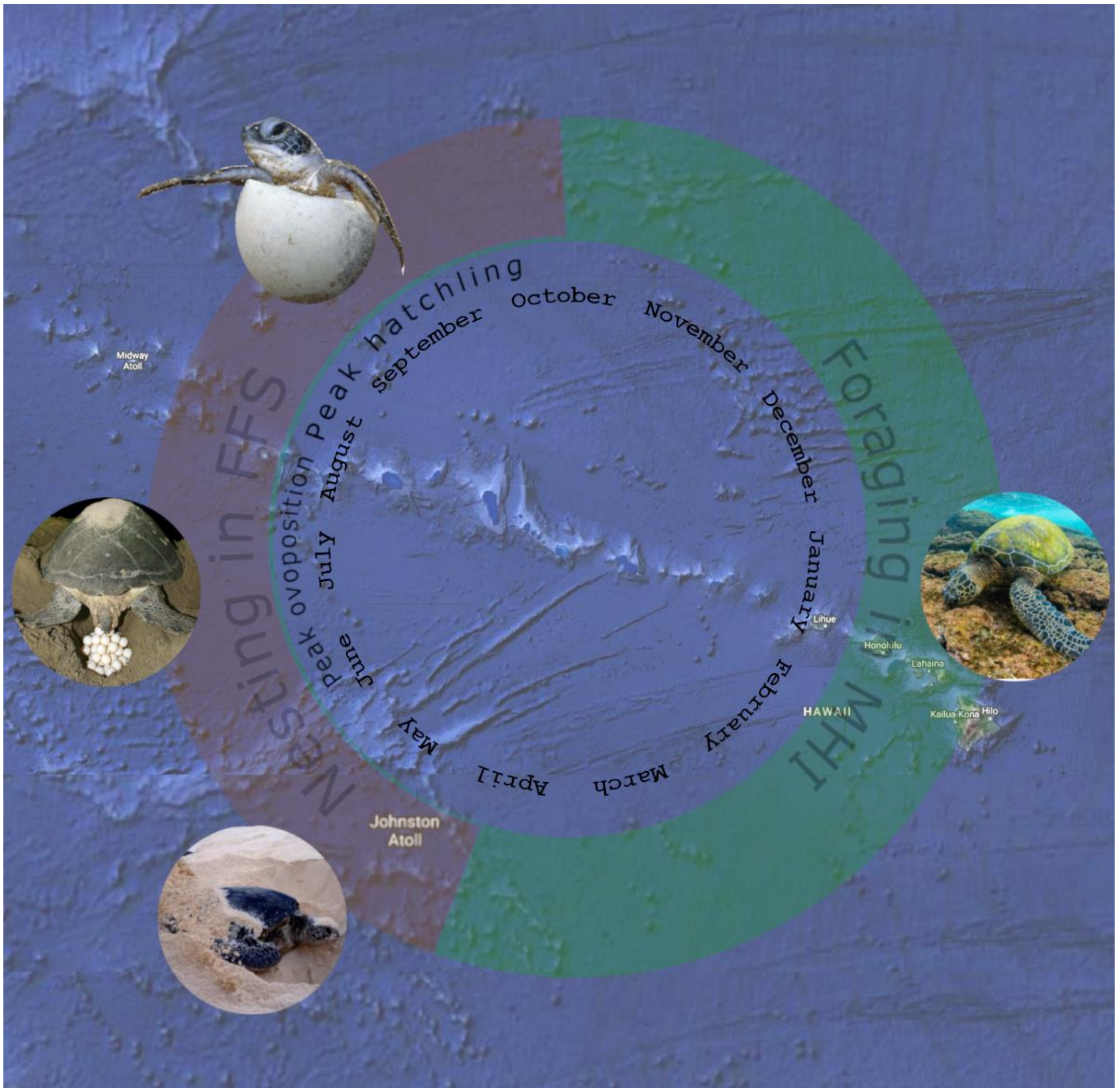

Figure 1 Life cycle of the Hawaiian green turtle *Chelonia mydas* superimposed on the map of the Hawaiian Archipelago, comprised of the inhabited main Hawaiian Islands (MHI) and the uninhabited reefs, banks, and atolls of the Northwestern Hawaiian Islands (NWHI). Images modified from original pictures taken by Andy Collins/NOAA Office of National Marine Sanctuaries, Sam Weber and M. Watson.

They nest on sandy, ocean-facing mainland and island beaches (Hirth 1997). In Hawaii, 90% of the nesting areas are located in the French Frigate Shoals (23.7489° N, 166.1461° W) in the northwestern Hawaiian Islands (Balazs 1976) (Figure *2*), where they find the high humidity, the type of sand that allows gas exchange and the ideal temperatures to hatch (Limpus et al. 2003). These human-uninhabited islands are the perfect place for them to nest because they have almost intact dune structures, native vegetation and no artificial lighting (Forsyth and Balazs 1989). Mean clutch size varies greatly among green turtle populations (Hirth 1997), and in Hawaii the mean is 104 eggs per clutch (Balazs et al. 2015).

Figure 2 Green turtle *Chelonia mydas* basking on a French Frigate Shoals beach. Photo by Marylou Staman, taken with permission under the USFWS TE-72088A-3 and PMNM Co-manager's permits.

Green turtle clutches (Figure 3 and Figure 4) incubate for variable periods of time (Mrosovsky and Yntema 1980), and in Hawaii is about 67 days (Balazs et al. 2015). Temperatures during the middle third of the incubation period determine the sex of hatchlings (Mrosovsky and Yntema 1980). Around 28 - 30 °C half of the nest would be females and the other half males. Temperatures near 32 °C would produce only females and near 26 °C the result would be only males. This is why global warming is also a concern in this regard (Eissa and Zaki 2011; Seminoff et al. 2015; Hays et al. 2022).

Figure 3 Green turtle *Chelonia mydas* nesting. Photo by Sam Weber.

Figure 4 Green turtle *Chelonia mydas* oviposition. Photo by M. Watson.

After incubation, the hatchlings pip and move upward and out of the nest over several days (Hendrickson 1958). They usually emerge at night and begin a frenzied walk towards the ocean (Figure 5), where they surf, swim (Figure 6) and are swept through the surf zone (Carr and Ogren 1960). It is believed that hatchlings use visual cues that orient them to the brightest horizon, which is over the ocean when there is no artificial lighting (Daniel and Smith 1947). This is also why uninhabited islands like the Northwestern Hawaiian Islands (NWHI) are ideal for them to nest, and in their absence, survival rates would be most likely lower in the highly populated MHI. After reaching the surf, the hatchlings use wave orientation in the nearshore and later, magnetic field orientation as they proceed further toward open water (Lohmann and Lohmann 1992), where they begin an oceanic juvenile phase.

Figure 5 Hatchlings of the green turtle *Chelonia mydas* walking towards the ocean in Hawaii immediately after hatching. Photo by Mark Sullivan.

Figure 6 Hatchling of the Hawaiian green turtle *Chelonia mydas* swimming in the French Frigate Shoals immediately after hatching. Photo by Koa Matsuoka/NOAA.

During this period, they are presumed to primarily inhabit areas where surface waters converge to form local downwelling, resulting in accumulations of floating material (Carr and Meylan 1980). During this stage, they feed on *Sargassum* sp. and its associated hydroids, bryozoans, polychaetes, gastropods, cnidarians, other pelagic invertebrates, fish eggs, and debris (Parker et al. 2011; Seminoff et al. 2015).

When they reach approximately 35 cm in curved carapace length (CCL) (Balazs 1980), they recruit to the neritic habitat, where they switch to a mostly herbivorous diet (Balazs 1980; Balazs et al. 1987; Russell and Balazs 2015; McCutcheon). During the earlier years of this neritic phase, green turtles are sometimes eating animals and moving periodically between the neritic and oceanic zone (Parker et al. 2011).

Several studies in Hawaii have shown that green turtles eat mostly red macroalgae during this phase (Figure 7), with seagrass and some terrestrial vegetation and to a lesser extent some animal items (Russell et al. 2003; McDermid et al. 2007; Russell and Balazs 2009).

Figure 7 Green turtle *Chelonia mydas* foraging in Oahu, Hawaii. Photo by Andy Collins/NOAA Office of National Marine Sanctuaries (2012).

Hawaiian green turtles have a slow growth rate (around 1 cm per year in juveniles), which is believed to be a consequence of their mostly herbivorous diet (Bjorndal 1985), limited food stock and cooler sea surface temperature compared to foraging grounds closer to the equator (Balazs and Chaloupka 2004a). They reach adulthood when around 92.2 cm straight carapace length (SCL) and the smallest nesting female documented was SCL 74.6 cm, and the largest was SCL 105.5 cm (Seminoff et al. 2015).

Their lifespan is unknown, but the maximum nesting lifespan that has been documented in Hawaii is 38 years, with most individuals nesting over a minimum of 25 - 35 years (Humburg and Balazs 2014). Considering that females travel to the nesting areas every 3 - 4 years (Seminoff et al. 2015), a female may nest around 12 seasons over their lifespan. On the other hand, adult males often migrate to breed on an annual basis (Balazs 1976).

Hawaiian green turtles are affected by several diseases, and in Hawaii, tumors associated with fibropapillomatosis are common and considered life threatening, because they can interfere with locomotion, vision, swallowing, and breathing (Jacobson 1990; Quackenbush et al. 1998; Work et al. 2001). Bacteraemia and immunosuppression have also been noted in Hawaiian green turtles affected by tumors (Work et al. 2001). It seems that the prevalence of fibropapillomatosis is correlated with the degradation of water quality, like water temperature changes, biotoxins, increased arginine in the turtles' natural dietary patterns, and excessive eutrophication (Dos Santos et al. 2010; Keller et al. 2014; Cárdenas et al. 2019).

Ecological role of the green turtle

Green turtles in the Hawaiian Archipelago are a geographically discrete population (Central North Pacific DPS), according to genetic data, range and movements (Seminoff et al. 2015). Once heavily exploited, the population has rebounded after forty decades of protection (Balazs and Chaloupka 2004b). Some research suggests that the increase in population has caused a decrease in their somatic growth rate for the past 40 years that has been linked to resource limitation, and not to genetic factors (Balazs and Chaloupka 2004a).

Early juvenile pelagic green turtles are mostly carnivorous with some omnivorous tendencies that forage within the first 100 m of the water column (Parker et al. 2011). After settlement in coastal habitats, they shift of a plant-dominated diet, although such ontogenic shift is not as strict as it was presumed in the past and omnivory is common (Arthur et al. 2008; Cardona et al. 2009; Cardona et al. 2010; Carman et al. 2012; Burgett et al. 2018). Furthermore, specimens as large as 70 cm CCL can be found in pelagic waters, suggesting that some turtles delay their recruitment to neritic habitats or move back and forth in both environments (Cardona et al. 2009; Parker et al. 2011).

The diet of Hawaiian adult green turtles is predominately based on macroalgae in anthropogenic disturbed sites such as Kāneʻohe Bay, Oahu (Arthur and Balazs 2008; Russell and Balazs 2015), where nitrogenous pollution has been implicated as a causal factor in phase shifts from coral-dominated to macroalgal-dominated ecosystems (Wabnitz et al. 2010). In more pristine places like the leeward side of the Island of Hawaii, where healthy populations of herbivorous fish keep macroalgae under control, they feed mostly on intertidal turf algae, instead (Wabnitz et al. 2010).

Green turtles are usually associated with seagrass meadows in the Greater Caribbean (Frazier 1971; Hearne et al. 2019; Christianen et al. 2021), Mediterranean (Cardona et al. 2010) and Indian Ocean (Stokes et al. 2019). In Hawaii, seagrasses are scarce and form only small and patchy meadows (McDermid et al. 2003), so most green turtles spend their adult lives in coastal foraging grounds associated to coral reef and coral rubble. Kāneʻohe bay, on windward Oahu, for example, is regularly visited by green turtles (Balazs et al. 2017).

Recent studies show that green turtle grazing is a major structuring force in the seagrass meadows of the Greater Caribbean and the Indian Ocean (Moran and Bjorndal 2005; Kelkar et al. 2013a). A reduction in the seagrass canopy can affect coastal protection (Christianen et al. 2013), irradiance levels for algae growth (Scott et al. 2020) and sediment retention (Moran and Bjorndal 2005), which may in turn affect the competitive advantages of certain seagrass or macroalgal species (Kelkar et al. 2013b). Furthermore, seagrass provide a habitat for other species, so their modification by green turtles can affect several species at the same time (Gartner et al. 2013). Overgrazing of seagrass meadows by green turtles can be explained partially by a decreased top-down control on green turtle populations due to global overfishing of large sharks (Heithaus et al. 2014).

The impact of green turtles on macroalgae is less known and sea urchin grazing is thought to be the major determinant of algal cover in Hawaiian coral reefs, with a relevant role for green turtles restricted only to intertidal rocky habitats (Wabnitz et al.

2010). Nevertheless, overgrazing by green turtles can occur in some areas (Bahr et al. 2018), which is not always detrimental, as it can control invasive species. For example, Bahr et al. (2018) found that the invasive red algae *Gracilaria salicornia* was eaten by green turtles in a short period of time in a marine reserve of Kāneʻohe Bay, Oahu, showing that the turtle was the primary driver of the rapid decline. This could help keep the reef healthy, preventing overgrowth of macroalgae that can ultimately kill the reef by smothering, shading, and abrasion (Vermeij et al. 2010). Understanding these foraging dynamics is important because the functional role of specific herbivore species can help maintain the balance in the reef. For example, smaller herbivorous fish preferentially consume the apices of *G. salicornia* mats before utilizing the thicker portions (Bierwagen et al. 2017), whereas the green turtle consumes the entire mat (Bahr et al. 2018), which can alter the successional trajectory of the coral reef community (Hixon and Brostoff 1996).

The knowledge about the ability of sea turtles to affect their ecosystem structure and function has been considered one of the most important objectives to be met in the study of the ecology of these species (Bjorndal et al. 2000), as well as understanding their home range, to be able to stablish critical habitats for their conservation.

Understanding their feeding ecology is integral to their conservation too, because diet quality and quantity not only influences growth, but also the variability in nesting numbers (Broderick et al. 2001) and is considered determinant in reproductive output and population survival (Arthur and Balazs 2008).

Overexploitation of the green turtle in Hawaii

The Hawaiian green turtle is listed as “threatened” under the Endangered Species Act (ESA) for the Central North Pacific distinct population segment (DPS) (Seminoff et al. 2015). Threatened is defined by the ESA as “any species which is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range.” The Hawaiian green turtle was listed as endangered due to past overexploitation of eggs and turtles (Van Houtan and Kittinger 2014), incidental mortality related to fisheries (Seminoff et al. 2015) and degradation of foraging and nesting habitats (Balazs and Chaloupka 2004b). Not only were they hunted for their meat, but the scutes and bones were used to create decorative ornaments, fishhooks, tattoo needles, and a variety of other implements (Balazs 1983).

The green turtle appears in tales and legends of Hawaii, and are considered sacred animals that ‘Ai’ai, the son of the God of fishermen Ku’ula, created when he overturned a rock on the sand (Thrum 1923). For the Hawaiians they embody good luck, protection, endurance and long life. They have also been used historically as ceremonial food and iconized in symbolism in other Pacific Islands (Rudrud et al. 2007) and traditional laws demonstrate cultural valuation of them above other food items (Luna 2003). These practices are consistent with modern conservation strategies, but that did not prevent the overexploitation of this species that almost brings it to extinction (Rudrud 2010). As a result of overexploitation, historical nesting areas distributed across all the archipelago vanished and now more than 90% of green turtle nesting occurs at the French Frigate Shoals, which is vulnerable to this mentioned sea level rise.

Three periods of sea turtle exploitation in Hawaii have been described (Kittinger et al. 2011; Kittinger et al. 2013; Van Houtan and Kittinger 2014): from 1250 to 1778 by indigenous Polynesian societies, from 1779 to 1945, between European contact and World War II, and between 1946 and 1974, when federal and state protection began. These periods affected different segments of the turtle’s population across its geographic range (Figure 8).

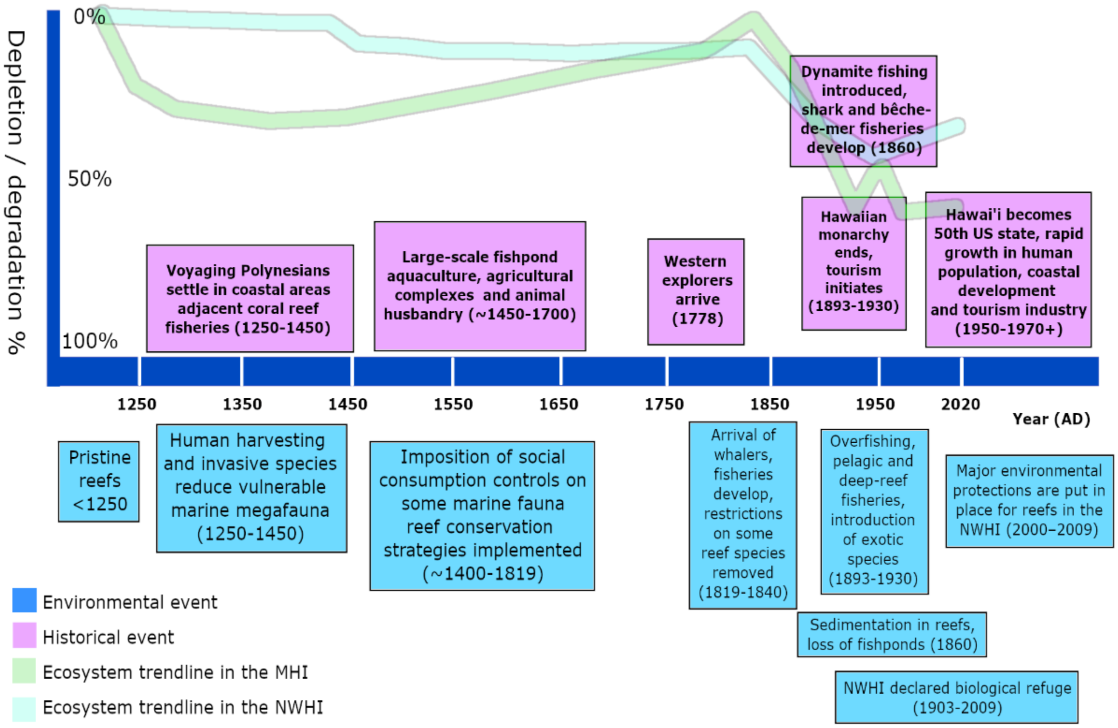

Figure 8 Major historical and environmental factors affecting coral reef ecosystems in the main Hawaiian Islands (MHI) and northwestern Hawaiian Islands (NWHI). Trendlines represent averages of changes in the relative abundance of seven trophic guilds (large carnivores, large herbivores, small carnivores, small herbivores) and sedentary species (corals, seagrasses, algae, suspension feeders and detritivores). Modified from Kittinger et al. (2011).

During the first period and according to archeological data, exploitation was widespread and the hunting pressure by the Polynesians destroyed important nesting areas in the main Hawaiian Islands (Kittinger et al. 2013).

During the second period, in the 1800s, the Hawaiian Islands were visited by Europeans, North Americans, and Asians who traveled on ships and visited the uninhabited Northwestern Hawaiian Islands (NWHI), making turtle harvests for subsistence and commercial trade (Kittinger et al. 2011). According to Van Houtan (2014), a hundred years later, in 1900, the green turtle was ubiquitous in Honolulu markets and restaurants, and by 1950 nesting areas were almost completely destroyed everywhere except a single remote atoll in the NWHI.

During the third period, there is more data available because the territory and state of Hawaii licensed a commercial turtle fishery and kept records of its operations, and it is estimated that 2.431 turtles were harvested during this period, with a peak production of 11.6 tons in 1973 (Van Houtan and Kittinger 2014). The harvest was dominated by juvenile turtles (Van Houtan and Bass 2007) and the business was centered around the major markets in Honolulu (Oahu), Kahului (Maui), and Hilo (Hawaii Island).

The end of the exploitation of turtles began in 1974, when turtles' harvest was prohibited, even though it took a few years to see a real increment in the abundance of nesting green turtles at the population's major rookery, in the NWHI (Balazs and Chaloupka 2006). Even though exploitation was high enough to bring the species close to extinction in the area, the revenue was not very high. Adjusted for inflation to 2022, the cumulative revenue over the 27-year span is $486.941 (around $18.000 annually), according to Van Houtan et al. (2014). Following protection, the population has rebuilt but is probably still below carrying capacity in most of the archipelago (Piacenza et al. 2016), which arises the question about how similar the current diet and trophic ecology to the original ones are.

Reconstruction of past animal diets

Understanding how the diet of the green turtle has changed over time and how humans have modified their habitat provides a baseline for contemporary conservation measures.

The composition of green turtle diet can be explored with several techniques (Jones and Seminoff 2013): direct observation using snorkel or SCUBA (Reisser et al. 2013), gut contents analysis from dead turtles (Mortimer 1981), esophageal lavage, fecal examination (Seminoff et al. 2002b), stable isotope analysis (Pearson et al. 2017), remote videography (Letessier et al. 2015), animal-borne cameras (Heithaus et al. 2002) and autonomous underwater vehicles (Dodge et al. 2018). Among these, only stable isotope analysis is useful to reconstruct historic changes in the diet of organism the analysis of the stable isotope ratios in the bones of specimens preserved in museum collections or recovered from the zooarchaeological record have been widely used (Schmidt et al. 2009; Saporiti et al. 2014; Van Rijssel et al. 2017; Conrad et al. 2018).

The stable isotopes of carbon and nitrogen are the most commonly used in ecological studies and the relative abundance of the heavy and the light isotope is reported using the delta notation as $\delta^{13}C$ and $\delta^{15}N$. Because different photosynthetic pathways and inorganic carbon acquisition strategies among other factors result in variable $\delta^{13}C$ values among different primary producers, these values can be used to trace the importance of different carbon sources to a consumer (DeNiro and Epstein 1978) and in Hawaii, it’s possible to classify them into at least three different categories: mangroves (lowest), algae (intermediate), and sea grasses (highest) (Marshall et al. 2007). When analyzing carbon stable isotope, it has to be considered that during the last three centuries, the content of ^{13}C-depleted in atmospheric CO_2 has been increasing rapidly due largely to burning of fossil fuel. This is called the Suess effect, and values in tissue have to be corrected to a specific year in order to be able to compare different periods.

On the other hand, consumer tissues have higher $\delta^{15}N$ values relative to their prey due to preferential retention of ^{15}N during metabolism and tissue maintenance, among other factors (DeNiro and Epstein 1981). As a result, there is predictable, stepwise ^{15}N enrichment with each trophic step, and thus, $\delta^{15}N$ values can be used to estimate an organism’s trophic position (Farrell et al. 1995; Post 2002; Newsome et al. 2007).

Another chemical element whose stable isotopes are becoming popular ecological tracers is sulfur. The $\delta\ ^{34}S$ values can vary between organisms mainly because they have different sources of inorganic sulfur, like sea-salt sulfate, sulfate ions from precipitation or sulfide from anoxic sediments (McCutchan Jr et al. 2003).

When the stable isotope ratios of two or more chemical elements are plotted, they represent the isotopic niche within the isospace, that integrates the stable isotope ratios in the diet sources of the consumer (Bearhop et al. 2004; Newsome et al. 2007). This isotopic niche is influenced by intrinsic differences in individual diet and habitat use, as well as extrinsic factors such as habitat diversity and local nutrient cycling regimes (Seminoff et al. 2021). It is useful to understand diet changes in response to habitat modification, shrinking or fragmentation (Layman et al. 2007; Resasco et al. 2018; Pagani-Núñez et al. 2019) and a decrease in trophic niche size is often interpreted as a trophic diversity loss.

2. OBJECTIVES

The main objective of this thesis is understanding the impact of human exploitation on the trophic ecology of Hawaiian green turtles. To do so, I aimed

1. To assess the current contribution of green turtles to the biomass of herbivores in the coastal habitats of the Hawaiian archipelago.
2. To characterize the isotopic niche of Hawaiian green turtles using epidermis samples.
3. To assess the suitability of stable isotope ratios in unprocessed samples of bone tissue to capture dietary information in green turtles.
4. To test the hypothesis that the isotopic niche of Hawaiian green turtles changed during the 20th and 21st centuries as a result of ecological and demographic changes.
5. To test the hypothesis that green turtle populations relying mostly on seagrasses differ in their skull morphology from populations relying mostly on seagrasses.

3. CHAPTER 1: CONTRIBUTION OF GREEN TURTLES *Chelonia mydas* TO TOTAL HERBIVORE BIOMASS IN SHALLOW TROPICAL REEFS OF OCEANIC ISLANDS

Abstract

Green turtles are megaherbivores with a key role in the dynamics of tropical seagrass meadows, but little is known about their relevance as herbivores in tropical reef habitats. We conducted underwater censuses of green turtles, herbivorous fishes and sea urchins in two distinct tropical regions: Fernando de Noronha (Western Atlantic Ocean) and the Hawaiian Archipelago (Central Pacific Ocean), to assess the contribution of green turtles to the total herbivore biomass in reef habitats of tropical oceanic islands. Juvenile green turtles ranging 40-60 cm were observed at most of the surveyed sites, and hence, could be considered typical components of the shallow reef fauna of tropical oceanic islands. Furthermore, they were usually one of the most abundant species of roving herbivores in many of the sites surveyed. However, the biomass of green turtles was usually much lower than the aggregated biomass of fishes or sea urchins, which usually constituted most of the total herbivore biomass. Green turtles made a major contribution to the total herbivore biomass only in sheltered sites with low rugosity, low coral cover and high algal cover. Further investigation on the trophic redundancy of the herbivore community is required to assess the actual relevance of green turtles in reef ecosystems of oceanic islands, compared to herbivorous fishes and sea urchins, because different herbivores may target different algal resources and complementarity may be needed to maintain ecosystem functioning across large, naturally varied reefscapes.

Introduction

Herbivory is a critical process in shallow marine ecosystems worldwide and changes in herbivore biomass may have profound effects on ecosystem structure (Steneck et al. 2002; Vinueza et al. 2006; Kelkar et al. 2013a). Tropical reefs are not an exception and the foraging activity of sea urchins, fishes and, to a lesser extent, crabs, create open spaces allowing the settlement of coral colonies (Fox and Bellwood 2007; Mumby et al. 2007; Mumby and Steneck 2008; Francis et al. 2019; Lefcheck et al. 2019). Although the actual abundance of macroalgae (Vroom and Braun 2010) and the exact relevance of individual herbivore species in healthy tropical reefs is debated (Choat et al. 2004; Mantyka and Bellwood 2007; Mumby et al. 2007; Bruno et al. 2019), the existence of an abundant and diverse assemblage of herbivores is thought to increase coral reef resilience and create a buffer for natural and human induced disturbances (Burkepile and Hay 2008; Mumby and Steneck 2008; Lefcheck et al. 2019).

Green turtles *Chelonia mydas* are megaherbivores occurring in tropical regions worldwide (Wallace et al. 2011). Some populations were decimated historically due to a combination of overharvesting, bycatch, loss or alteration of nesting habitat, degradation and loss of foraging habitat, and entanglement in or ingestion of marine debris (Seminoff et al. 2015). Nevertheless, most populations are currently increasing thanks to conservation actions implemented during the past decades (Chaloupka et al. 2008). Recovery has been particularly successful in the Western South Atlantic and the Hawaiian Archipelago (Kittinger et al. 2013; Silva et al. 2017), where subpopulations have recently been classified as not threatened by the International Union for Conservation of Nature (Broderick and Patricio 2019; Chaloupka and Pilcher 2019).

Recent evidence shows that green turtle grazing is a major structuring force in seagrass meadows once populations are rebuilt (Moran and Bjorndal 2005; Burkholder et al. 2013; Kelkar et al. 2013a) and the same could be true in other habitats. Tropical reefs are the main habitat of green turtles in the Western South Atlantic and most of the tropical Pacific (Goatley et al. 2012; Santos et al. 2015; Balazs et al. 2017; Becker et al. 2019), where extended sea grass meadows are scarce (Short and Frederick 2003). As a result, macroalgae and turf algae, but not seagrasses, represent the unprocessed of green turtle diet in those regions (Russell and Balazs 2009; Santos et al. 2015).

On these grounds, Goatley and coworkers (Goatley et al. 2012) hypothesized a relevant role for green turtles in the dynamics of algal communities in the Great Barrier Reef if the populations reached pre-exploitation levels. Conversely, ecosystem modeling suggests that sea urchin grazing is the major determinant of algal cover in Hawaiian reefs, with a relevant role for green turtles only in intertidal rocky habitats (Wabnitz et al. 2010). Nevertheless, there is a paucity of data about the abundance of green turtles in tropical reef habitats and little is known about their contribution to the total biomass of herbivores. This paper aims to assess the potential contribution of green turtles to the total herbivore biomass of shallow tropical reefs of oceanic islands in the Western South Atlantic and the Central Pacific Ocean, by means of underwater censuses of green turtles, herbivorous fishes and sea urchins.

Material and methods

Study sites

Underwater surveys were conducted by snorkeling in September 2017 at seven sites in Fernando de Noronha (Western South Atlantic Ocean) and September 2018 at eight sites in the islands of Hawaii and Oahu (Hawaiian Archipelago, Central Pacific Ocean) as depicted in Figure 1.

Surveys coincided with the end of the dry season in both areas and were conducted always at high tide. Each site was visited at least twice. The starting point and bearing of each transect (see below) were selected during the first visit to allow evenly spaced transects within the area. The censuses were conducted during the second visit, although sometimes a third visit was required to complete them. The coordinates and characteristics of these sites are detailed in Table 1 and the methods used to assess habitat descriptors are detailed below.

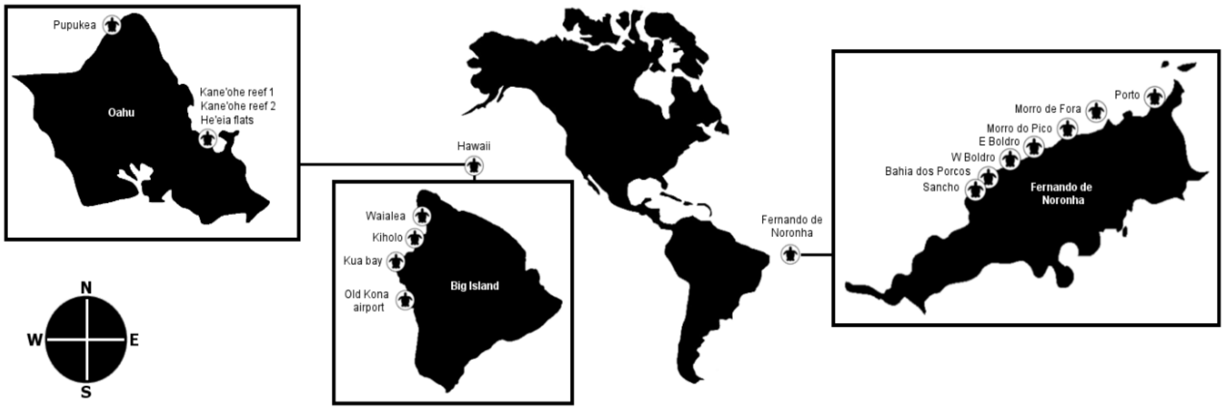

Figure 1 Location of sampling sites in Fernando de Noronha and Hawaii. Map not to scale. Figure is similar but not identical to the original image from Earth Resources Observatory and Science Center and is for illustrative purposes only.

Table 1. Major characteristics of sampling sites in the Western South Atlantic Ocean (Fernando de Noronha) and the Central Pacific Ocean (Hawaiian Archipelago) according to the data collected during the present study. See text for details on methods. Data reported as mean ± standard deviation. Habitat type: vermetid reef (V), rocky reef (R), rocky reef with scattered coral (RC); coral reef (C), coral rubble (CR).

MPA (protection from fishing): spearing and set nets forbidden (Y), spear fishing and set nets allowed (N)

Site	Location coordinates (lat./long.)	Habitat	MPA	Depth (m)	Rugosity index	Live coral (% cover)	Turf (% cover)	Macroalgae (% cover)
Fernando de Noronha								
Porto	-3.835/32.402	V	Y	1.9 ± 0.5	1.4 ± 0.3	0.0 ± 0.0	19.2 ± 3.1	5.1 ± 2.9
Morro de Fora	-3.838/-2.416	RC	Y	1.8 ± 0.7	2.4 ± 0.5	1.0 ± 0.1	19.2 ± 3.5	4.0 ± 2.0
Morro do Pico	-3.842/-2.422	V	Y	1.9 ± 0.5	1.3 ± 0.3	0.0 ± 0.0	76.3 ± 1.9	5.9 ± 1.9
E. Boldro	-3.844/-2.426	R	Y	1.8 ± 0.7	2.3 ± 0.3	0.0 ± 0.0	44.9 ± 4.4	14.5 ± 5.5
W. Boldro	-3.846/-2.429	V	Y	1.8 ± 0.3	1.4 ± 0.3	0.0 ± 0.0	22.3 ± 1.8	2.7 ± 8.9
Baia dos Porcos	-3.851/-2.442	RC	Y	1.8 ± 0.6	2.1 ± 0.5	0.2 ± 0.5	24.3 ± 0.7	0.5 ± 0.4
Sancho	-3.854/-2.444	RC	Y	2.0 ± 0.6	2.4 ± 0.5	3.2 ± 1.8	18.2 ± 3.6	3.2 ± 1.7
Hawaiian Archipelago/island of Hawaii-Kona coast								
Old Kona Airport	19.641/-56.008	C	Y	5.0 ± 0.8	3.0 ± 0.2	22.4 ± 16.8	8.0 ± 2.8	0.0 ± 0.0
Kua bay	19.810/-56.007	RC	N	3.0 ± 0.4	1.5 ± 0.5	6.0 ± 1.2	13.6 ± 14.4	0.0 ± 0.0
Kīholo	19.852/-155.932	RC	N	2.5 ± 0.1	1.0 ± 0.1	31.6 ± 2.8	4.4 ± 5.2	0.0 ± 0.0
Waialea	19.981/-155.829	RC	Y	3.0 ± 0.7	2.0 ± 0.5	1.6 ± 1.6	26.8 ± 23.6	0.0 ± 0.0
Hawaiian Archipelago/island of Oahu								
Pūpūkea	21.656/-158.062	RC	Y	2.0 ± 0.6	2.5± 0.5	13.2 ± 1.2	36.4 ± 30.0	0.0 ± 0.0
He’eia flats	21.442/-157.808	CR	N	1.0 ± 0.0	1.0 ± 0.0	0.0 ± 0.0	48.0 ± 10.5	6.2 ± 4.3
Kāne‘ohe reef 1	21.459/-157.798	C	N	2.5 ± 0.2	3.0 ± 0.2	93.6 ± 3.6	0.0 ± 0.0	6.5 ± 4.5
Kāne‘ohe reef 2	21.462/-157.798	C	N	2.0 ± 0.3	3.0 ± 0.1	96.4 ± 2.0	0.0 ± 0.0	8.4 ± 4.8

Underwater census

Green turtles, herbivorous fishes and sea urchins use habitat at different scales and hence the biomass of each group should be assessed at different scales using different methods (Friedlander et al. 2003; Friedlander et al. 2005; Williams et al. 2008; Jouffray et al. 2015).

Herbivorous fishes were censused visually using four independent and non-overlapping transects of 50 m x 5 m (Friedlander et al. 2003; Friedlander et al. 2005; Williams et al. 2008; Jouffray et al. 2015) parallel to the shore and positioned randomly. Fish were counted on site and only the following truly herbivorous roving fish species were censused: *Kyphosus sectatrix*, *Sparisoma amplum, Sparisoma axillare*, *Sparisoma* *frondosum* and *Sparisoma radians* at Fernando de Noronha (Bonaldo et al. 2006) and *Acanthurus achilles, Acanthurus blochii, Acanthurus guttatus, Acanthurus leucopareius, Acanthurus nigricans, Acanthurus nigroris, Acanthurus triostegus, Calotomus carolinus, Calotomus zonarchus, Kyphosus spp., Naso unicornis, Naso lituratus, Scarus dubius, Scarus perspecillatus, Scarus psittacus, Scarus rubroviolaceus, Scarus sordidus*, *Zebrasoma flavescens* and *Zebrasoma veliferum* at the Hawaiian Archipelago (Jones 1968; Choat et al. 2002; Choat et al. 2004; Crossman et al. 2005). Other species of Acanthuridae rely primarily on detritus or zooplankton. Territorial herbivores (damselfish and blennies) were not considered.

Each fish in the transect was identified to the species level, included in a 5 cm length class, and counted. Fish size was then converted to fish biomass using the equation weight = a x length^b with a and b values for that species from FishBase When the total length (TL) was not available, we converted the fork length (FL) to TL. If the weight-length equation was not available for the species, the genus equation was used.

Once the fish were counted, depth was recorded at 0, 10, 20, 30, 40, and 50 m from the starting point of the transect, to calculate the average. Habitat rugosity was assessed using a relative scale ranging from 1 (flat sea bead) to 4 (seabed with large rocks or coral heads). The coverage (%) of erect algae, turf-forming alga and live coral and the abundance of sea urchins were measured along the fish transects (roughly at 10, 20, 30, 40, and 50 m from the starting point) using 0.5 x 0.5 m PVC quadrants (25 quadrants per transect). Quadrants were positioned randomly at flat areas contiguous to the 50 m belt delimiting the central part of the transect. All sea urchins found inside each quadrant were measured with plastic calipers (horizontal test diameter without spines) and counted. The horizontal test diameter was converted to biomass following McClanahan (McClanahan 1988). The coverage (%) of erect algae, turf-forming alga and live coral with each quadrant was estimated using the internal 25-cell grid of the quadrant (Friedlander et al. 2003; Friedlander et al. 2005; Williams et al. 2008; Jouffray et al. 2015).

Finally, the abundance of green turtles was assessed in four 100 m x 10 m transects parallel to the shore (Roos et al. 2005; Ballorain et al. 2010; Gitirana and Souza 2012; Becker et al. 2019). Turtle transects overlapped with those used for fish censuses. Each turtle was counted and was included in a 10 cm length class and its behavior (foraging, resting, swimming) was noted. Carapace length was later converted to biomass using the following equation: $W = -35.823 + 0.966CCL$, where W is weight in kg and *CCL* is length in cm (R^2= 0.887, p<0.001). This equation has been calculated previously by two of the authors (LC and PC) for juvenile green turtles at the Tamar field station in Ubatuba (Brazil).

Statistical analysis

The coverage of erect algae, turf-forming alga and live coral and the sea urchin biomass at each site is reported as the average of a hundred quadrants. Fish biomass and green turtle biomass are reported as the average of four transects at each site. The average biomass of sea urchins, herbivorous fishes and green turtles at each site was expressed as tons per square kilometer (tons/km^2) to allow comparison (Wabnitz et al. 2010).

Normality was checked with the Lilliefors test and data were transformed as $\log_{10}(x + 1)$ or the $\sin^{-1}(x)$ when necessary. MATLAB Simulink Student Suite R2019a was used to analyze the correlations between green turtle, sea urchins and fish biomasses with the environmental descriptors measured at each location (depth, rugosity, coral, algae and turf coverage) with the Pearson correlation test. Simple or multiple linear regressions were plotted using a robust bisquare fit to characterize these correlations and find the best model to predict herbivore biomass with those environmental descriptors. Model selection was based on corrected r^2 and p values ($\alpha<0.05$). Student's t-test were used for pairwise comparisons.

Results

The survey was conducted at sites less than 5 m deep and with a habitat rugosity less than 3, both in the Fernando de Noronha and the Hawaiian Archipelago (Table 1). Depth and rugosity were uncorrelated (p=0.208, n=15). Live coral was scarce and covered less than 3.6% of the seafloor in Fernando de Noronha sites. Hawaiian sites were more variable and live coral cover ranged 0-96%. Live coral cover was positively correlated with depth (r=0.748, p=0.001, n=15) and habitat rugosity (r=593, p=0.020, n=15). Turf cover was generally high at Fernando de Noronha (18-76%) and less than 48% at the Hawaiian Archipelago) (Table 1). Macroalgae cover was low in both areas: 0.5-15% at Fernando de Noronha and ≤8% at the Hawaii Archipelago (Table 1).

The most common erect macroalgae species in the quadrants at Fernando de Noronha were *Caulerpa racemosa*, *Dictyopteris plagiogramma* and *Sargassum* spp. *Dictiosphaeria cavernosa* was the only macroalgae observed in the quadrants at the Hawaiian Archipelago, although other species were observed outside the quadrants. Turf and macroalgae cover were pooled for later analysis. Total algae cover was uncorrelated with habitat rugosity (p=0.181, n=15) and negatively correlated with depth (r=-0.595, p=0.019, n=15) and live coral cover (r=-0.817, p<0.001, n=15).

Sea urchins were virtually absent from Fernando de Noronha, with only two specimens of two different species (*Diadema antillarum* and *Tripnestes ventricosus*) observed, none of them inside any sampling quadrant. Sea urchins, mainly *Echinometra mathaei*, occurred at most sites in the Hawaiian Archipelago, at an average density of 3.7 urchins/m^2. Nevertheless, sea urchins were absent from the two coral heads and He'eia flats in Kāne‘ohe Bay. Sea urchin biomass ranged 0-283.44 tons/km^2 at the Hawaiian Archipelago (Figure 2) and the best predictor was the model derived from a multiple linear regression including depth and rugosity (R^2=0.608, p=0.003).

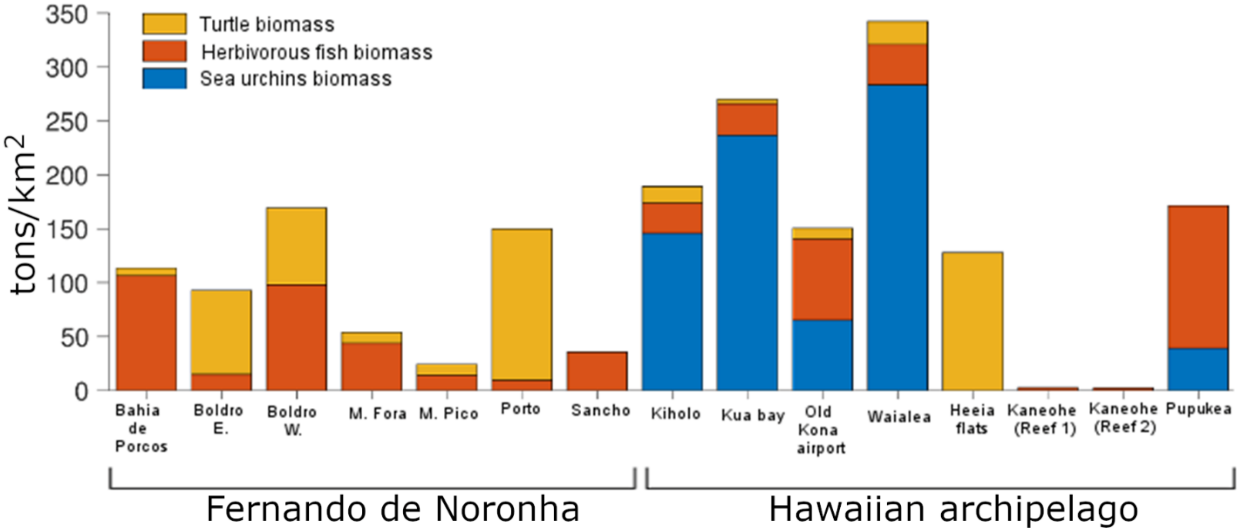

Figure 2 Biomass of herbivores (green turtles, sea urchins and fishes) at reef habitats in Fernando de Noronha and Hawaii.

Herbivorous fishes were found everywhere, except at the He'eia flats (Oahu). They were present in very low numbers in the two coral heads in Kāne‘ohe. Biomass ranged 13.9-106.6 tons/km^2 at Fernando de Noronha and 0-132.4 tons/km^2 at the Hawaiian Archipelago (Figure 2). Fish biomass was uncorrelated with depth (p=0.204, n=15), rugosity (p=0.724, n=15) or total algal cover (p=0.550, n=15). Correlation with live coral cover was marginally significant (r=-0.476; p=0.073, n=15).

We observed 103 green turtles in Fernando de Noronha and 47 in the Hawaiian Archipelago. They ranged 40-60 cm and were observed at most sites except Sancho in Fernando de Noronha and Pūpūkea and the two coral heads in Kāneʻohe Bay. One hawksbill turtle (*Eretmochelys imbricata*) was observed at E. Boldro (Fernando de Noronha) but was not considered for later analysis. Green turtles ranging 40-50 cm prevailed in both regions. Green turtles >50 cm were observed only at sites with a rugosity index lower than 1.5. Accordingly, green turtle biomass was negatively correlated with the rugosity index (r=-0.649, p=0.009, n=15) and the live coral cover (r=-0.573, p=0.026). Green turtle biomass was uncorrelated with depth (p=0.492, n=15) and total algae cover (p=0.186, n=15). Most of the green turtles observed while foraging were grazing intertidal pastures in the Hawaiian Archipelago (intertidal: 24, subtidal: 4), but the opposite was true in Fernando de Noronha (intertidal: 4, subtidal 16).

The biomass of green turtles was uncorrelated with that of herbivorous fishes (p=0.675, n=15) and sea urchins (p=0.653, n=8 for the Hawaiian Archipelago only). However, the biomass of herbivorous fishes and sea urchins were positively correlated in the Hawaiian Archipelago only (r=0.839, p<0.001, n=8). The range of total herbivore biomass was broad in both regions (Fernando de Noronha: 24.2-169.4 tons/km^2 and the Hawaiian Archipelago: 2.3-342.3 tons/km^2), but total herbivore biomass was significantly higher in the Hawaiian Archipelago than in Fernando de Noronha (t=-2.71, df =9, p=0.024).

In addition, differences existed between the two regions in the contribution of green turtles, fishes and sea urchins to total herbivore biomass (Figure 2). Comparable rocky reef with scattered coral (RC in Table 1) supported a much lower total herbivore biomass (t=3.4, df =5, p=0.017) in Fernando de Noronha (67.7 ± 40.73 tons/km^2) than in the Hawaiian Archipelago (243.2 ± 78.7 tons/km^2), likely because of the absence of sea urchins in the former. Indeed, fishes were the major herbivores in most of the rocky reefs with scattered coral off Fernando de Noronha, except for E. Boldro. In contrast, sea urchins were the dominant herbivores in most Hawaiian sites. Green turtles represented less than 8.5% of total herbivore biomass at any Hawaiian site, except in the He'eia flats, where green turtles were the only herbivores present and accounted for a higher biomass (127.7 tons/km^2). Green turtles were also the dominant herbivores at two sites with a low rugosity index (Porto and W. Boldro) in Fernando de Noronha.

Indeed, the contribution of green turtles to the total biomass of herbivores was negatively correlated with the habitat rugosity (r=-0.515, p=0.05, n=15) and positively correlated with total algal cover (r=0.526, p=0.044, n=15).

Discussion

To our knowledge, this is the first study to assess simultaneously the biomass of sea urchins, herbivorous fishes and green turtles in tropical reef habitats. The results reported here suggest that currently the aggregated biomass of fishes or sea urchins make up most of the total herbivore in the reef habitats of tropical oceanic islands. Green turtles make a major contribution to the total herbivore biomass only in flat areas with low live coral cover and high algal cover, a pattern resulting from the contrasting habitat requirements of green turtles and herbivorous fishes and sea urchins. Nevertheless, it should be noted that the biomass of green turtles is much higher than that of any other species of roving herbivore at many sites, although it´s much lower than the aggregated biomass of herbivorous fishes.

The distribution of sea urchins in tropical reefs is strongly determined by water movement, substrate type and sedimentation rate (Russo 1977; Ogden et al. 1989; Johansson et al. 2013). This could explain the virtual absence of sea urchins from the sheltered sites of the Hawaiian Archipelago (the He’eia flats and the two coral heads at Kāneʻohe Bay) and a much higher abundance at deeper, exposed sites (Old Kona Airport, Kua bay, Kīholo, Waialea and Pūpūkea). Sea urchins were virtually absent from all the sampling sites at Fernando de Noronha, as in 1985, when the first comprehensive survey of benthic habitats was conducted (Eston et al. 1986). This suggests that the population of *D. antillarum* might have collapsed at a similar time to that in the Caribbean (Mumby et al. 2007; Mumby and Steneck 2008), although the collapse was unnoticed because of the absence of previous research.

Habitat complexity and wave exposure are the major determinants of fish biomass both in Fernando de Noronha (Krajewski and Floeter 2011) and the Hawaiian Archipelago, although protection from fishing is also relevant in the latter (Friedlander et al. 2003; Friedlander et al. 2007; Williams et al. 2008; Helyer and Samhouri 2017). Most of the sites included in the study were no-take zones, although fishing was allowed at five of the Hawaiian sites (Table 1). Therefore, it is not surprising that the lowest biomass of herbivorous fishes was recorded at those sites open to fishing, thus confounding the role of environmental determinants on the distribution of fish biomass.

In contrast, green turtles are legally protected both in Fernando de Noronha and the Hawaiian Archipelago and hence, patterns of biomass distribution are determined by natural factors such as habitat rugosity and live coral cover. The reasons why green turtles, and particularly specimens >50 cm, concentrate at flat, sheltered areas are unknown, but might be related to food availability and predator avoidance. Morro Pico, in Fernando de Noronha, was the only shallow and flat site where the biomass of green turtles was low, likely because of the strong currents that sweep the area.

Previous studies in oceanic islands across the Central and Western Pacific Ocean reported a patchy distribution of green turtles both at regional and local scales, with sea surface temperature, chlorophyll levels and human disturbance as the main drivers (Becker et al. 2019). Nutrient availability is one of the major determinants of algal cover and primary productivity in tropical reef systems (Vermeij et al. 2010; Jantzen et al. 2013) and hence a higher green turtle biomass was expected at sites with a higher algal productivity. Although green turtle biomass was uncorrelated with total algal cover in the present study, the contribution (%) of green turtles to the total herbivore biomass was positively correlated with total algal cover, which stresses the relevance of green turtles as herbivores in areas of enhanced primary productivity.

It should be noted that total algal cover was usually low in subtidal habitats in the Hawaiian Archipelago, likely because of intense sea urchin grazing (Wabnitz et al. 2010). The highest algal cover of all the sites surveyed in the Hawaiian Archipelago during this study was recorded at the He’eia flats, an intertidal area covered with coral rubble and devoid of sea urchins and roving herbivorous fishes at high tide. Therefore, it is not surprising that large numbers of green turtles aggregated there at high tide. The existence of a *Halophila* spp. meadow at the nearby Kāneʻohe Sandbar (~1 km away), may facilitate the presence of green turtles there.

Low algal cover in subtidal habitats in the Hawaiian Archipelago may explain why most of the turtles observed while foraging in the Hawaiian sites were scraping turf from intertidal rocks or coral rubble. This behavior has been previously reported at Kaloko-Honokōhau, on the west coast of Hawaii (Wabnitz et al. 2010) and could be common throughout the Hawaiian Archipelago. In contrast to the situation observed in the Hawaiian Archipelago, green turtles were observed usually foraging in subtidal habitats in Fernando de Noronha. This is likely because of the much higher vegetation cover at subtidal habitats in Fernando de Noronha compared with the Hawaiian Archipelago. Certainly, the distribution of green turtles varies along the tide cycle and they may forage in subtidal habitats as well at low tide. But the relevant point here is that green turtles exploited intensely intertidal habitats in the Hawaiian Archipelago when available, but not in Fernando de Noronha. Differences in the algal cover of subtidal habitats in both regions offer the best explanation as to the difference.

Nevertheless, food availability alone cannot explain the preference of green turtles for the flat and sheltered areas of W. Boldro and Porto in Fernando de Noronha, as they have only modest algal cover and high turtle biomass. Sharks (*Carcharhinus perezi* and *Negaprion bervirostris*) were spotted at all the sampling sites in Fernando de Noronha, except W. Boldro, Porto and Morro do Pico. This is evidence that predatory avoidance may explain the preference of green turtles at W. Boldro and Porto. Nevertheless, complex interactions exist between body condition, forage quality and predation risk (Heithaus et al. 2007; Burkholder et al. 2013). It is worth noting that green turtles >60 cm prevail in deeper habitats (~15 m) in the Hawaiian Archipelago (Becker et al. 2019), which could reveal reduced predation risk at larger body size. Certainly, further research is required to better understand habitat selection by green turtles in tropical reef habitats in relation to predator avoidance.

In any case, our results show that only sheltered, flat areas with a low coral cover support a high biomass of green turtles in the shallow reefs of tropical oceanic islands. This is an expected result, considering the similarity of those sheltered, flat algal pastures, with seagrass meadows that represent the favored habitat of green turtles in most of their distribution range (Burkholder et al. 2013). It should be noted that green turtles could be less dependent on these flat algal pastures in the reefs of tropical continental regions, as they usually support a much higher primary algal productivity than the reefs of oceanic islands (Adey and Steneck 1985; Vermeij et al. 2010; Jantzen et al. 2013).

Furthermore, coral reefs and seagrass meadows are often intermingled in continental regions, whereas seagrass meadows are poorly developed in most oceanic islands (Short and Frederick 2003). As green turtles can use both seagrasses and algae concurrently (André et al. 2005; Fuentes et al. 2007; Howell et al. 2016), nearby seagrass meadows may subsidize green turtle population in continental reef habitats, thus resulting in a much higher green turtle biomass in reef habitats. Further research on habitat use, diet selection, grazing rates and connectivity between seagrass meadows and reefs is required to improve our understanding of green turtle role in the dynamics of underwater vegetation in tropical reef ecosystems.

Finally, the legacy of the past human exploitation of green turtles should be considered, because current population size is probably much lower than in the pre-harvest period, despite population increase in the past decades. The recovery of green turtle populations began worldwide in the 1980´s (Chaloupka et al. 2008), and has been particularly successful in the Western South Atlantic Ocean and the Hawaiian Archipelago (Chaloupka et al. 2008; Kittinger et al. 2013; Silva et al. 2017; Broderick and Patricio 2019; Chaloupka and Pilcher 2019) Currently, the encounter rate of green turtles in underwater surveys at Fernando de Noronha is twice that reported a decade ago (Gitirana and Souza 2012), but the pre-harvest population density is unknown. Estimates of past green turtle abundance in the nearby Greater Caribbean suggest that a decade ago, the population of green turtles was three orders of magnitude lower than their historic numbers (McClenachan et al. 2006). If this was also true for the Western South Atlantic, green turtle density in Fernando de Noronha is still well below carrying capacity and green turtle could have been the dominant herbivores in pre-harvest times, although this is highly speculative.

Green turtles in the Hawaiian Archipelago have one the lowest population densities reported from oceanic islands in the Central and Western Pacific Ocean, due to a combination of low sea surface temperature, low primary productivity and high human impact (Becker et al. 2019). Nevertheless, the green turtle population in the Hawaiian Archipelago exhibits a high growth rate thanks to legal protection (Chaloupka et al. 2008; Kittinger et al. 2013; Becker et al. 2019) and green turtles have been close to carrying capacity on the west coast of the island of Hawaii for more than two decades (Balazs and Chaloupka 2004a). Ecosystem modeling indicates that the dynamics of subtidal vegetation at Kaloko-Honoköhau, on the west coast of the island of Hawaii, is ruled by sea urchin grazing, with green turtles playing a relevant role only at intertidal rocky habitats (Wabnitz et al. 2010). Considering the similarity in the biomass and make-up of the herbivore community at Kaloko-Honoköhau and the other sites analyzed in this study, green turtles are likely to play a minor role in the dynamics of subtidal vegetation in these places. Further increase in green turtle biomass would be unlikely in that part of the Hawaiian Archipelago if the green turtle population there is approaching carrying capacity (Balazs and Chaloupka 2004a), and hence, sea urchins and roving herbivorous fishes will likely continue to dominate the herbivore biomass on the west coast of the island of Hawaii in the future.

The situation could be different at more productive sites, such as Kāneʻohe Bay, in Oahu. This is one of the most productive areas in the Hawaiian Archipelago (Drupp et al. 2011). Green turtles exhibit a much higher rate of somatic growth there than on the west coast of the island of Hawaii (Balazs and Chaloupka 2004a) and the algal pastures at the He'eia flats support the highest green turtle biomass reported in this study for the Hawaiian Archipelago. There is no evidence that green turtles have reached carrying capacity at Kāneʻohe Bay (Balazs and Chaloupka 2004a), and hence, biomass could increase in the future in areas with a low cover of live coral. Nevertheless, nothing can be said about the original situation in pre-harvest times, due to the dramatic modification of the bay during the 20th century.

Finally, a better understanding of trophic redundancy within the community of herbivores is required to assess the role of green turtles in tropical reef habitats. This is because different species may target different algal resources, thus creating the potential for strong complementarity in reef habitats, where more species might be needed to maintain ecosystem functioning across large, naturally varied reefscapes than suggested by small-scale studies (Jouffray et al. 2015; Helyer and Samhouri 2017; Lefcheck et al. 2019). This study shows that green turtle biomass is often much smaller than the aggregated biomass of herbivorous fishes or sea urchins. Furthermore, the latter two groups have much higher daily feeding rates than green turtles: 29% of body weight for parrotfish and surgeonfish (Mill et al. 2007) and 13% of body weight for the sea urchin *E. mathaei* (Hiratsuka and Uehara 2007) compared to <1% of body weight for green turtles (Moran and Bjorndal 2005). Nevertheless, green turtles may still play a relevant role in the dynamics of the underwater vegetation of tropical reefs if they used resources differently from fishes and sea urchins. However, the specificity of this kind of data is not yet available, so in in order to gain further insight into these specific questions, further research is needed.

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4. CHAPTER 2: RELATIONSHIP BETWEEN SKULL MORPHOLOGY AND DIET IN GREEN TURTLES

Abstract

Here, we use geometric morphometrics to compare the skull shape of green turtles from six regions to assess the hypothesis that variability in skull and mandible morphology is related to diet. Results revealed no differences in the skull morphology of the two populations relying largely on seagrasses (Greater Caribbean and the western Indian Ocean), whereas the skulls from the Greater Caribbean differed from those of all the populations relying largely on macroalgae (the Mexican Pacific, Hawaii, the Central Pacific, and the tropical Atlantic). When specimens were grouped according to diet, significant differences were observed in the morphology of the skull and mandible of seagrass and macroalgae consumers because macroalgae eaters had narrower skulls with shorter supraoccipital bone but longer anterior skulls than seagrass eaters. This suggests that populations of green turtles inhabiting regions devoid of extensive seagrass meadows retain some of the characteristics typical of the skull of juveniles that allow selective browsing, whereas populations relying on seagrasses develop skulls better suited for grazing.

Introduction

Interspecific variability in skull morphology is tightly linked to diet in vertebrates, and skull shape differs according to the hardness of prey in shrews (Tse and Calede 2021), browsing versus grazing in ungulates (Mendoza et al. 2002), levels of carnivory in dogs (Friscia et al. 2007), and grazing versus grasping in sea turtles (Chatterji et al. 2022). Following the same reasoning, interpopulation variability in skull morphology might also be adaptative and relate to dietary differences (Parsons et al. 2020), but most of the research on the topic focused on delineating independent taxonomic units (Hohl et al. 2020).

Green turtles (*Chelonia mydas*) are megaherbivores (Esteban et al. 2020) broadly distributed in tropical and warm temperate regions worldwide (Wallace et al. 2010), where they inhabit seagrass meadows (Rezaie-Atagholipour et al. 2021; Scott et al. 2021; Vanderklift et al. 2021), coral reefs (Makowski et al. 2006; Reisser et al. 2013; Smithers and Dawson 2023), mangrove forests (López-Mendilaharsu et al. 2005; Pillans et al. 2021) and rocky reefs (Reisser et al. 2013; Cardona et al. 2020a;

Quiñones et al. 2022). Early juvenile green turtles are pelagic carnivores (Reich et al. 2007; Parker et al. 2011) and have large heads relative to carapace length and a relatively longer supraoccipital bone and a small temporal bone and infratemporal fossae compared to adults, which results in a weak bite force (Marshall et al. 2014) and a skull morphology best suited for suction feeding (Nishizawa et al. 2010). On the contrary, late juveniles and adults have a short, rounded snout, jaws with propalineal as well as arcilineal movement, a much stronger bite force, and a rhamphotheca with serrated, sharply ridged edges, all of them considered adaptations to herbivory (Marshall et al. 2014; Figgener et al. 2019; Chatterji et al. 2022).

The ontogenetic dietary shift from carnivory to herbivory experienced by green turtles after settlement in neritic habitat (Reich et al. 2007; Cardona et al. 2009) is linked to the changes in skull morphology reported above (Nishizawa et al. 2010), but the pattern of ontogenetic changes probably varies across populations. First, significant differences exist in the skull shape of neritic juveniles from different natal populations sharing the same foraging grounds off Brazil, with a trade-off between bite force and suction feeding (Coelho et al. 2018). Second, remarkable variability exists in the skull dimensions of the adult green turtles from several populations, with those from the Greater Caribbean and the western Indian Ocean having much deeper, broader, and longer skulls than those from the Galapagos Islands, Japan, and Guiana (Kamezaki and Matsui 1995). Interestingly, skull height and width are the major determinants of bite force in green turtles, and high shearing forces are probably required to grind tough seagrasses using propalineal jaw movements (Marshall et al. 2014). Seagrasses dominate the diet of the adult green in the Greater Caribbean, the western Indian Ocean, the Mediterranean, and most of Australia, whereas macroalgae dominate the diet of adult green turtles elsewhere (Esteban et al. 2020). It is worth noting that the populations from the Greater Caribbean and the western Indian Ocean belong to different evolutionary lineages, and hence each of them is more related respectively to other populations relying on macroalgae (Jensen et al. 2019). This suggests that the interpopulation variability in the dimensions and morphology of green turtles’ skulls revealed by traditional morphometrics (Kamezaki and Matsui 1995) might be linked to intrapopulation differences in diet, a hypothesis not yet explored in the literature, although the interspecific differences in skull morphology of marine turtles as a group are thought to be related with diet (Figgener et al. 2019; Chatterji et al. 2022).

Unfortunately, the only information published to date on the interpopulation variability of skull morphology in green turtles is based on linear measurements and traditional morphometric analysis, which often fail to capture subtle differences in shape and cannot correct for differences in skull size (Adams et al. 2004). Here, we use geometric morphometrics to compare the skull morphology of green turtles from six populations differing in diet and evolutionary origin to test the hypothesis that green turtles with seagrass-based diets and macroalgae-based diets differ in their skull morphology, independently of their evolutionary lineage, after controlling for differences in size.

Materials and methods

Turtle sample collection

Museum samples (Table 1) were obtained from the Smithsonian Natural History Museum, San Diego Natural History Museum, and Bishop Museum. Specimens were classified into six regions (Figure 1): Greater Caribbean (Florida, Gulf of Mexico, Yucatan, Cayman Islands, Jamaica, and Cuba), Tropical Atlantic (Ascension and Suriname), Western Indian Ocean (Maldives, Kenya, Tanzania, Comoros, Aldabra), central Pacific (Marshall Islands, Philippines, Mariana Islands, Phoenix Islands, Line Islands, Tuamotu, Gambier Islands), Hawaii (Oahu, Island of Hawaii, Maui, French Frigate Shoals, Pearl atoll, other Northwestern Hawaiian Islands) and Mexican Pacific (Baja California, San Diego, Channel Islands). According to Esteban et al. (2020), green turtles from the Greater Caribbean and the Western Indian Ocean rely mostly on seagrasses, and the other populations have mixed diets dominated by macroalgae (Seminoff et al. 2015). On the other hand, the populations from the Greater Caribbean and the Tropical Atlantic belonged to the same evolutionary lineage, and those from the western Indian Ocean, the Central Pacific, Hawaii, and the Mexican Pacific to a different one, with further structuring within each major lineage (Jensen et al. 2019). Most museum specimens were represented by the skull and mandible but the latter was missing in some of them.

Table 1 Turtle specimens from museums with location where they were captured/ stranded according to museum documentation and their main diet item

Museum	Turtle	Location	Diet
San Diego Natural History Museum	56568	Hawaii	Algae
San Diego Natural History Museum	56569	Hawaii	Algae
San Diego Natural History Museum	56570	Hawaii	Algae
San Diego Natural History Museum	56572	Hawaii	Algae
San Diego Natural History Museum	46799	Mexican Pacific	Algae
San Diego Natural History Museum	56571	Mexican Pacific	Algae
San Diego Natural History Museum	56574	Mexican Pacific	Algae
San Diego Natural History Museum	56575	Mexican Pacific	Algae
San Diego Natural History Museum	56576	Mexican Pacific	Algae
San Diego Natural History Museum	56582	Mexican Pacific	Algae
San Diego Natural History Museum	56584	Mexican Pacific	Algae
San Diego Natural History Museum	56585	Mexican Pacific	Algae
San Diego Natural History Museum	56586	Mexican Pacific	Algae
San Diego Natural History Museum	56587	Mexican Pacific	Algae
San Diego Natural History Museum	56588	Mexican Pacific	Algae
San Diego Natural History Museum	56589	Mexican Pacific	Algae
San Diego Natural History Museum	47299	Greater Caribbean	Seagrass
San Diego Natural History Museum	47300	Greater Caribbean	Seagrass
San Diego Natural History Museum	56557	Greater Caribbean	Seagrass
San Diego Natural History Museum	56558	Greater Caribbean	Seagrass
San Diego Natural History Museum	56559	Greater Caribbean	Seagrass
San Diego Natural History Museum	56560	Greater Caribbean	Seagrass
San Diego Natural History Museum	56561	Greater Caribbean	Seagrass
San Diego Natural History Museum	56562	Greater Caribbean	Seagrass
San Diego Natural History Museum	56563	Greater Caribbean	Seagrass
San Diego Natural History Museum	56564	Greater Caribbean	Seagrass
San Diego Natural History Museum	56565	Greater Caribbean	Seagrass
San Diego Natural History Museum	56566	Greater Caribbean	Seagrass
San Diego Natural History Museum	56577	Greater Caribbean	Seagrass
San Diego Natural History Museum	56578	Greater Caribbean	Seagrass
San Diego Natural History Museum	56581	Greater Caribbean	Seagrass
San Diego Natural History Museum	56590	Greater Caribbean	Seagrass
San Diego Natural History Museum	59648	Greater Caribbean	Seagrass
San Diego Natural History Museum	59649	Greater Caribbean	Seagrass
San Diego Natural History Museum	59650	Greater Caribbean	Seagrass
San Diego Natural History Museum	59651	Greater Caribbean	Seagrass
San Diego Natural History Museum	59653	Greater Caribbean	Seagrass
San Diego Natural History Museum	59655	Greater Caribbean	Seagrass
San Diego Natural History Museum	59656	Greater Caribbean	Seagrass

San Diego Natural History Museum	59658	Greater Caribbean	Seagrass
San Diego Natural History Museum	59659	Greater Caribbean	Seagrass
San Diego Natural History Museum	59660	Greater Caribbean	Seagrass
San Diego Natural History Museum	59661	Greater Caribbean	Seagrass
San Diego Natural History Museum	59663	Greater Caribbean	Seagrass
Bernice Pauahi Bishop Museum	1371	Central Pacific	Algae
Bernice Pauahi Bishop Museum	1372	Central Pacific	Algae
Bernice Pauahi Bishop Museum	1373	Central Pacific	Algae
Bernice Pauahi Bishop Museum	5645	Central Pacific	Algae
Bernice Pauahi Bishop Museum	43327	Central Pacific	Algae
Bernice Pauahi Bishop Museum	6737	Hawaii	Algae
Bernice Pauahi Bishop Museum	10672	Hawaii	Algae
Bernice Pauahi Bishop Museum	11572	Hawaii	Algae
Bernice Pauahi Bishop Museum	43287	Hawaii	Algae
Bernice Pauahi Bishop Museum	43290	Hawaii	Algae
Bernice Pauahi Bishop Museum	43295	Hawaii	Algae
Bernice Pauahi Bishop Museum	43298	Hawaii	Algae
Bernice Pauahi Bishop Museum	43306	Hawaii	Algae
Bernice Pauahi Bishop Museum	43310	Hawaii	Algae
Bernice Pauahi Bishop Museum	43321	Hawaii	Algae
Bernice Pauahi Bishop Museum	43323	Hawaii	Algae
Bernice Pauahi Bishop Museum	43324	Hawaii	Algae
Bernice Pauahi Bishop Museum	43325	Hawaii	Algae
Bernice Pauahi Bishop Museum	43326	Hawaii	Algae
Bernice Pauahi Bishop Museum	43332	Hawaii	Algae
Bernice Pauahi Bishop Museum	43338	Hawaii	Algae
Bernice Pauahi Bishop Museum	43339	Hawaii	Algae
Bernice Pauahi Bishop Museum	43341	Hawaii	Algae
Bernice Pauahi Bishop Museum	43342	Hawaii	Algae
Bernice Pauahi Bishop Museum	43343	Hawaii	Algae
Bernice Pauahi Bishop Museum	43345	Hawaii	Algae
Bernice Pauahi Bishop Museum	43346	Hawaii	Algae
Bernice Pauahi Bishop Museum	43347	Hawaii	Algae
Bernice Pauahi Bishop Museum	43351	Hawaii	Algae
Bernice Pauahi Bishop Museum	43354	Hawaii	Algae
Bernice Pauahi Bishop Museum	43396	Hawaii	Algae
Bernice Pauahi Bishop Museum	43397	Hawaii	Algae
Bernice Pauahi Bishop Museum	43507	Hawaii	Algae
Bernice Pauahi Bishop Museum	47942	Hawaii	Algae
Smithsonian National Museum of Natural History	29854	Central Pacific	Algae
Smithsonian National Museum of Natural History	132544	Central Pacific	Algae
Smithsonian National Museum of Natural History	156983	Central Pacific	Algae
Smithsonian National Museum of Natural History	158320	Central Pacific	Algae

Smithsonian National Museum of Natural History	163548	Central Pacific	Algae
Smithsonian National Museum of Natural History	163549	Central Pacific	Algae
Smithsonian National Museum of Natural History	163550	Central Pacific	Algae
Smithsonian National Museum of Natural History	197868	Central Pacific	Algae
Smithsonian National Museum of Natural History	220777	Central Pacific	Algae
Smithsonian National Museum of Natural History	220779	Central Pacific	Algae
Smithsonian National Museum of Natural History	279322	Central Pacific	Algae
Smithsonian National Museum of Natural History	279325	Central Pacific	Algae
Smithsonian National Museum of Natural History	67357	Hawaii	Algae
Smithsonian National Museum of Natural History	67358	Hawaii	Algae
Smithsonian National Museum of Natural History	67359	Hawaii	Algae
Smithsonian National Museum of Natural History	231635	Mexican Pacific	Algae
Smithsonian National Museum of Natural History	291937	Mexican Pacific	Algae
Smithsonian National Museum of Natural History	300390	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300391	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300392	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300393	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300394	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300395	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300397	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300398	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300399	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300400	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300402	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300403	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	300404	Tropical Atlantic	Algae
Smithsonian National Museum of Natural History	59059	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	59060	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	90886	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	167525	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	220795	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	252440	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313713	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313714	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313715	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313717	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313719	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313720	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313721	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313723	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313724	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313725	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	313726	Greater Caribbean	Seagrass

Smithsonian National Museum of Natural History	313727	Greater Caribbean	Seagrass
Smithsonian National Museum of Natural History	79480	West Indian	Seagrass
Smithsonian National Museum of Natural History	132732	West Indian	Seagrass
Smithsonian National Museum of Natural History	132733	West Indian	Seagrass
Smithsonian National Museum of Natural History	220774	West Indian	Seagrass
Smithsonian National Museum of Natural History	220775	West Indian	Seagrass
Smithsonian National Museum of Natural History	220776	West Indian	Seagrass
Smithsonian National Museum of Natural History	231560	West Indian	Seagrass
Smithsonian National Museum of Natural History	231564	West Indian	Seagrass
Smithsonian National Museum of Natural History	231572	West Indian	Seagrass
Smithsonian National Museum of Natural History	231573	West Indian	Seagrass
Smithsonian National Museum of Natural History	231574	West Indian	Seagrass
Smithsonian National Museum of Natural History	231575	West Indian	Seagrass
Smithsonian National Museum of Natural History	231576	West Indian	Seagrass
Smithsonian National Museum of Natural History	231577	West Indian	Seagrass
Smithsonian National Museum of Natural History	231578	West Indian	Seagrass
Smithsonian National Museum of Natural History	231579	West Indian	Seagrass
Smithsonian National Museum of Natural History	231582	West Indian	Seagrass
Smithsonian National Museum of Natural History	231587	West Indian	Seagrass
Smithsonian National Museum of Natural History	231589	West Indian	Seagrass
Smithsonian National Museum of Natural History	231592	West Indian	Seagrass
Smithsonian National Museum of Natural History	231598	West Indian	Seagrass
Smithsonian National Museum of Natural History	231599	West Indian	Seagrass
Smithsonian National Museum of Natural History	231600	West Indian	Seagrass
Smithsonian National Museum of Natural History	231601	West Indian	Seagrass
Smithsonian National Museum of Natural History	231602	West Indian	Seagrass
Smithsonian National Museum of Natural History	231603	West Indian	Seagrass
Smithsonian National Museum of Natural History	231605	West Indian	Seagrass
Smithsonian National Museum of Natural History	231606	West Indian	Seagrass
Smithsonian National Museum of Natural History	231609	West Indian	Seagrass
Smithsonian National Museum of Natural History	231610	West Indian	Seagrass
Smithsonian National Museum of Natural History	231611	West Indian	Seagrass
Smithsonian National Museum of Natural History	231612	West Indian	Seagrass
Smithsonian National Museum of Natural History	231678	West Indian	Seagrass
Smithsonian National Museum of Natural History	231685	West Indian	Seagrass
Smithsonian National Museum of Natural History	231689	West Indian	Seagrass
Smithsonian National Museum of Natural History	231706	West Indian	Seagrass
Smithsonian National Museum of Natural History	231719	West Indian	Seagrass
Smithsonian National Museum of Natural History	235858	West Indian	Seagrass
Smithsonian National Museum of Natural History	235872	West Indian	Seagrass
Smithsonian National Museum of Natural History	235885	West Indian	Seagrass
Smithsonian National Museum of Natural History	235886	West Indian	Seagrass
Smithsonian National Museum of Natural History	235895	West Indian	Seagrass

Smithsonian National Museum of Natural History	235897	West Indian	Seagrass
Smithsonian National Museum of Natural History	269991	West Indian	Seagrass

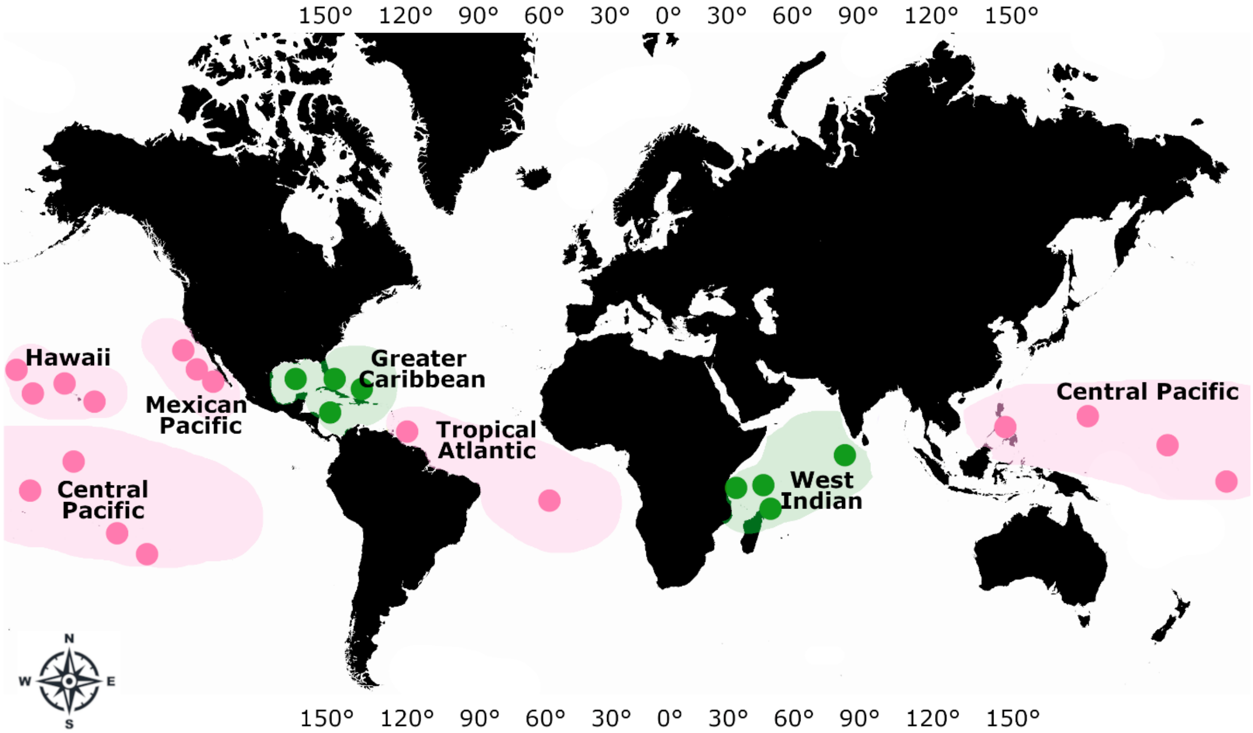


Figure 1 Sampling locations of *Chelonia mydas*. Each dot represents a location, not an individual specimen. Pink dots and area denote locations where the green turtle feeds mostly on macroalgae and green ones where they feed primarily on seagrass.

Each skull or mandible was placed at the same location with a ruler as a scale reference on the side, and images of dorsal, lateral and ventral views were taken perpendicular to the plane using an iPhone 13 mini with 12-megapixel resolution. Two dimensional landmarks, modified from Nishizawa et al. (2010), were digitized using ImageJ (Figure 2), adding them as ROIs (region of interest) and then measured to obtain the x and y coordinates to be used on Geomorph in R (Adams and Otárola-Castillo 2013). In total, 22 landmarks were used for the dorsal and ventral view as well as for the mandible and 26 for the lateral view (Nishizawa et al. 2010). The landmark coordinates were superimposed using the Generalized Procrustes Analysis (GPA), based on the criterion of least squares (Rohlf 1990; Adams et al. 2004) using Geomorph in R. Then, skull size was estimated for each view using the centroid size, which was calculated as the square root of the sum of squares of the distance between each landmark and center of the configuration (Adams and Otárola-Castillo 2013). For the mandibles, the oral cavity size was calculated using ImageJ as the area of the triangle formed inside the mandible (Figure 2).

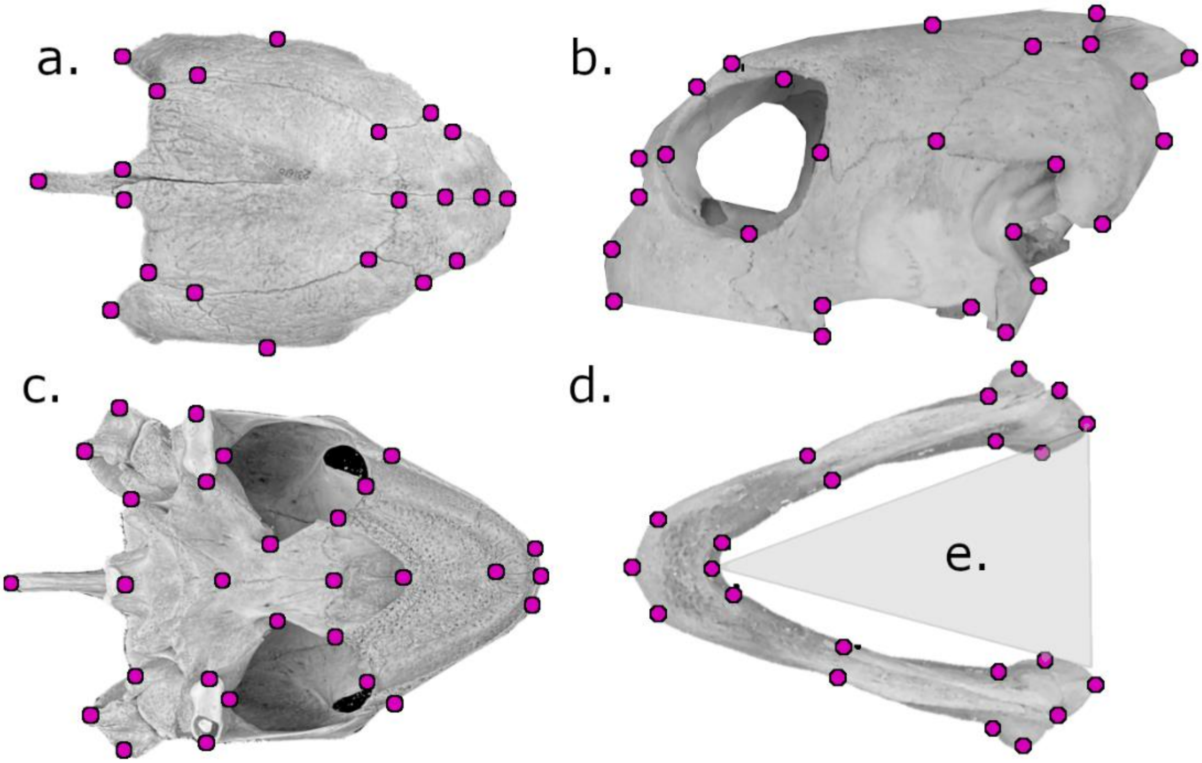


Figure 2 Landmarks for dorsal (a), lateral (b) and ventral (c) skull views and mandible (c). Landmarks were modified from Nishizawa et al. (2010). Oral cavity was measured as the area of the triangle formed inside the mandible (e).

Statistical analysis

The Geomorph package in R (Adams and Otárola-Castillo 2013) was used to perform the multivariate analysis of variance (MANOVA) and to compare skull and mandible shapes between groups.

Later, the Procrustes ANOVA test (Adams et al. 2004) were used for pair-wise comparison with the same software. These differences are evaluated through permutation (999 in this case), where the vectors of residuals are randomized among groups. To account for allometry in comparison for location, centroid size was included in the equation. To quantify these differences between two groups, the partial Procrustes distance was used. ANOVA was used to compare size ratios calculated with traditional morphometrics between diets.

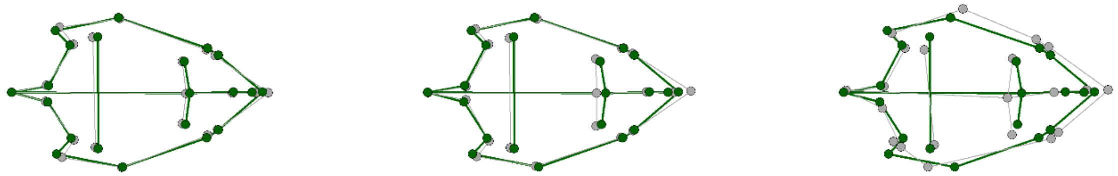
Results

Significant interpopulation differences in skull and mandible shape were observed when accounting for allometry (skull dorsal view: MANOVA, F_{151} = 5.0, p=0.001; skull ventral view: MANOVA, F_{152} = 4.8, p=0.001; skull lateral view: MANOVA, F_{138} = 3.0, p=0.001; mandible: MANOVA, F_{103} = 5.7, p=0.001). However, most of the variability was due to the populations from the Mexican Pacific, Hawaii, the Greater Caribbean and the tropical Atlantic. Variability on the dorsal view (Figure 3), was driven by green turtles from the Mexican Pacific, whose skulls were wider than the ones from the Greater Caribbean and Hawaii. Conversely, variability on the ventral view (Figure 4) was driven by green turtles from Hawaiian, whose skulls were narrower than the ones from the Greater Caribbean, Mexican Pacific and Western Indian Ocean. Variability on the lateral view (Figure 5) was driven by green turtles from the Greater Caribbean, whose skulls were broader on the anterior part of the skull (premaxilla and prefrontal bones) than those on the Central Pacific and had longer squamosal bone than the ones from the Central Pacific, Hawaii, and tropical Atlantic. Finally, variability on mandible shape (Figure 6) was driven by the Greater Caribbean and the tropical Atlantic, which differed to each other. The former had a mandible shorter than the western Indian Ocean and the latter narrower than those from the central Pacific.

It is remarkable that the shape of the skulls from the two populations relying largely on seagrasses (Greater Caribbean and the western Indian Ocean) did not differ in any view, whereas the skulls from the Greater Caribbean differed in at least one view from all the populations relying largely on macroalgae (the Mexican Pacific, Hawaii, the Central Pacific and the tropical Atlantic). Furthermore, the skulls of green turtles from Hawaii and the Mexican Pacific differed both in dorsal and ventral view. On the other hand, the mandibles of the two populations relying largely on seagrasses (Greater Caribbean and the western Indian Ocean) did not differ.

When specimens were grouped according to diet and allometry was controlled, significant differences were observed in the morphology of the skull and mandible of seagrass and macroalgae consumers, for either the dorsal, ventral and lateral views (Figure 7). The mean skull shape from algae eaters was narrower than the one from the seagrass eaters, both on the dorsal and ventral views (dorsal: MANOVA, F_{151} = 3.7, p=0.001; ventral: MANOVA, F_{152} =2.6, p=0.018). When the lateral images were analyzed, there was also a significant difference between the skull shape of seagrass and the algae eaters, but in this case, it was due to the algae eaters having a shorter supraoccipital and longer anterior skulls (premaxilla, prefrontal and frontal bones) than the seagrass eaters (MANOVA, F_{138} = 5.3, p=0.001). Correspondingly, the mandible of the algae eaters was narrower than the seagrass eaters (MANOVA, F_{103} = 6.2, p=0.001).

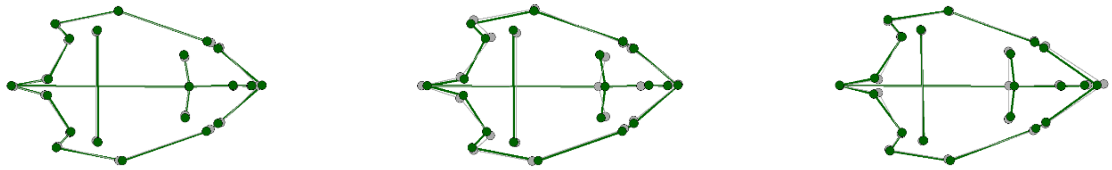
The length of the supraoccipital bone was also assessed using traditional morphometrics, and even though algae eaters also had a shorter one, it was not statistically significant when using skull area, skull height or mandible area for the ratio (Figure 8). On the other hand, the oral cavity size was larger in algae eaters compared to seagrass eaters, both when using the skull area and the skull height in the ratio (Student’s t-test, t_{82}=2.428, p=0.016; t_{82}=-22.08, p<0.001 respectively), due to a narrower and longer mandible, according to geometric morphometrics, as stated above.



Central Pacific vs Greater Caribbean
ANOVA p= 0.65

Central Pacific vs Hawaii
ANOVA p= 0.79

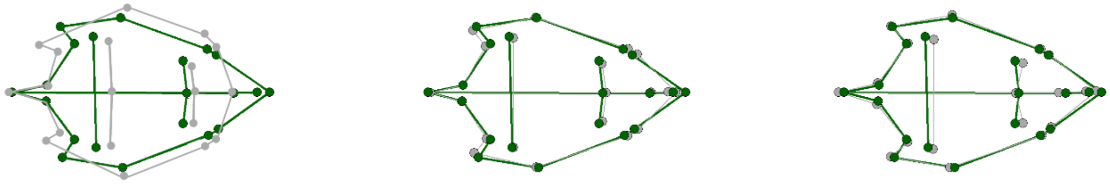
Central Pacific vs Mexican Pacific
ANOVA p= 0.06



Central Pacific vs West Indian
ANOVA p= 0.72

Central Pacific vs. Tropical Atlantic
ANOVA p= 0.37

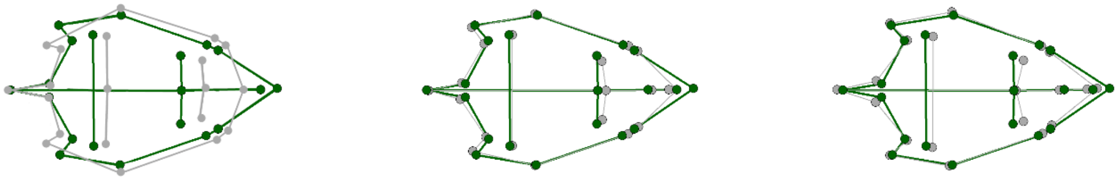
Greater Caribbean vs. Hawaii
ANOVA p= 0.38



Greater Caribbean vs. Mexican Pacific
ANOVA **p=0.02**

Greater Caribbean vs. West Indian
ANOVA p= 0.96

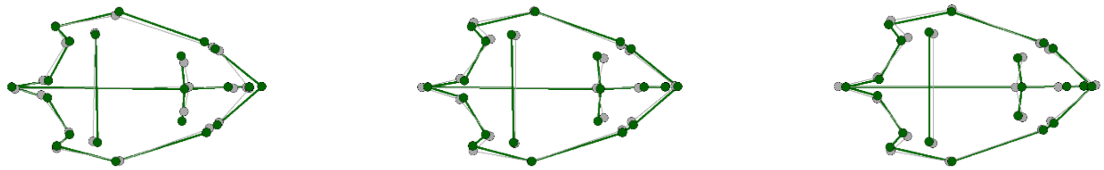
Greater Caribbean vs. Tropical Atlantic
ANOVA p= 0.48



Hawaii vs.Mexican Pacific
ANOVA **p=0.01**

Hawaii vs. West Indian
ANOVA p= 0.47

Hawaii vs. Tropical Atlantic
ANOVA p= 0.18

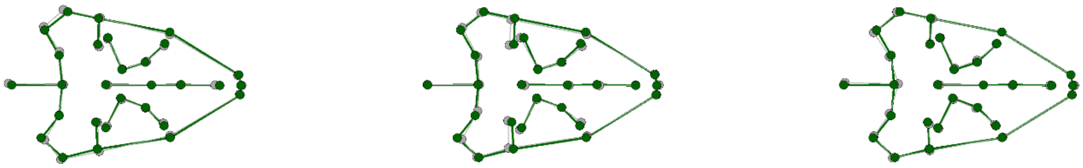


Mexican Pacific vs. West Indian
ANOVA p= 0.07

Mexican Pacific vs. Tropical Atlantic
ANOVA p= 0.29

West Indian vs. Tropical Atlantic
ANOVA p= 0.49

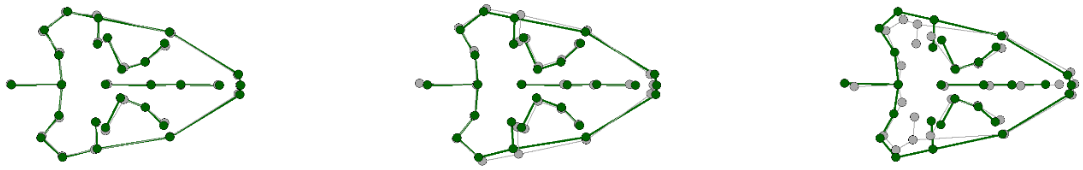
Figure 3 Shape differences (Procrustes ANOVA) between locations for dorsal view of green turtle *Chelonia mydas* skulls using geometric morphometrics. Images were generated using Geomorph for R (Adams and Otárola-Castillo 2013). The green outline is the mean shape for the first location named at the bottom of the image. The grey one is the mean shape of the second name location mentioned below each image.



Central Pacific vs Greater Caribbean
MANOVA p= 0.85

Central Pacific vs Hawaii
MANOVA p= 0.15

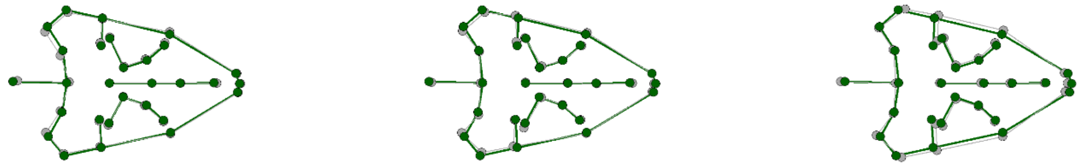
Central Pacific vs Mexican Pacific
MANOVA p= 0.16



Central Pacific vs West Indian
ANOVA p= 0.61

Central Pacific vs. Tropical Atlantic
ANOVA p= 0.54

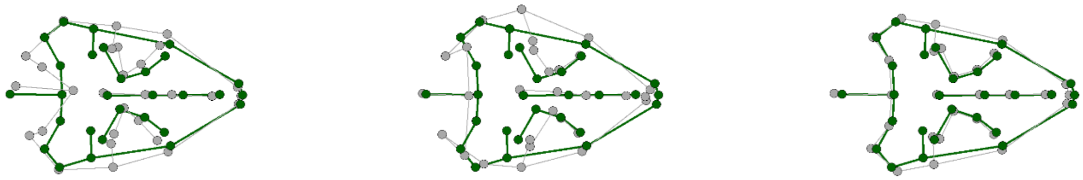
Greater Caribbean vs. Hawaii
ANOVA **p=0.04**



Greater Caribbean vs. Mexican Pacific
ANOVA p= 0.13

Greater Caribbean vs. West Indian
ANOVA p= 0.67

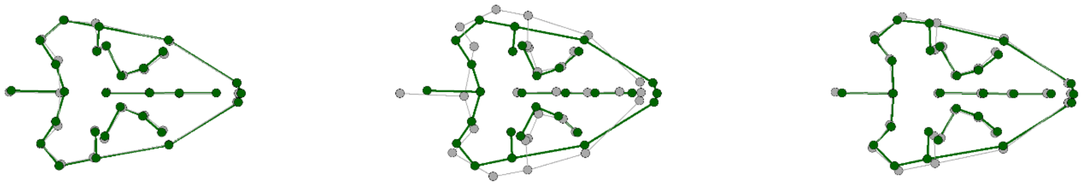
Greater Caribbean vs. Tropical Atlantic
ANOVA p= 0.43



Hawaii vs.Mexican Pacific
ANOVA **p=0.01**

Hawaii vs. West Indian
ANOVA **p=0.01**

Hawaii vs. Tropical Atlantic
ANOVA p= 0.55

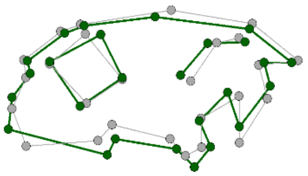


Mexican Pacific vs. West Indian
ANOVA p= 0.29

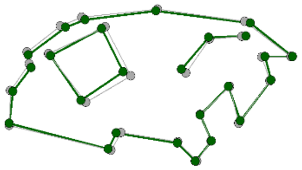
Mexican Pacific vs. Tropical Atlantic
ANOVA p= 0.06

West Indian vs. Tropical Atlantic
ANOVA p= 0.26

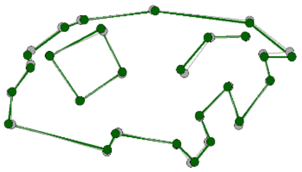
Figure 4 Shape differences between locations (Procrustes ANOVA) for the ventral view of green turtle *Chelonia mydas* skulls using geometric morphometrics. Images were generated using Geomorph for R (Adams and Otárola-Castillo 2013). The green outline is the mean shape for the first location named at the bottom of the image. The grey one is the mean shape of the second location mentioned below each image.



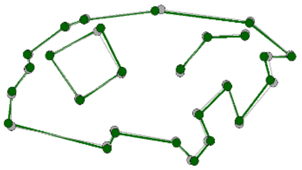
Central Pacific vs Greater Caribbean
ANOVA **p=0.04**



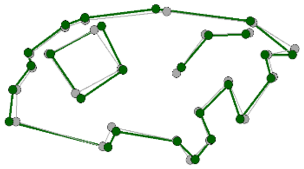
Central Pacific vs Hawaii
ANOVA p= 0.82



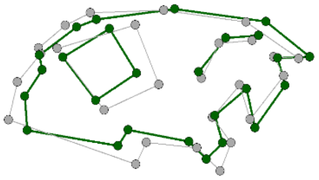
Central Pacific vs Mexican Pacific
ANOVA p= 0.32



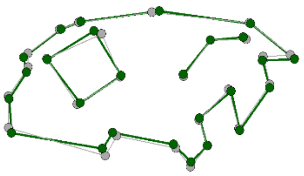
Central Pacific vs West Indian
ANOVA p= 0.61



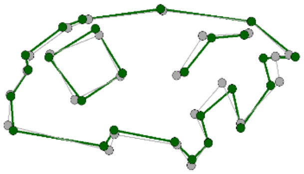
Central Pacific vs. Tropical Atlantic
ANOVA p= 0.13



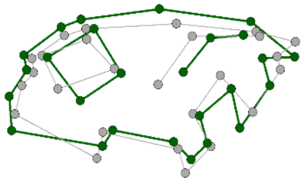
Greater Caribbean vs. Hawaii
ANOVA **p=0.02**



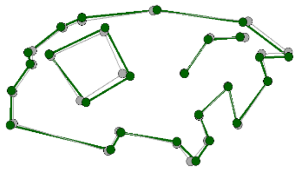
Greater Caribbean vs. Mexican Pacific
ANOVA p= 0.48



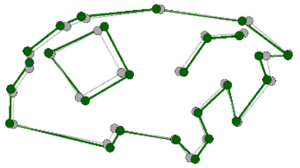
Greater Caribbean vs. West Indian
ANOVA p=0.05



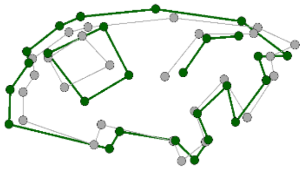
Greater Caribbean vs. Tropical Atlantic
ANOVA **p=0.01**



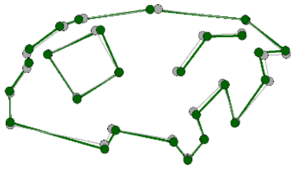
Hawaii vs.Mexican Pacific
ANOVA p= 0.33



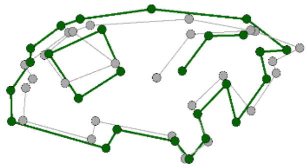
Hawaii vs. West Indian
ANOVA p= 0.65



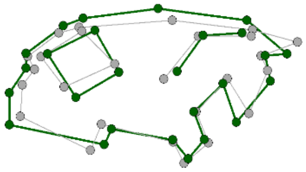
Hawaii vs. Tropical Atlantic
ANOVA p= 0.05



Mexican Pacific vs. West Indian
ANOVA p= 0.56

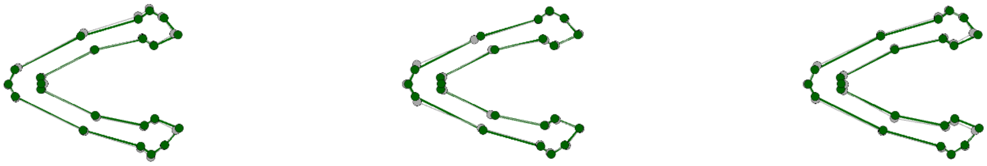


Mexican Pacific vs. Tropical Atlantic
ANOVA **p=0.03**



West Indian vs. Tropical Atlantic
ANOVA **p=0.04**

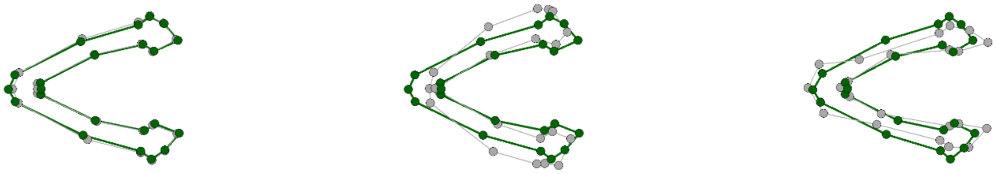
Figure 5 Shape differences between locations (Procrustes ANOVA) for the lateral view of green turtle *Chelonia mydas* skulls using geometric morphometrics. Images were generated using Geomorph for R (Adams and Otárola-Castillo 2013). The green outline is the mean shape for the first location named at the bottom of the image. The grey one is the mean shape of the second location mentioned below each image.



Central Pacific vs Greater Caribbean
ANOVA p= 0.64

Central Pacific vs Hawaii
ANOVA p= 0.24

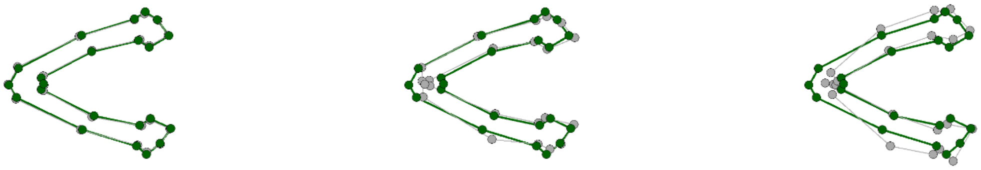
Central Pacific vs Mexican Pacific
ANOVA p= 0.34



Central Pacific vs West Indian
ANOVA p= 0.13

Central Pacific vs. Tropical Atlantic
ANOVA **p=0.02**

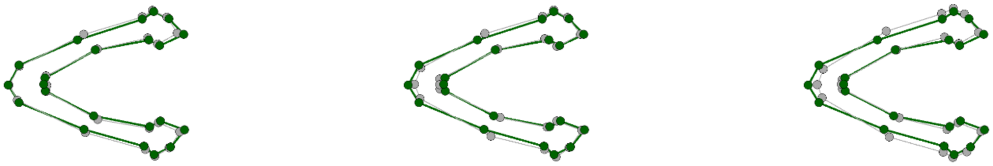
Greater Caribbean vs. Hawaii
ANOVA p= 0.05



Greater Caribbean vs. Mexican Pacific
ANOVA p= 0.13

Greater Caribbean vs. West Indian
ANOVA **p=0.02**

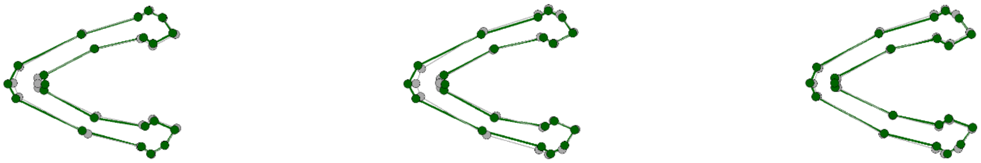
Greater Caribbean vs. Tropical Atlantic
ANOVA **p=0.01**



Hawaii vs.Mexican Pacific
ANOVA p= 0.99

Hawaii vs. West Indian
ANOVA p= 0.61

Hawaii vs. Tropical Atlantic
ANOVA p= 0.11



Mexican Pacific vs. West Indian
ANOVA p= 0.70

Mexican Pacific vs. Tropical Atlantic
ANOVA p= 0.18

West Indian vs. Tropical Atlantic
ANOVA p= 0.32

Figure 6 Shape differences between locations (Procrustes ANOVA) for the mandible view of green turtle *Chelonia mydas* skulls using geometric morphometrics. Images were generated using Geomorph for R (Adams and Otárola-Castillo 2013). The green outline is the mean shape for the first location named at the bottom of the image. The grey one is the mean shape of the second location mentioned above each image.

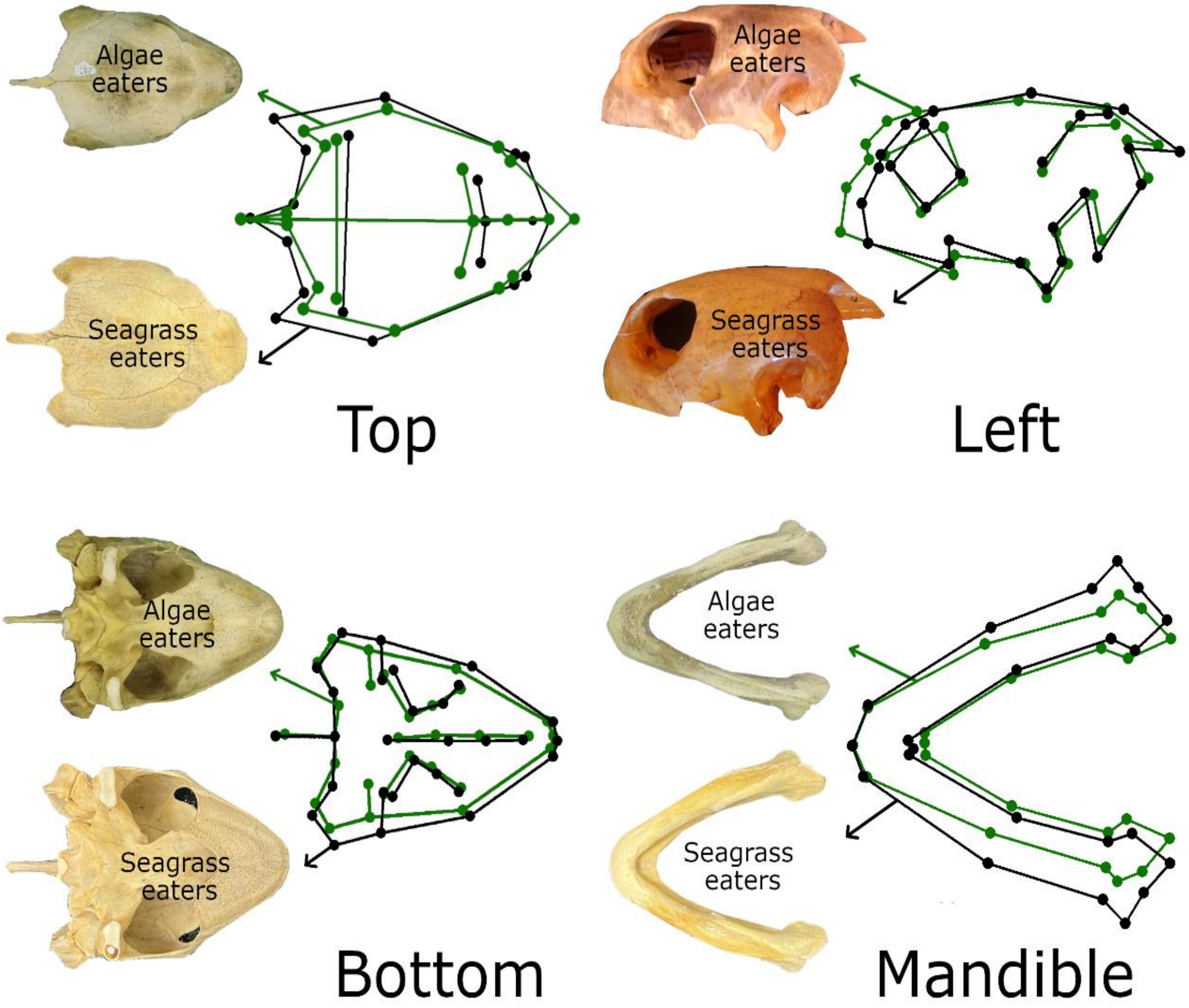


Figure 7 Comparison between green turtle *Chelonia mydas* skulls from algae (green outline) and seagrass eaters (black outline/). The outlines were generated with Geomorph in R (Adams and Otárola-Castillo 2013).

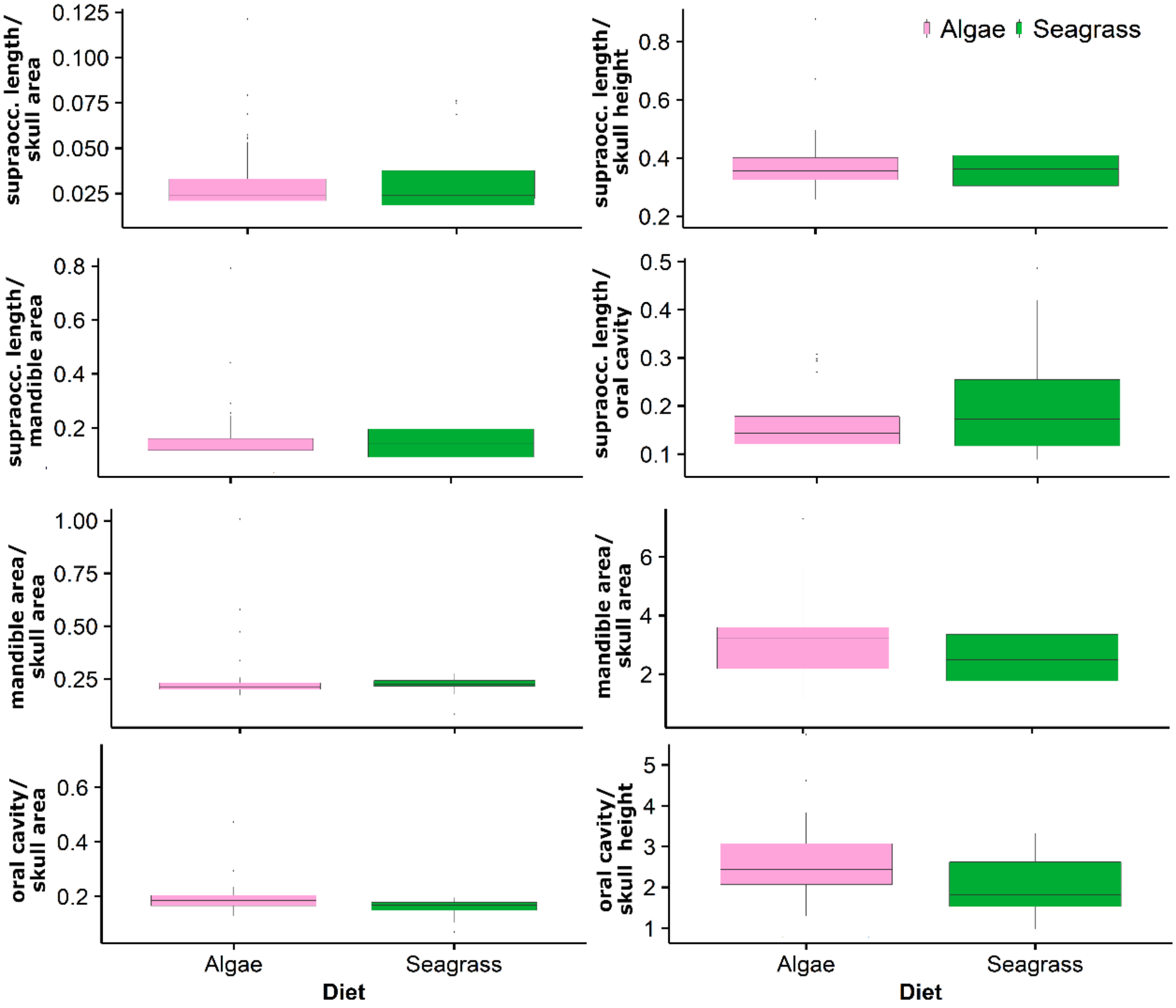


Figure 8 Ratios of supraoccipital bone length (cm), skull area (cm^2), skull height (cm) and oral cavity size (cm^2) ratios between green turtle *Chelonia mydas* algae and seagrass eaters.

Discussion

The results reported here reveal significant interpopulation variability in the shape of the skull and mandible of green turtles. The two seagrass eating populations considered here did not differ in skull morphology, whereas one of them differed from several populations of macroalgae consumers. On this ground, we pooled together all the specimens with similar diets at the population level (seagrasses vs. macroalgae) and compared their skulls and mandibles. The results confirmed the existence of differences in the skull and mandible morphology between seagrass and macroalgae consumers, although the idealized macroalgae consumer resulting from this pooling do not exist anywhere, because Hawaiian and Mexican populations differed consistently in skull shape. It is worth noting that the skull morphology of the average macroalgae consumers is more similar to that of juveniles (long, narrow, with weaker bite force and more suited for suction feeding) than that of adults. This is consistent with the smaller skull size of algae consumers already noted by Kamezaki and Matsui (1995) and suggests that differences emerged independently through neoteny in different populations inhabiting regions with a scarcity of seagrasses. This hypothesis is supported by the pattern of ontogenetic diet change reported for green turtles in the Greater Caribbean, where recently settled juveniles forage mostly on macroalgae and increased the consumption of seagrasses as they grow older (Foley et al. 2007).

Seagrasses and macroalgae differ largely in nutrient composition and digestibility for green turtles, with red macroalgae standing as the most profitable source, brown macroalgae as the less valuable source and seagrasses in between (Bjorndal 1980; McDermid et al. 2007; Campos and Cardona 2020). They also differ in ecology, as seagrasses usually occur in monospecific meadows (Hemminga and Duarte 2000), whereas macroalgae form complex communities, including many species. This suggests that unselective grazing is the most efficient way to exploit monospecific seagrass meadows, whereas careful picking of the most nutritious species is required to exploit species-rich macroalgal communities. This trade-off correlates well with the morphological dichotomy reported here, as short, rounded snouts and broad mouths are well suited for unselective grazing, and more elongated snouts are better suited for selectively browsing (Chatterji et al. 2022). However, green turtles are not completely unselective while grazing on sea grasses, as they prefer shorter blades with a lower lignin content, and by re-grazing the same plot, they promote the growth of short blades with a higher nutritional quality (Bjorndal 1980).

Little is actually known about the foraging behavior of green sea turtles, but available evidence reveals continuous foraging during light hours of green turtles browsing on algal pastures (Makowski et al. 2006) and discontinuous foraging for those grazing on seagrass meadows, where they usually exhibit two foraging peaks, each one lasting about 2 hours (Okuyama et al. 2013). Furthermore, video recording reveals that once in a suitable seagrass patch, green turtles forage continuously, with distinct head movements to bite and chew seagrasses (Okuyama et al. 2013). Conversely, green turtles browsing on macroalgae spend long periods examining the seabed before taking a bite, and hence food consumption is highly discontinuous (Seminoff et al. 2006; Burkholder et al. 2011; Fukuoka et al. 2019). Although more studies describing in detail the underwater foraging behavior of green turtles are necessary, the available evidence suggests a more selective food choice by green turtles in algal pastures compared to seagrass meadows, consistent with the browsing/grazing dichotomy revealed by skull morphology. This would also suggest that food intake per time unit is lower in macroalgal pastures than in seagrass meadows, which results in continuous foraging during light hours in the former and the existence of two daily foraging bouts in the latter.

An interesting result of video recording of food handling by green turtles is the evidence of lateral (propalineal) jaw movements (Okuyama et al. 2013; Marshall et al. 2014). This is unparalleled in other herbivorous reptiles (Fritz et al. 2010) and may explain why the bite force in green turtles is much higher than that necessary to cut the blades of seagrasses (Marshall et al. 2014). If this hypothesis is correct, increasing bite force with turtle size would facilitate the processing of tough seagrass fiber to break blades into smaller pieces, which would enhance digestion (Fritz et al. 2010) and would explain why seagrass pieces in the stomach of green turtles have a similar length across localities (Gulick et al. 2021).

In conclusion, the results reported here revealed that interpopulation variability in the skull shape of the green turtle is related to diet and that those populations relying heavily on macroalgae retain some characteristics typical of the skull of juvenile green turtles that make it suitable for browsing selectively.

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5. CHAPTER 3: ISOTOPIC ECOLOGY OF HAWAIIAN GREEN TURTLES AND RELIABILITY OF STABLE ISOTOPES ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) IN BONE SAMPLES FOR DIETARY STUDIES

Abstract

Stable isotope analysis of tissues from specimens preserved in museum collections is particularly interesting in historical ecology because it allows for reconstructing temporal changes in resource use. However, tissues differ in their turnover rates. Their stable isotope ratios might not be informative of the diet immediately prior to death, particularly in species with complex ontogenetic diet changes. This study assessed the effect of body size and sampling locality on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of the epidermis and two skeletal elements with a thick core of trabecular bone (rib and squamosal bones) of Hawaiian green turtles (*Chelonia mydas*). Bone samples were obtained from East Oahu (21.449° N, 157.801° W) and Kona/Kohala coast (19.603° N, 156.009° W) from 2018-2020, along with prey samples in 2018 and 2019 from the same localities. Results revealed a similar effect of body size and sampling locality on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ of the three tissues, thus suggesting the stable isotope ratios in the squamosal and rib of green turtles settled in neritic habitats are informative of diet prior to death. On the other hand, mixing models combining the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of the epidermis with the TDF derived experimentally for epidermis indicated a diet make-up consistent with that described in previously published gut content analysis. However, mixing models combining the stable isotope ratios in rib and squamosal and the TDF derived experimentally for cortical bone failed to do so. This result suggests that the TDF of cortical bone probably differs from that of trabecular bone and should be derived experimentally before the stable isotope ratios from trabecular bone can be used in mixing models.

Introduction

Green turtles (*Chelonia mydas*) are megaherbivores occurring in the coastal ecosystems of tropical and warm temperate regions worldwide (Wallace et al. 2010). Most populations are currently rebounding after centuries of over-harvest and decades of conservation efforts (Chaloupka et al. 2008; Kondo et al. 2017; Casale et al. 2018), but little is known about the impact of overharvesting on the trophic ecology of green turtles (Turner Tomaszewicz et al. 2022). Green turtles are oceanic carnivores during their first years of life (Reich et al. 2008; Parker et al. 2011; Vélez-Rubio et al. 2015;

Bjorndal 2017) but shift to a plant-based diet after settlement to near-shore habitats (Reich et al. 2008). Nevertheless, the diet of neritic green turtles is more flexible than initially thought and ranges from pure herbivory to plant-based omnivory, depending on food availability and environmental temperature (Turner Tomaszewicz et al. 2018; Esteban et al. 2020). Demographic changes in green turtle populations might have modified the intensity of intraspecific competition and hence altered habitat use patterns, as reported for other species of marine vertebrates, such as the California sheepshead (Braje et al. 2017), the Antarctic fur seal (Hanson et al. 2009), the South American sea lion (Zenteno et al. 2015b) or the Atlantic cod (Ólafsdóttir et al. 2021).

For sea turtles, the stable isotope analyses (SIA) of skull bone and carapace keratin offer the possibility of studying historical diet changes after settlement. Still, current knowledge about the temporal integration of dietary information in the stable isotope ratios of bone tissue in sea turtles is limited to the cortical portion of the humerus bone, which is widely used for skeletochronology (Snover et al. 2011; Turner Tomaszewicz et al. 2016). Moreover, stable isotope ratios fractionate during metabolism, which results in animal tissues having higher values than their diet (DeNiro and Epstein 1978). This difference is called trophic discrimination factor (TDF), and it is tissue-specific because each tissue has a different metabolism, biochemical composition, and routing pathways (Bearhop et al. 2002). This is why experimentally determined TDF values have to be incorporated into models aiming to reconstruct the diet of animals using stable isotope ratios (Stock et al. 2018).

The skeletal elements of turtles consist of a core of trabecular bone surrounded by an outer layer of cortical bone of variable thickness, as in mammals (Bjorndal et al. 2003; Snover and Hohn 2004; Snover et al. 2007b). However, turtles grow seasonally throughout their lives (Omeyer et al. 2017) and layers of new cortical bone, delimited by lines of arrested growth, are formed annually in the humerus and other long bones (Bjorndal et al. 2003; Snover and Hohn 2004; Snover et al. 2007b; Snover et al. 2011). Because cortical bone experiences little remodeling in sea turtles, stable isotope analysis and skeletochronology can be combined in longitudinal studies to assess ontogenetic dietary changes (Snover et al. 2010; Avens and Snover 2013; Ramirez et al. 2015; Turner Tomaszewicz et al. 2016; Turner Tomaszewicz et al. 2017; Turner Tomaszewicz et al. 2018) which allows a detailed reconstruction of historical changes in the trophic ecology of sea turtles. Unfortunately, humeri are less common in scientific collections, where skulls and carapaces prevail (e.g., https://vertnet.org).

The cortical layer of the skull and carapace skeletal elements is much thinner than the humeri. It cannot be sub-sampled along the lines of arrested growth with current technology. Bones such as the squamosal (in the skull) and ribs have a large core of trabecular bone (Arencibia et al. 2006; Jones et al. 2012), which is constantly remodeled (Castanet and Smirina 1990) and in mammals, integrates dietary information during a relatively brief period prior to death (Skedros et al. 2013; Fahy et al. 2017). If this also stands for sea turtles, the stable isotope ratios of skeletal elements' cross-sections with a large core of trabecular bone and a thin layer of cortical bone, like the skull and rib used in this study, could be informative about this recent period, with a minor contribution of the diet from earlier life stages.

Here, we sampled dead stranded green turtles in two areas of the main Hawaiian Islands to compare the dietary information derived from the stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ from squamosal and rib bones with the isotope ratios of the same isotopes in the epidermis. The epidermis of sea turtles integrates dietary information over several months (Reich et al. 2008), and hence, it is the preferred tissue to study the recent trophic ecology of modern green turtles using stable isotope analysis (Arthur and Balazs 2008; Lemons et al. 2011; Prior et al. 2016; Vélez-Rubio et al. 2016; Gillis et al. 2018; Pagès Barceló et al. 2021). If stable isotope ratios in rib and squamosal show the same geographic and ontogenetic variability as the epidermis from the same individuals, the bones could be used to assess the green turtle diet during that brief period before death that is typically revealed from SIA of epidermis tissue. Furthermore, we tested if the TDF values found in the literature and derived from the cortical bone of green turtles (Turner Tomaszewicz et al. 2017) are appropriate to apply to stable isotope ratios of squamosal and rib bone in mixing models to assess the contribution of different sources to the diet of green turtles (Boecklen et al. 2011; Layman et al. 2012; Gillis et al. 2018).

Materials and methods

Turtle sample collection

Samples were taken from 15 dead-stranded green turtles from East Oahu and 18 dead-stranded green turtles from the Kona/Kohala coast in the west of the island of Hawaii (Figure 1 and Table 1). East Oahu supports a diversity of coastal ecosystems, including non-native mangroves, coral reefs, and coastal lagoons with seagrass meadows and coral rubble covered by macroalgae (Allen 1998; Cheroske et al. 2000; McDermid et al. 2002; McDermid et al. 2003; McDermid and Stuercke 2003; McDermid et al. 2007; Friedlander et al. 2008; Williams et al. 2008). The region also supports a large human population (37.430 persons according to the U.S Census Bureau 2020), and Kāneʻohe Bay suffered eutrophication due to improper management of the wastewater collection from 1951 until 1978 (Laws and Taguchi 2018) and the presence of inorganic nutrients in industrial, urban, and agricultural wastes (Lesser 2021). In contrast, rocky shores with fringing reefs prevail on the Kona/Kohala coast, where macroalgae are scarce, and seagrass occurs primarily in water deeper than 20 m depth (McDermid and Stuercke 2003; Friedlander et al. 2008; Wabnitz et al. 2010; Cardona et al. 2020a). Furthermore, Kona/Kohala coast receives much less precipitation and runoff than East Oahu, and thus it is oligotrophic (Adolf et al. 2019).

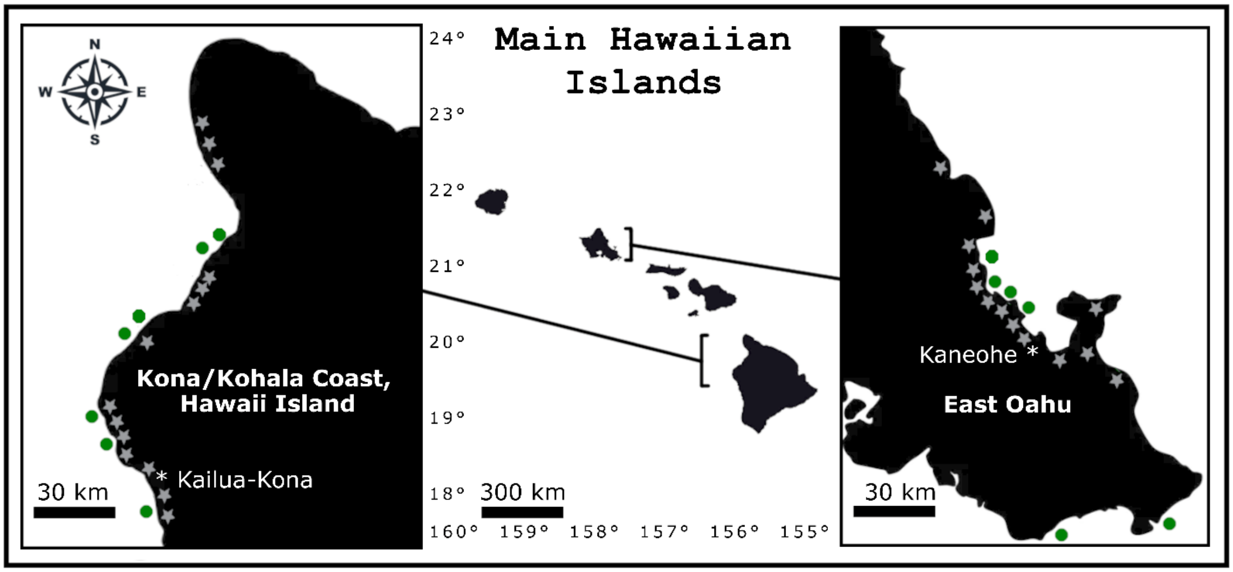

Figure 1 Sampling locations on East Oahu and Kona/Kohala coast, Hawaii. The green circles indicate places where turtles were found dead-stranded. A grey star represents locations for prey sampling. Prey sampling locations on East Oahu were: Wailupe, Kāneʻohe flats, Kāneʻohe reef, Kāneʻohe sandbar, Wawamalu; and on the Kona/Kohala coast: Kiholo, Waialea, Kaloko-Honokōhau National Historical, Waialea, Honokōhau Kalaoa small harbor. Maps modified from R tmap (Tennekes 2018)

Table 1 Mean stable isotope ratios and standard deviation for squamosal, rib, and epidermis of green turtles from East Oahu and Kona/+Kohala coast. Carbon ratios were corrected for acidification and lipid extraction (Eq. 1, see Methods). CCL for each island is shown as the mean and standard deviation

East Oahu (CCL=72 ± 13.4 cm)			**Kona/Kohala coast (CCL=53 ± 17.4 cm)**		
Squamosal (n=15)			**Squamosal (n=18)**		
$\delta^{13}C$	**$\delta^{15}N$**	**$\delta^{34}S$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{34}S$**
-11 ± 1.2	7 ± 1.2	14 ± 1.3	-12 ± 1.9	9 ± 1.3	16 ± 1.1
Rib (n=15)			**Rib (n=18)**		
$\delta^{13}C$	**$\delta^{15}N$**	**$\delta^{34}S$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{34}S$**
-12 ± 1.9	7 ± 2.0	15 ± 1.3	-11 ± 2.1	9 ± 1.5	15 ± 1.8
Epidermis (n=13)			**Epidermis (n=12)**		
$\delta^{13}C$	**$\delta^{15}N$**	**$\delta^{34}S$**	**$\delta^{13}C$**	**$\delta^{15}N$**	**$\delta^{34}S$**
-13 ± 2.6	8 ± 1.6	18 ± 1.5	-13 ± 1.7	10 ± 1.2	17 ± 1.6

Upon recovery, the dead-stranded green turtles were frozen at -20ºC. Necropsies were conducted by the Pacific Islands Fisheries Science Center (NOAA) staff in Oahu, Hawaii. Curved carapace length (CCL) was measured, and a piece of the skull (squamosal bone) and rib bone was cut from each turtle, washed and, air dried. A cross-section of bone was then drilled with Dremel 0.1-inch drill bits. The collected powder (approximately 50 mg) was dried at 50 ºC for 24 h and sent to the Isotopes laboratory at Washington State University for SIA. Epidermis samples were taken from the shoulder region using a razor blade, except for turtles in advanced decomposition, from which no samples were taken. Epidermis samples were preserved in 2-ml cryovials with saturated salt solution and were stored at -20°C (Seminoff et al. 2021). Before analysis, epidermis samples were rinsed with deionized water, dried at 50ºC for 24 h, ground to a fine powder, and sent to the same laboratory mentioned above for SIA.

Prey sample collection

Previous gut content studies had 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 decipens,* and *Halophila hawaiiana* supplying less than 15% of the ingested material (Russell et al. 2003; Arthur and Balazs 2008; Russell and Balazs 2009; Russell and Balazs 2015). Conversely, the diet of green turtles foraging off the Kona/Kohala coast was dominated by red macroalgae in the genus *Pterocladiella* (Arthur and Balazs 2008). Hawaiian green turtles also consume lesser amounts of animal prey (Arthur and Balazs 2008; Russell et al. 2011). Those on the Kona/Kohala coast are observed regularly scavenging fish scraps at the Honokōhau Small Boat Harbor (G. Balazs, personal observation). Accordingly, we sampled the macroalgae *A. spicifera*, *G. salicornia*, and *H. musciformis*, the seagrass *H. hawaiiana*, and turf algae from East Oahu, and the macroalgae *G. salicornia*, *H. musciformis,* and *P. capillacea* and turf algae from the Kona/Kohala coast. 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 and Vermeij 2016). Both areas (East Oahu and the Kona/Kohala coast) were sampled at the end of the dry (September 2018) and rainy seasons (February 2019). Red mangrove leaves (*Rhizophora mangle*) and scraps of mahi-mahi (*Coryphaena hippurus*) were collected at Wawamalu (East Oahu) and Honokōhau Small Boat Harbors (Kona/Kohala coast), respectively, in March 2021. Samples were cleaned of sand and debris, rinsed with deionized water, dried at 50ºC for 24 h, and ground to a fine powder.

Stable Isotope Analysis

In this study we did not use chemical acidification or lipid extraction since both treatments require larger amounts of sample that were not available. In order to correct the samples, we analyzed 94 bone samples of green turtles from the tissue collection at the University of Barcelona and calculated a calibration equation that could be used to correct mathematically the $\delta^{13}C$ values of our samples based on the C : N ratios (Turner Tomaszewicz et al. 2015). Bone samples used for SIA are typically demineralized with HCl, and lipids are removed with organic solvents before the analysis of C and N stable isotope ratios (Bas and Cardona 2018; Bas et al. 2020) to remove lipids (highly depleted in ^{13}C compared to protein) and non-collagen proteins, which are more variable in composition and hence in $\delta^{15}N$ values than collagen (Koch et al. 2007; Turner Tomaszewicz et al. 2015). However, acidification modifies the $\delta^{34}S$ values (Connolly and Schlacher 2013), and the measurement of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ requires sub-sampling, hence a larger amount of sample.

To calculate this equation, the green turtle bone samples from the University of Barcelona were ground to a fine powder with mortar and pestle and split into two subsamples of approximately 20 mg each. Unprocessed subsamples were processed for stable isotope analysis without further treatment. The other subsamples were first demineralized (dml hereafter) by soaking in 0.5 N HCl for 24 h, rinsed with deionized water, dried again at 50°C for 24 h, and lipids were removed through sequential rinses with 2:1 chloroform:methanol until the solution was clear (Folch et al. 1957). After lipid extraction, subsamples were dried at 50°C for 24 h. Powder from each type of subsample was weighed (~0.3 mg) in tin capsules at CCiT (Centres Científics i Tecnològics de la Universitat de Barcelona), combusted at 900°C, gases separated and analyzed in a continuous flow isotope ratio mass spectrometer (Flash 1112 IRMS Delta C Series EA, Thermo Finnigan, Bremen). Isotopic reference materials were interspersed with samples for calibration, and the contribution of ^{17}O was corrected by the IRMS software using the Santrock correction (Santrock et al. 1985). Carbon isotopic results are reported in parts per thousand (‰) relative to VPDB (Vienna Peedee Belemnite), and N isotope ratios are reported ‰ relative to N_2 in air.

Similarly, for carbon and nitrogen isotopic analysis, green turtle skin and bone samples from Hawaii were ground to fine powder, weighed (5 mg), and encapsulated in tin cups at the Stable Isotope Core Laboratory at Washington State University, combusted in an elemental analyzer (ECS 4010, Costech Analytical Valencia, CA), gases separated with a 3-m GC 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. The contribution of ^{17}O was corrected by the IRMS software using the Santrock correction (Santrock et al. 1985). Carbon isotopic results are reported in parts per thousand (‰) relative to VPDB (Vienna Peedee Belemnite) using NBS 19 and L-SVEC as anchor points. Nitrogen isotope ratios are reported in ‰ in relative to N_2 in air.

Sulfur isotopic analysis of Hawaiian green turtles' samples was conducted, independently on the same samples. The powder was weighed (10 mg in bone and epidermis and 5 mg in algae) and encapsulated at the Stable Isotope Core Laboratory at Washington State University, combusted with the same elemental analyzer as C and N samples, and SO_2 gases were separated with a 0.8-m GC column (100°C) and analyzed with a continuous flow isotope ratio mass spectrometer (Delta Plus XP, Thermo Finnigan, Bremen) (Qi and Coplen 2003). The final determination of ^{34}S was based on the collection of ions 64 and 66. 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, Alpha Aesar BaSO4, Salt Lake Medals Ag_2S, Acros elemental S). A grizzly bear hair (*Ursus arctos horribilis*) sample from Pullman, WA, where the stable isotopes laboratory is located, was also run with all samples as a blind QC. Isotopic results are expressed in delta (δ) notation relative to VCDT (Vienna-Canyon Diablo Troilite) in parts per thousand (‰).

Statistical analysis

Paired Student's t-tests were run in R version 4.1.1 (2021-08-10) (R Core Team 2018) to analyze if there were differences in the $\delta^{13}C$ values between untreated samples and the same samples after acidification and lipid extraction. Pearson correlation coefficients were calculated for the individual values of each stable isotope ratio in each tissue, separately for each island, to explore ontogenetic diet changes. Then, generalized linear models were run in IBM SPPS v. 25 to assess if locality (East Oahu and Kona/Kohala coast) and tissue type (squamosal, rib, and epidermis separately) had any significant effect on the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of turtles after accounting for TDF (see below). MixSIAR (Stock et al. 2018) was used in R to run Bayesian mixing models to analyze $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in green turtles and their putative prey, following the MixSIAR model framework, which estimates the proportion contributions of the source (prey) to the mixture (green turtle). All JAGS models (Just Another Gibbs Sampler) were run at normal configuration (chain length = 100000, burn = 50000, thin = 50, chains = 3). The TDF values used were as follows: $X \pm SE = +1.9 \pm 0.3$ ‰ for $\delta^{13}C_{epidermis}$ and $+4.1 \pm 0.4$ ‰ for $\delta^{15}N_{epidermis}$, $+1.7 \pm 0.6$ ‰ for $\delta^{13}C_{bone}$ and $+5.1 \pm 1.1$ ‰ for $\delta^{15}N_{bone}$, according to Turner Tomaszewicz et al. (2017). TDF for $\delta^{34}S$ was assumed to be 0 (McCutchan Jr et al. 2003).

Results

The difference between the $\delta^{13}C$ values of the paired unprocessed samples and the same samples after acidification and lipid extraction ranged from 1.6 ‰ to 5.7 ‰ (X ± SE = 4.1 ± 1.1 ‰), and the difference between the untreated samples and the treated samples was statistically significant (paired Student's t-test; $t = 12.754$, $p<0.001$). The relationship between the $\delta^{13}C$ values of these paired unprocessed samples and the same samples after acidification and lipid extraction was best described by the following equation:

$$\delta^{13}C_{dml} = \delta^{13}C_{unp} - 3.658 + (3.372 \times \ln (C{:}N)_{unp}) \quad (r^2 = 0.805, p<0.001) \quad \text{(Eq. 1)}$$

Where $\delta^{13}C_{dml}$ denotes the $\delta^{13}C$ value after acidification and lipid extraction, $\delta^{13}C_{unp}$ denotes the $\delta^{13}C$ value in unprocessed samples, and $(C{:}N)_{unp}$ is the atomic ratio of unprocessed samples. Accordingly, this equation was used to calibrate the $\delta^{13}C_{unp}$ values of all bone samples for green turtles from Hawaii.

The green turtles from Hawaii ranged 45.1-102.5 cm CCL (X ± SE = 64 ± 17 cm, n = 13) at East Oahu and 39.0-98.6 cm CCL (X ± SE = 57 ± 16 cm, n = 14) at Kona/Kohala coast. Rib samples were collected from all turtles included in this study. Squamosal and epidermis samples were missing from two turtles due to severe decomposition or because the head (skull bone) was missing when individuals were found. The mean stable isotope ratios of each tissue from green turtles on each island are reported in Table 1, and the results of the Pearson correlation analysis are reported in Table 2. Correlations were calculated for the individual values of each stable isotope ratio in each tissue, separately for each island. The $\delta^{13}C$ and $\delta^{15}N$ values were negatively correlated, and $\delta^{13}C$ and $\delta^{34}S$ were uncorrelated in the three tissues when all turtles were considered. However, $\delta^{15}N$ and $\delta^{34}S$ were positively correlated in the squamosal bone but uncorrelated in the epidermis and the rib bone (Table 2).

Table 2 Pearson correlations between the stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in the epidermis (epi), squamosal bone (sq), and rib bone of green turtles from Hawaii.

Only significant correlations between the stable isotope ratios of the same element across tissues or different elements within the same tissue are shown

Tissue	**$\delta^{13}C_{rib}$**	**$\delta^{13}C_{epi}$**	**$\delta^{15}N_{sq}$**	**$\delta^{15}N_{rib}$**	**$\delta^{15}N_{epi}$**	**$\delta^{34}S_{sq}$**	**$\delta^{34}S_{rib}$**	**$\delta^{34}S_{epi}$**
$\delta^{13}C_{sq}$	r=0.93 p=<0.001 n=33	r=0.54 p=<0.006 n=25	r=-0.69 p=<0.001 n=33					
$\delta^{13}C_{rib}$		r=0.68 p=<0.001 n=25		r=0.65 p=<0.001 n=33				
$\delta^{13}C_{epi}$					r=-0.47 p=<0.015 n=25			
$\delta^{15}N_{sq}$				r=0.91 p=<0.001 n=33	r=0.67 p=<0.001 n=25	r=0.44 p=<0.010 n=33		
$\delta^{15}N_{rib}$					r=0.63 p=<0.001 n=25			
$\delta^{15}N_{epi}$								
$\delta^{34}S_{sq}$								
$\delta^{34}S_{rib}$								

No significant difference was found between the δ^{13}C values of green turtles from East Oahu (X ± SE = -12 ± 0.9 ‰ in skull, X ± SE = -12 ± 1.2 ‰ in rib, X ± SE = -13 ± 1.9 ‰ in epidermis) and Kona/Kohala coast (X ± SE = -12 ± 1.9 ‰ in skull, X ± SE = -11 ± 2.0 ‰ in rib, X ± SE = -13 ± 1.7 ‰ in epidermis) (Figure 2) for any of the three tissues or between the δ^{13}C values of squamosal and rib bone, although epidermis was significantly depleted in ^{13}C compared to bone (GLM, F (5,92) = 3.048, p = 0.014), tissue (GLM, F (2,92) = 6.985, $p<0.002$), island (GLM, F (1,92) = 0.305, p = 0.582) and their interaction (GLM, F (2,92) = 0.415, p = 0.662).

The three tissues were significantly more enriched in ^{15}N in green turtles from Kona/Kohala coast (X ± SE = 9 ± 1.3 ‰ in skull, X ± SE = 9 ± 1.4 ‰ in rib, X ± SE = 10 ± 1.1 ‰ in epidermis) relative to those from East Oahu (X ± SE = 8 ± 1.2 ‰ in skin, X ± SE = 8 ± 1.3 ‰ in skull, X ± SE = 8 ± 1.4 ‰ in rib), and no significant differences were found among the δ^{15}N values of the three tissues within each area (GLM, F (5,92) =

5.146, $p<0.001$), tissue (GLM, F (2,92) = 1.073, p = 0.346, island (GLM, F (1,92) = 23.508, $p<0.001$ and interaction (GLM, F (2,92) = 0.198, p = 0.820).

Finally, $\delta^{34}S$ values did not differ between green turtles at the two islands for any tissue (X ± SE = 14 ± 1.3 ‰ in skull, X ± SE = 15 ± 1.5 ‰ in rib, X ± SE = 17 ± 1.9 ‰ in epidermis in Oahu and X ± SE = 16 ± 1.1 ‰ in skull, X ± SE = 15 ± 1.7 ‰ in rib, X ± SE = 17 ± 1.6 ‰ in epidermis in the Kona/Kohala coast).

The epidermis was significantly enriched in ^{34}S compared to squamosal and rib bone. However, the tissue and island interaction was significant because the average $\delta^{34}S$ value of the squamosal of the green turtles from East Oahu was lower than that of the Kona/Kohala coast. No difference existed between the average $\delta^{34}S$ value of the two areas for rib or epidermis (GLM, F (5,92) = 8.700, $p<0.001$), tissue (GLM, F (5,92) = 15.882, $p<0.001$), island (GLM, F (1,92) = 1.257, p = 0.265), interaction (GLM, F (5,92) = 5.718, p = 0.005).

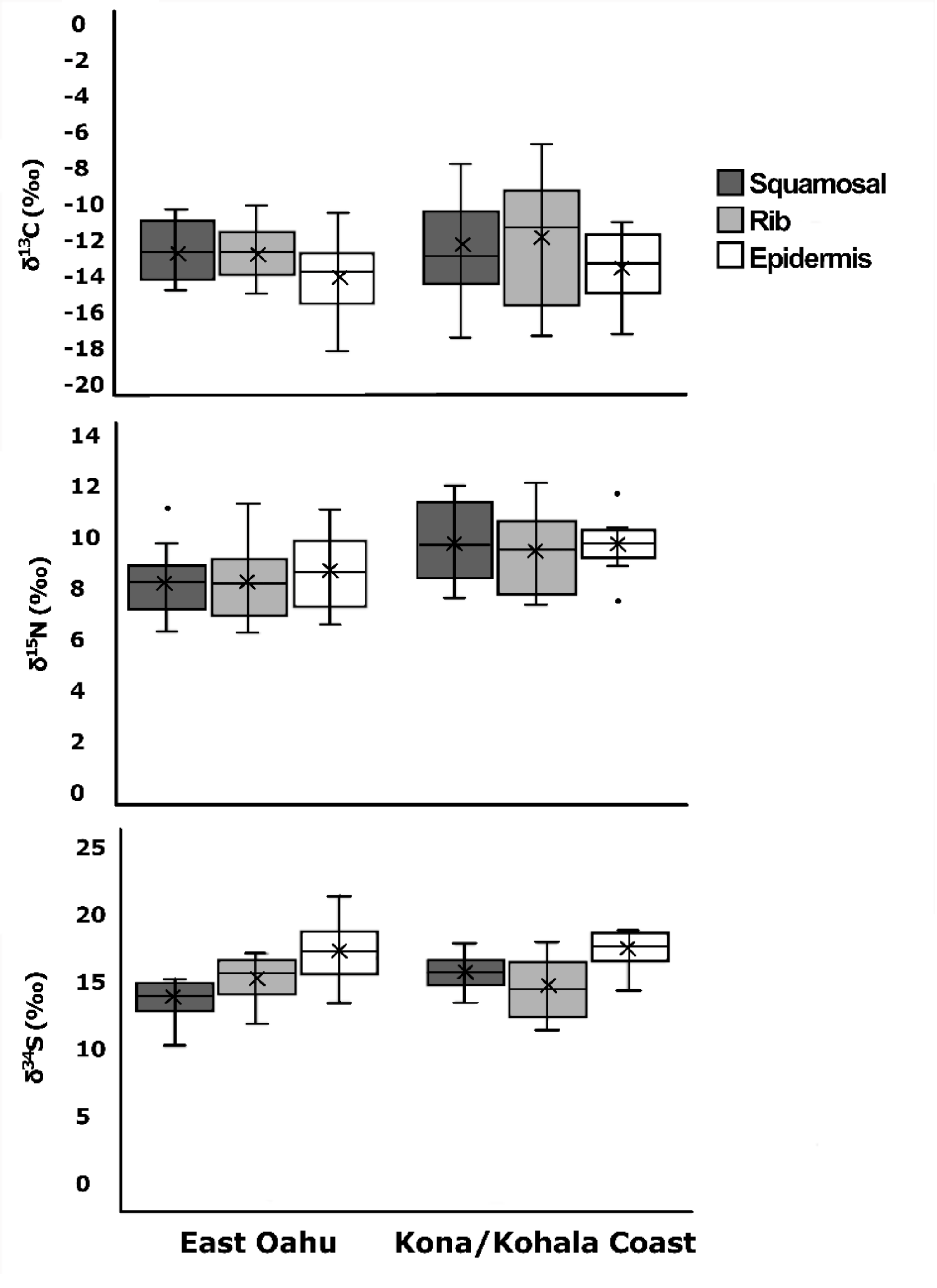

Figure 2 Stable isotope ratios of carbon, nitrogen and sulfur in squamosal, rib, and epidermis samples from the green turtle on East Oahu and Kona/Kohala coast. The whiskers represent the minimum and maximum values, and the dots the outliers. The mean is shown as an x, and the median as a line: the box represents the interquartile range (the bottom line of the box represents the median of the bottom half or 1st quartile, and the top line of the box represents the median of the top half or 3rd quartile).

The CCL of the green turtles from East Oahu was uncorrelated to $\delta^{13}C$ and $\delta^{15}N$ for all tissues, but it was negatively correlated to the $\delta^{34}S$ in rib (Table 3). The correlation between CCL and $\delta^{34}S$ in the squamosal approached significance (Pearson, r(13) = -0.54, p = 0.059). Conversely, CCL of green turtles from the Kona/Kohala coast was positively correlated with $\delta^{13}C$ of the epidermis, but correlation was not significant for the $\delta^{13}C$ values of squamosal (Pearson, r(16) = 0.46, p = 0.076) and rib (Pearson, r(13) = 0.48, p = 0.063). Turtle size (CCL) was negatively correlated to the $\delta^{15}N$ value of squamosal and epidermis but was not significant for rib (Pearson, r(16) = -0.47, p = 0.064). Finally, CCL was uncorrelated to the $\delta^{34}S$ values of all tissues.

Table 3 Pearson correlations between turtle size (CCL) and the stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in the squamosal bone (sq), rib bone, and epidermis (epi) of green turtles from East Oahu and the Kona/Kohala coast. Only significant correlations are shown

East Oahu									
	$\delta^{13}C_{sq}$	$\delta^{13}C_{rib}$	$\delta^{13}C_{epi}$	$\delta^{15}N_{sq}$	$\delta^{15}N_{rib}$	$\delta^{15}N_{epi}$	$\delta^{34}S_{sq}$	$\delta^{34}S_{rib}$	$\delta^{34}S_{epi}$
CCL								r=-0.73 p=0.003 n=14	
$\delta^{13}C_{sq}$		r=0.81 p=<0.001 n=15		r=-0.72 p=0.002 n=15	r=-0.74 p=0.002 n=15				
$\delta^{13}C_{rib}$				r=-0.78 p=<0.00 1 n=15	r=-0.84 p=<0.001 n=17				
$\delta^{13}C_{epi}$						r=-0.78 p=0.002 n=13			
$\delta^{15}N_{sq}$					r=0.92 p=<0.001 n=15	r=0.63 p=0.020 n=13			
$\delta^{15}N_{rib}$						r=0.66 p=0.015 n=13			
$\delta^{15}N_{epi}$									
$\delta^{34}S_{sq}$									
$\delta^{34}S_{rib}$									
Kona/Kohala coast									

CCL		r=0.66 p=0.027 n=11	r=-0.59 p=0.016 n=16		r=0.66 p=0.029 n=11
$\delta^{13}C_{sq}$	r=0.98 p=<0.001 n=18	r=0.70 p=0.010 n=12	r=-0.83 <0.001 n=18	r=-0.80 p=<0.001 n=18	
$\delta^{13}C_{rib}$		r=0.72 p=0.008 n=12	r=-0.83 p=<0.00 1 n=18	r=-0.78 p=<0.001 n=18	
$\delta^{13}C_{epi}$			r=-0.82 p=<0.00 1 n=12	r=-0.82 p=<0.001 n=12	r=-0.67 p=<0.017 n=12
$\delta^{15}N_{sq}$				r=0.89 p=<0.001 n=18	
$\delta^{15}N_{rib}$					
$\delta^{15}N_{epi}$					
$\delta^{34}S_{sq}$					
$\delta^{34}S_{rib}$					

Potential prey from East Oahu were pooled in four groups for later analysis: red mangrove leaves, seagrass (*H. hawaiiana*), macroalgae, and turf (Table 4). Red mangrove leaves were characterized by very low $\delta^{13}C$ and $\delta^{34}S$ values and very high $\delta^{15}N$ values compared to any other potential prey (Figure 3 and Figure 4). The second group included the seagrass *H. hawaiiana*. It was characterized by remarkably high $\delta^{13}C$ values, very low $\delta^{15}N$ values, and intermediate $\delta^{34}S$ values. Macroalgae (*A. spicifera*, *G. salicornia,* and *H. musciformi*s) and turf had $\delta^{13}C$ and $\delta^{15}N$ values intermediate between red mangrove leaves and *H. hawaiiana* and were highly enriched in ^{34}S compared to both. Turf was more enriched in ^{15}N than macroalgae and hence was included in a group different from macroalgae.

Table 4 Stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in green turtles' prey. Macroalgae include *Acanthophora spicifera*, *Gracilaria salicornia,* and *Hypnea musciformis*, and seagrass is *Halophila hawaiiana*

	$\delta^{13}C$	Stdv. $\delta^{13}C$	$\delta^{15}N$	Stdv. $\delta^{15}N$	$\delta^{34}S$	Stdv. $\delta^{34}S$	n
			East Oahu				
Macroalgae	-15.1	0.4	3.8	0.3	21	1.6	5
Seagrass	-8.1	0.6	2.1	0.3	18	2.3	10
Turf	-15.3	0.5	4.5	0.3	22	1	5

***Rhizophora* leaves**	-30.3	0.3	6.2	0.3	12	1.1	10
Kona/Kohala coast							
Gracilaria	-9.9	0.7	3.8	0	21.5	0.3	2
Hypnea-Pterocladiella	-15.4	0.6	3.9	0.2	22	1.5	4
Turf	-13.2	0.9	3.5	0.9	21	1.9	26
Mahi-mahi	-18.5	0.2	13.7	0.2	17	1	5

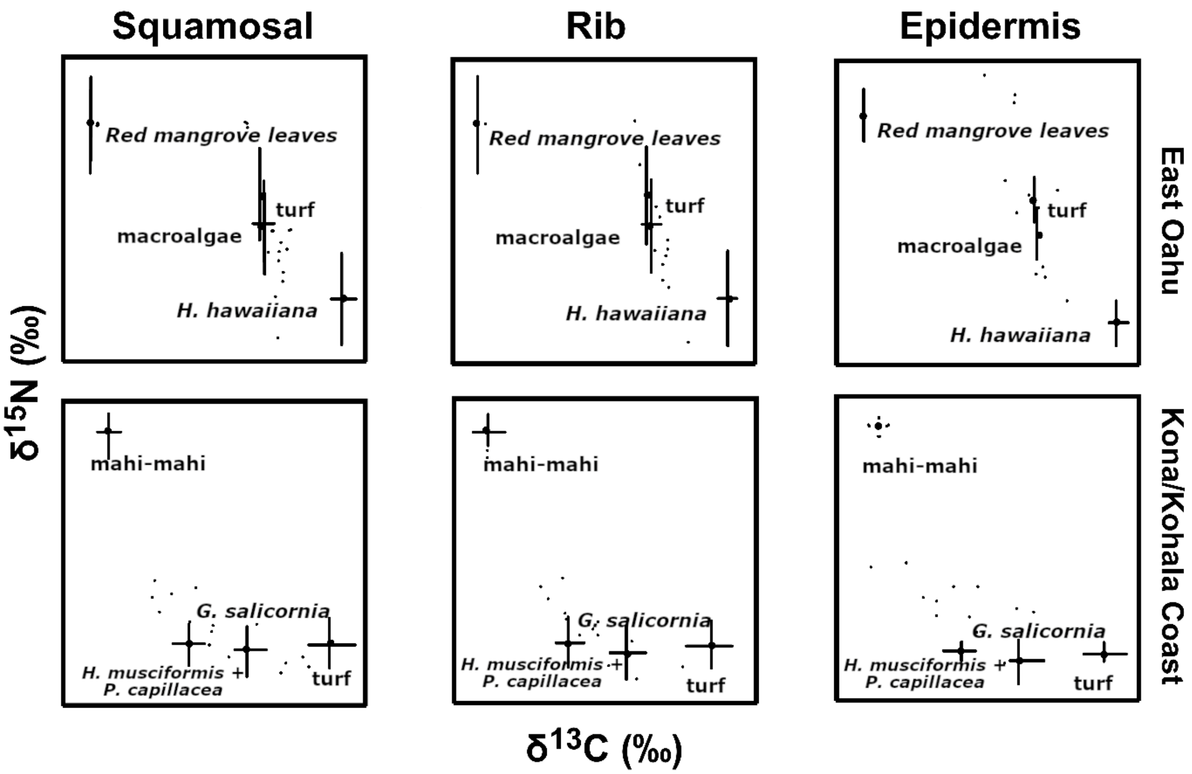

Figure 3 Isospace for δ^{13}C and δ^{15}N in green turtle bone (squamosal and rib) and epidermis on East Oahu and Kona/Kohala coast after correcting for the TDF (trophic discrimination factor). The TDF values were as follows: X ± SE = +1.9 ± 0.3 ‰ for δ^{13}C in bone and +4.1 ± 0.4 ‰ for δ^{15}N in the skin, and +1.7 ± 0.6 ‰ for δ13C and +5.1 ± 1.1 ‰ for δ^{15}N in bone, according to Turner Tomaszewicz et al. (2017) (2017).

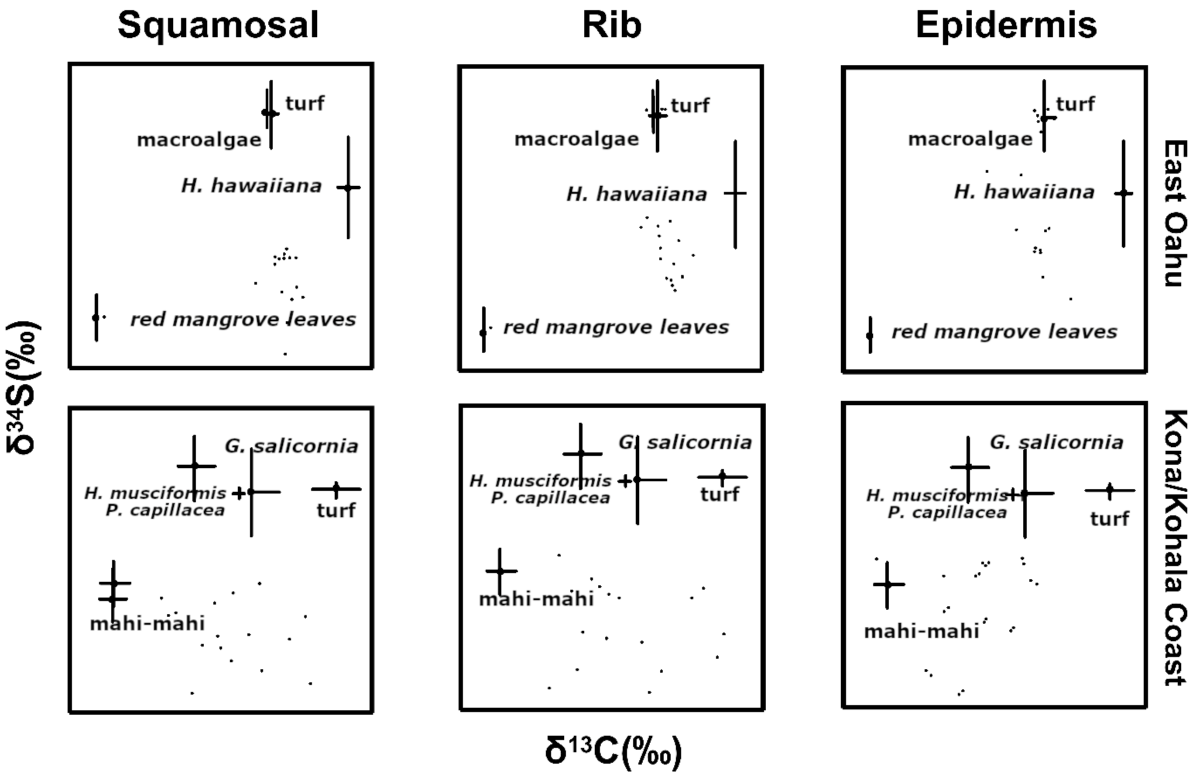

Figure 4 Isospace for $\delta^{13}C$ and $\delta^{34}S$ in green turtle bone (squamosal and rib) and epidermis on East Oahu and Kona/Kohala coast after correcting for the TDF (trophic discrimination factor). The TDF values were as follows: X ± SE = +1.9 ± 0.3 ‰ for $\delta^{13}C$ in skin and +1.7 ± 0.6 ‰ for $\delta^{13}C$ in bone, according to Turner Tomaszewicz et al. (2017). TDF for $\delta^{34}S$ was assumed to be 0 (McCutchan Jr et al. 2003)

Potential prey from the Kona/Kohala coast also differed in their $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values (Table 2). Mahi-mahi was the most isotopically distinct prey and was characterized by high $\delta^{15}N$ values and low $\delta^{13}C$ and $\delta^{34}S$. Algae and turf differed in $\delta^{13}C$ but not in $\delta^{15}N$ or $\delta^{34}S$ values. *G. salicornia* was more enriched in ^{13}C than any other potential prey, and turf was more enriched in ^{13}C than *H. musciformis* or *P. capillacea* (Figure 2). The latter two were pooled for statistical analysis and MixSIAR models.

Most of the squamosal, rib, and epidermis samples from East Oahu laid within the mixing polygon formed by turf, macroalgae, and *H. hawaiiana* in the $\delta^{13}C$ - $\delta^{15}N$ isospace after correcting for the TDF (Figure 3). However, $\delta^{34}S$ values in those three tissues were much lower than those of the three sources mentioned (Figure 4 and Figure 5) if TDF = 0. Only when red mangrove leaves were considered a potential food source did all the squamosal, rib, and epidermis samples from East Oahu lay within the mixing polygon. Hence, MixSIAR estimated that red mangrove leaves contributed approximately 15% to the assimilated diet of green turtles on East Oahu, independently of the tissue considered (Table 5). However, MixSIAR estimated disparate contributions of the remaining prey groups to the diet of green turtles from East Oahu depending on the tissue considered. According to $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ mixing model analyses for the epidermis, macroalgae dominated the diet of green turtles (X ± SE = 42.1 ± 33.5%), followed by *H. hawaiiana* (X ± SE = 29.3 ± 27.6%). However, credible intervals were loose for both. Conversely, $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ in bone, both squamosal and rib, identified *H. hawaiiana* as the main dietary source (rib: X ± SE = 74.8 ± 6.7%, squamosal: X ± SE = 73.4 ± 8.2%) and indicated a much lower contribution for macroalgae (rib: X ± SE = 3.8 ± 7.0%, squamosal: X ± SE = 5.8 ± 8.7%).

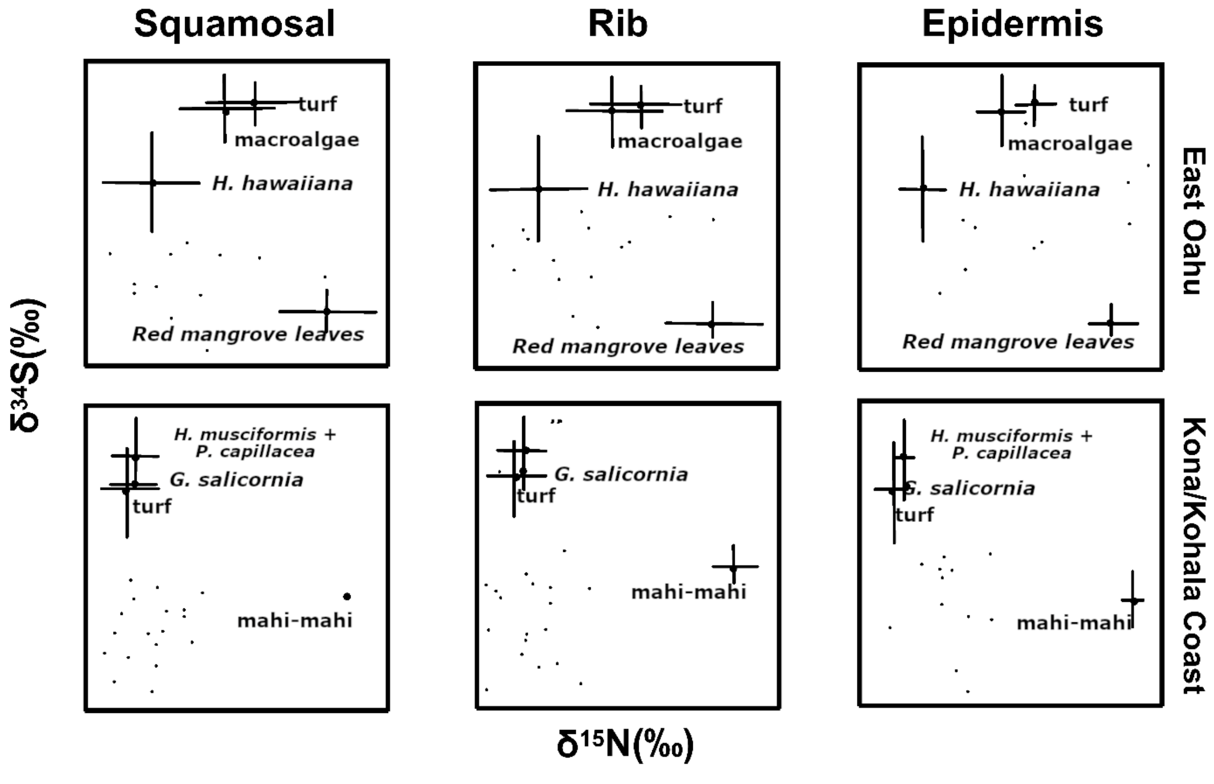

Figure 5 Isospace for $\delta^{15}N$ and $\delta^{34}S$ in green turtle bone (squamosal and rib) and epidermis on East Oahu and Kona/Kohala coast after correcting for the TDF (trophic discrimination factor). The TDF values were as follows: X ± SE = +1.9 ± 0.3 ‰ for $\delta^{13}C$ and +4.1 ± 0.4 ‰ for $\delta^{15}N$ in the skin, and +1.7 ± 0.6 ‰ for $\delta^{13}C$ and +5.1 ± 1.1 ‰ for

$\delta^{15}N$ in bone, according to Turner Tomaszewicz et al. (2017). TDF for $\delta^{34}S$ was assumed to be 0 (McCutchan Jr et al. 2003)

Table 5 Prey contribution to green turtle diet from the squamosal, rib, and epidermis tissue on East Oahu and Kona/Kohala coast estimated using MixSIAR

Tissue	Prey	Mean	Stdv.	5.0%	95.0%
		Windward Oahu			
Rib	***Halophila***	0.75	0.07	0.61	0.80
	Macroalgae	0.04	0.07	0.00	0.17
	***Rhizophora* leaves**	0.20	0.03	0.15	0.23
	Turf	0.02	0.03	0.00	0.07
Epidermis	***Halophila***	0.29	0.28	0.02	0.71
	Macroalgae	0.42	0.34	0.01	0.85
	***Rhizophora* leaves**	0.13	0.10	0.03	0.28
	Turf	0.16	0.23	0.00	0.73
Squamosal	***Halophila***	0.73	0.08	0.56	0.80
	Macroalgae	0.06	0.09	0.00	0.25
	***Rhizophora* leaves**	0.18	0.03	0.12	0.21
	Turf	0.03	0.05	0.00	0.12
		Kona/Kohala coast			
Rib	***Gracilaria***	0.15	0.10	0.01	0.34
	Hypnea-Pterocladiella	0.34	0.22	0.02	0.70
	Mahi-mahi	0.04	0.02	0.01	0.09
	Turf	0.47	0.27	0.04	0.86
Epidermis	***Gracilaria***	0.05	0.04	0.01	0.14
	Hypnea-Pterocladiella	0.54	0.19	0.07	0.76
	Mahi-mahi	0.18	0.03	0.12	0.23
	Turf	0.23	0.21	0.03	0.73
Squamosal	***Gracilaria***	0.15	0.09	0.02	0.31
	Hypnea-Pterocladiella	0.48	0.18	0.12	0.74
	Mahi-mahi	0.06	0.03	0.02	0.11
	Turf	0.31	0.22	0.02	0.73

Several samples of the epidermis, squamosal, and rib bone from the Kona/Kohala coast laid outside the mixing polygon formed by turf, and the three species of macroalgae in the δ^{13}-δ^{15}N isospace because they were highly depleted in ^{13}C and enriched in ^{15}N (Figure 3). Only when mahi-mahi was included as a potential source did all the samples lay within the mixing polygon. However, some of the squamosal, rib, and epidermis samples from the Kona/Kohala coast were depleted in ^{34}S compared to macroalgae, turf, and mahi-mahi and hence laid outside the mixing polygons formed by those four sources in the δ^{13}C - δ^{34}S (Figure 4) and the δ^{15}N - δ^{34}S (Figure 5) isospaces. Hence the importance of having at least three different isotopes that show if prey items are missing from the diet composition. According to MixSIAR results, *H. musciformis-P. capillacea* was the primary source of assimilated nutrients for the green turtles inhabiting the Kona/Kohala coast when using both squamosal (X ± SE = 47.9 ± 18.3%) and epidermis samples (X ± SE = 54.2 ± 19.4%). Turf was the secondary source (squamosal: X ± SE = 31.1 ± 22.2%, epidermis: X ± SE = 22.8 ± 20.7%).

Stable isotope ratios in ribs also showed a high contribution of *H. musciformis-P. capillacea* to the green turtle diet (X ± SE = 34.2 ± 22.1%), but only second most important relative to turf (X ± SE = 46.5 ± 27.4%). Nevertheless, discriminating between the actual contribution of *H. musciformis-P. capillacea* and turf to the diet of green turtles is difficult considering the loose credible intervals. In any case, the stable isotope ratios in the three tissues suggest a minor role for *G. salicornia* and mahi-mahi (Table 5).

Discussion

Results revealed differences in the δ^{13}C, δ^{15}N, and δ^{34}S values of the epidermis compared to the two skeletal elements of green turtles (squamosal and rib), but no differences between the two skeletal elements. Furthermore, body size and sampling locality had similar effects on the stable isotope ratios of squamosal, rib, and epidermis, although the effect was stronger in the latter. This suggests that the δ^{13}C, δ^{15}N, and δ^{34}S values in the squamosal and rib of green turtles from neritic habitats are informative of diet prior to death, similarly to the epidermis.

Gut content analysis indicates that settlement to neritic habitats and transition to a plant-based diet happen in Hawaiian green turtles at approximately 40 cm CCL, although larger turtles may still consume some animal prey (Russell et al. 2003; Arthur and Balazs 2008; Russell and Balazs 2009; Russell and Balazs 2015). The epidermis integrates diet over a few months (Reich et al. 2008), and hence the $\delta^{13}C$ and $\delta^{15}N$ of the epidermis should be uncorrelated to turtle size if the ontogenetic diet shift was abrupt and only neritic individuals were considered; the exception being if those turtles were sampled or stranded very soon after arrival. This is the pattern observed on East Oahu but not on the Kona/Kohala coast, where $\delta^{13}C$ increases and $\delta^{15}N$ decreases as carapace length increases, thus suggesting a more gradual ontogenetic dietary shift in the latter locality. Notably, the same patterns were found when analyzing the epidermis, squamosal, or rib bone.

Previous research has suggested that the pace and timing of the ontogenic diet shift in green turtles are highly dependent on the relative availability of animal and plant food sources (Cardona et al. 2009; González Carman et al. 2014), and this might be the case for the localities considered here. East Oahu supports meadows of *H. hawaiiana* and *H. decipens* (McDermid et al. 2002; McDermid et al. 2003; McDermid and Stuercke 2003), extensive red mangrove areas (Allen 1998), and areas of coral rubble with high algal coverage (Cheroske et al. 2000), where green turtles are the most abundant large herbivores (Cardona et al. 2020a). Algal availability was even higher in the recent past when the area suffered a high nutrient load (Cheroske et al. 2000; Drupp et al. 2011; Lesser 2021). Conversely, subtidal algal coverage is low along most of the Kona/Kohala coast (Cardona et al. 2020a), where sea urchins (mainly *Echinometra mathae*i) and several species of surgeon (Acanthuridae) and parrot fish (Scaridae) are the prevailing subtidal herbivores (Wabnitz et al. 2010; Cardona et al. 2020a), and green turtles forage mostly on intertidal turf and macroalgae (Wabnitz et al. 2010). Green turtles inhabiting the Kona/Kohala coast also have access to the fish discarded by anglers at Honokōhau Small Boat Harbor (G. Balazs, personal observation) and fish flesh has also been reported in the gut contents of some green turtles dead-stranded along the Kona/Kohala coast (Russell et al. 2011). Regular fish consumption may explain the high $\delta^{15}N$ values observed in the epidermis, squamosal, and rib bone of the green turtles from the Kona/Kohala coast compared to those from East Oahu. However, the negative correlation between $\delta^{15}N$ and carapace length suggests declining fish consumption with body size. The same negative correlation is found when analyzing the epidermis, squamosal, and rib bone.

Compared to $\delta^{13}C$ and $\delta^{15}N$, the patterns of variability in the $\delta^{34}S$ values of the three tissues were harder to interpret, but the inclusion of this third element provided relevant new information. Marine primary producers relying only on marine sulfate as a S source are expected to be highly enriched in ^{34}S, whereas those using sulfides formed by bacterial activity in reduced sediments are expected to be depleted in ^{34}S (Fry and Smith 2002; Connolly et al. 2004). The results reported here support this marine-terrestrial gradient, as macroalgae and turf algae from both study areas were enriched in ^{34}S compared to the seagrass *H. hawaiiana* from East Oahu, which in turn was enriched in ^{34}S compared to mangrove *R. mangle* from the same area. Terrestrial vegetation, which green turtles sometimes consume in Hawaii, according to gut contents analysis and observations (Russell et al. 2011; McDermid et al. 2015; McDermid et al. 2018), is also depleted in ^{34}S (Rodrigues et al. 2011).

Previous gut content analyses identified the red macroalgae *A. spicifera*, *Gracilaria spp.,* and *H. musciformis* as the primary food source of green turtles on East Oahu, where *Halophila* seagrasses were the second most important prey (Russell et al. 2003; Arthur and Balazs 2008; Russell and Balazs 2009; Russell and Balazs 2015). Similarly, the diet of green turtles on the Kona/Kohala coast has been found to be dominated by red macroalgae in the genera *Pterocladiella* and *Hypnea*, followed by *G. salicornia* (Arthur and Balazs 2008). However, green turtles have been observed regularly scavenging fish scraps at the Honokōhau Small Boat Harbor (G. Balazs, personal observation). However, the δ^{34}S values of the analyzed prey in all those sources are too high to explain the δ^{34}S values of many of the squamosal and rib bone samples and some skin samples. Hence ,at least one additional yet unsampled ^{34}S-depleted source is likely being consumed by green turtles, particularly on East Oahu.

At first glance, red mangrove leaves are a convenient and accessible ^{34}S-depleted source for green turtles on East Oahu because they are abundant and are consumed by green turtles elsewhere (Limpus and Limpus 2000; Amorocho and Reina 2007; Arthur et al. 2009; Carrión-Cortez et al. 2010; Nagaoka et al. 2012). According to MixSIAR, red mangrove leaves might contribute 13% to the diet of green turtles on East Oahu, although its consumption has not been reported previously by green turtles in Hawaii. Alternatively, leaves of unidentified terrestrial grasses often occur in the gut contents of green turtles from Oahu (Russell et al. 2011) and might be the primary ^{34}S-depleted source. Similarly, senescent leaves of *Heliotropium foertherianum* are consumed by green turtles along the Kona/Kohala coast (McDermid et al. 2018), thus offering a ^{34}S-depleted source in a region where *Halophila* seagrasses are scarce and occur only deeper than 20 m (McDermid et al. 2002; Russell et al. 2003).

On the other hand, red mangroves were always scarce (Allen 1998) and have been eradicated recently along the Kona/Kohala coast (G. Balazs, personal observation). Further research is necessary to identify better the ^{34}S-depleted prey consumed by green turtles at each locality. However, it is still relevant to note here that analyzing the stable isotopes of S has shed new light on the potential role of red mangroves and terrestrial vegetation in the diet of Hawaiian green turtles.

Despite the uncertainty about the actual identity of the ^{34}S-depleted prey consumed by green turtles at each locality, results revealed that mixing models combining the δ^{13}C, δ^{15}N, and δ^{34}S values of the epidermis using the TDF derived experimentally for green turtle epidermis adequately reflected the gross composition of green turtles' diet, as described above. Conversely, mixing models failed to do so when the δ^{13}C, δ^{15}N, and δ^{34}S of rib and squamosal bone were combined with the TDF derived experimentally for cortical bone. This is likely because cortical and trabecular bone metabolism differences result in different TDF values. It should also be noted that all the turtles from Kona/Kohala coast laying outside the mixing polygon delimited by macroalgae and turf were smaller than 47 cm CCL, so they could have been eating a carnivorous diet.

The stable isotope ratios of δ^{13}C and δ^{15}N vary across the skeletal elements of several mammals (Clark et al. 2017; Fahy et al. 2017; Bas et al. 2020; Smith et al. 2020) and fish species (Bas and Cardona 2018) because of differences in assimilation pathways, the turnover rate, and the degree of bone remodeling of their skeletal elements. The relative contribution of cortical and trabecular bone to the structure of the elemental isotope considered (Fahy et al. 2017; Bas et al. 2020) and regional heterothermy (Bas et al. 2020; Smith et al. 2020) explain such variability in mammals. The skeletal elements of sea turtles have a structure like that of mammals (Bjorndal et al. 2003; Snover and Hohn 2004; Arencibia et al. 2006; Snover et al. 2007a; Snover et al. 2011; Jones et al. 2012), and green turtles may also exhibit regional endothermy (Standora et al. 1982), so differences in the stable isotope ratios of δ^{13}C and δ^{15}N might also vary across their skeletal elements. However, no differences were observed between squamosal and rib bone, probably because both belong to core areas along the body's central axis and have similar anatomic structures, endothermal dynamics, and likely similar turnover rates.

Conversely, the humerus bone, the focus of most of the previous research on the skeletal elements of sea turtles, is a component of the axial skeleton and has a thick layer of cortical bone (Bjorndal et al. 2003; Arencibia et al. 2006; Snover et al. 2007b; Snover et al. 2011; Jones et al. 2012; Medeiros et al. 2015). The cortical layer of the humerus bone has been used to estimate TDF values for green turtle bone (Turner Tomaszewicz et al. 2017), which certainly has a much slower isotope turnover rate than the trabecular bone (Castanet and Smirina 1990).

Given this, it was expected that the cortical and trabecular bone of green turtles would differ in their TDF values (Medeiros et al. 2015), which may explain why MixSIAR failed to recover the diet make up of green turtles in the two study areas when using the δ^{13}C, δ^{15}N, and δ^{34}S values in the squamosal and rib bone. In contrast, MixSIAR using the δ^{13}C, δ^{15}N, and δ^{34}S values in the epidermis of green turtles and the TDF derived experimentally for the same tissue successfully identified the main diet sources of green turtles in both areas, according to gut content analysis (Russell et al. 2003; Arthur and Balazs 2008; Russell and Balazs 2009; Russell and Balazs 2015).

This highlights the need for further research on the TDF of trabecular bone before the stable isotope ratios in skeletal elements with a prevalence of this tissue can be used for diet reconstruction using mixing models. Nevertheless, the results reported here revealed that the stable isotope ratios in squamosal and rib bone capture much of the variability observed in the epidermis and hence are useful to make inferences on the trophic ecology of green turtles.

In conclusion, the results reported here indicate that the stable isotope ratios of δ^{13}C, δ^{15}N, and δ^{34}S from skeletal elements with a thick core of trabecular bone integrate dietary information similarly to the epidermis, although probably over more extended periods, and that the identity of the skeletal elements analyzed is not a significant source of variability if they have a similar anatomical structure, physiology, and endothermal tendencies. Given these findings, the δ^{13}C, δ^{15}N, and δ^{34}S values in skeletal elements of green turtle specimens from scientific collections, mainly skulls and carapaces, could be used to infer potential historical changes in the trophic ecology of green turtles following the overharvest and recovery cycle of most populations. However, the use of mixing models such as MixSIAR is not appropriate for these bone tissues until a TDF can be experimentally derived specifically for this type of trabecular bone.

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6. CHAPTER 4: HISTORICAL CHANGES IN THE TROPHIC ECOLOGY OF HAWAIIAN GREEN TURTLES AS REVEALED BY STABLE ISOTOPE RATIOS (C, N, AND S) IN THE BONES OF MUSEUM SPECIMENS

Abstract

This study analyzed the stable isotopes of C, N, and S in the bone tissue of modern and museum specimens of green turtles collected from 1901 to 2020 in Oahu and the Northwestern Hawaiian Islands to understand how their isotopic niche has changed through time. The effect of turtle size and collection year on the stable isotope ratios was assessed using general linear models and Pearson correlations. Furthermore, we calculated 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. Results revealed that the value of stable isotope ratios has not changed throughout time and that green turtles had a macroalgae-based diet even before the introduction of exotic species coupled with eutrophication promoted the development of algal pastures in Kāneʻohe. However, the breadth of the isotopic niche decreased in the most recent period, indicating that ancient green turtles exhibited a broader diversity of individual foraging strategies, with some individuals relying mostly on seagrasses and others consuming substantial amounts of animal matter. These trophic specialists are currently gone, and green turtles have converged on the use of the most abundant resource, red macroalgae, probably because of the homogenization and simplification of coastal habitats.

Introduction

Human activity dramatically reduced the abundance of many marine species worldwide during the 20th century (McCauley et al. 2015), and only recently, some populations have started to increase as a result of protection (Duarte et al. 2020). Human-induced changes in population size are often associated with diet shifts (Hanson et al. 2009; Zenteno et al. 2015a; Hanson et al. 2018; Cardona et al. 2020b; Moranta et al. 2020), which in turn may modify the structure of anthropogenically impacted food webs (Saporiti et al. 2014; Bas et al. 2019; Ólafsdóttir et al. 2021). Such ecosystem effect can be exacerbated by the uneven recovery of strongly interacting species following biased conservation schemes (Heithaus et al. 2014) and by the introduction of exotic species, as they become major predators, competitors, or prey for native species and hence modify food web structure (Roemer et al. 2002; Anton et al. 2020).

Green turtles *Chelonia mydas* are tropical megaherbivores rebounding worldwide after several decades of conservation efforts (Chaloupka et al. 2008; Kondo et al. 2017; Casale et al. 2018). The recovery of green turtles has been particularly vigorous in the Hawaiian Archipelago (Balazs and Chaloupka 2006; Kittinger et al. 2013; Piacenza et al. 2016), inhabited by a geographically discrete population (Seminoff et al. 2015). Nothing is known about the Hawaiian green turtle's population size in pre-Polynesian times, but green turtle numbers are thought to decline since human settlement in the archipelago (Balazs and Chaloupka 2006; Kittinger et al. 2011; Van Houtan and Kittinger 2014). The trend was reversed after green turtles were listed under the endangered species act in 1978 (43 FR 32800), and the Hawaiian population has been growing steadily since then (Balazs and Chaloupka 2006; Balazs et al. 2015).

However, the coastal ecosystems inhabited by this thriving population of green turtles has been intensely modified by human activity because of the introduction of mangroves and several species of macroalgae (Russo 1977; Allen 1998) the decline of roving herbivorous fishes except at marine protected or remote areas (Friedlander et al. 2003; Williams et al. 2008; Jouffray et al. 2015; Helyer and Samhouri 2017) and the nutrient enrichment resulting from population growth and overfertilization of farmland (Conklin and Stimson 2004; Drupp et al. 2011; Jouffray et al. 2015; Murray et al. 2019). All these processes operated synergistically to cause a reduction in the abundance of coral in favor of turf or macroalgae during the second half of the 20th century, which in turn might have been beneficial for the recovery of green turtles.

Diet analysis conducted since the 1970s revealed that Hawaiian green turtles rely largely on exotic macroalgae, particularly at Kāneʻohe Bay, on the island of Oahu (Russell et al. 2003; Arthur and Balazs 2008; Russell and Balazs 2009; Balazs et al. 2015; Russell and Balazs 2015). However, the original diet of green turtles in the Hawaiian archipelago remains completely unknown and the potential contribution of the native seagrass *Halophila hawaiiana* is particularly intriguing, considering its 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 (Schmidt et al. 2009; Saporiti et al. 2014;

Van Rijssel et al. 2017; Conrad et al. 2018). 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 to the diet of major prey, if isotopically distinct (Peterson and Fry 1987; Bearhop et al. 2004; Layman et al. 2012; Gillis et al. 2018; Swan et al. 2020). Trophic discrimination factors are tissue and species specific and hence detailed diet reconstruction from stable isotope analysis is possible only if trophic discrimination factors have been assessed experimentally which is not always the case (Turner Tomaszewicz et al. 2017).

Despite this limitation, stable isotope analysis allows, at least, detecting changes in the type of resources used (coastal vs oceanic; benthic vs. pelagic; macroalga vs. seagrasses) and trophic position. Furthermore, stable isotope analysis allows measuring the degree of individual specialization and the breadth of the isotopic niche, which in turns may change in response to intraspecific and interspecific competition and ecological opportunity (Layman et al. 2007; Araújo et al. 2011; Rigolet et al. 2015; Resasco et al. 2018; Pagani-Núñez et al. 2019). The breadth of the isotopic niche can be measured as the standard ellipse area (SEA) and the convex hull area (TA), both calculated with the Stable Isotope Bayesian Ellipses in R (SIBER) SEA and TA in tissues with a high turnover rate are good indicators of how varied the diet is (Araújo et al. 2011), but values in tissues with a low turnover rate also depend on the degree of individual specialization (Drago et al. 2017).

In this study, we analyzed specimens from three museum collections to assess changes in the isotopic niche of green turtles from Oahu through the 20th century and the first two decades of the 21st century. More precisely, we are assessing changes through time in the $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values of green turtles and in the isotopic niche breadth as revealed by SEA and TA, to test the hypothesis that reliance on macroalga has increased and that trophic niche breadth has decreased since the 1970s due to increased green turtle population and macroalgae availability.

Materials and methods

We sampled green turtle skulls from museum samples and from dead-stranded green turtles in Hawaii to compare the dietary information derived from the stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) with the same isotopes in three different periods (1901-1951, 1992-2008 and 2018-2020).

Turtle sample collection

Samples from 1901-2008 were taken from 39 Hawaiian green turtle specimens from three museums in the United States: Smithsonian National History Museum (n = 7), San Diego Natural History (n = 5), and Bishop Museum (n = 13). Additional samples were collected from 14 dead-stranded green turtles from Oahu in 2018-2020. According to museum documentation, samples from museums were collected in Oahu, northwestern Hawaiian Islands, and French frigate shoals. The ones from 2018-2020 were all dead-stranded in Oahu (Figure 1). Most of the turtles in museums and the modern ones came from the Kāneʻohe Bay area in Oahu. Some samples from museums came from the Northwestern Hawaiian Islands, including the French Frigate Shoals, the main nesting area in Hawaii (Balazs 1976).

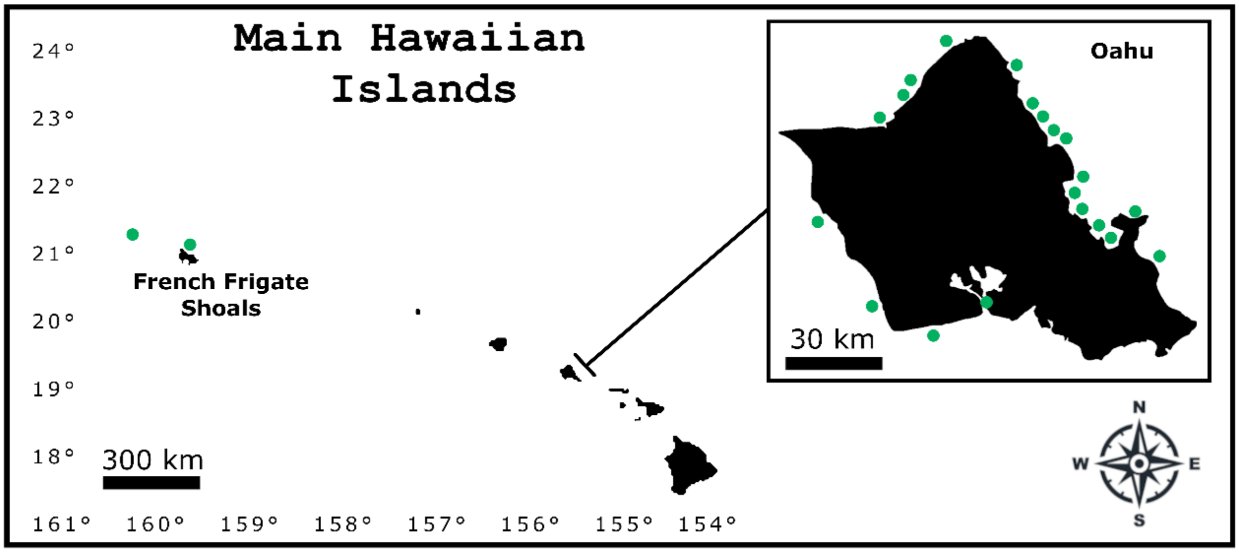

Figure 1 Locations where the turtles were found in Hawaii. The green dots indicate where turtles were found dead-stranded or are reported as the location in the museum records. Locations in Oahu are Barbor's point, Coconut Island, Hickham air force base, Kahaluu, Kai boat ramp, Kāneʻohe Bay, Kawailoa, Koolina, Kualoa regional park, Laniakea, Lanikai, Makaha, Malaekahana, Mokuleia, Nimitz beach, Waialea, and Waialua. Locations in other islands are: French Frigate Shoals and described in other files as Northwestern Hawaiian Islands. Each dot doesn't indicate a specific specimen, but locations where they were found. Maps modified from R tmap (Tennekes 2018).

Museum collections had mostly skulls, but some specimens had only ribs or vertebrae available (6 specimens had only ribs and one only a vertebrae). A previous study (Velasquez-Vacca, under review) showed no significant differences in the stable isotope values between those bones, so they could be combined in the same study. The contemporary, dead-stranded turtles were frozen at -20ºC when found, and then necropsies were conducted by the Pacific Islands Fisheries Science Center (NOAA) staff in Oahu, Hawaii. Curved carapace length (CCL) was measured, and a piece of the skull (squamosal bone) or rib (when the skull was missing) was cut from each turtle, washed, and air-dried. A cross-section of bone for all samples was then drilled with Dremel 0.1-inch drill bits.

To obtain the CCL from museum samples, pictures were taken from the skulls and its length was measured using ImageJ. Then, an equation (Eq. 1) to obtain CCL was calculated with the skulls of dead-stranded turtles from 2008 to 2020 for which CCL was measured directly on the turtle. The resulting equation is as follows:

$$\text{CCL} = 4.3257 \times \text{skull length} - 0.09 \quad \text{(Eq. 1)}$$

Prey sample collection

Previous gut content studies had 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 decipens,* and *Halophila hawaiiana* supplying less than 15% of the ingested material (Russell et al. 2003; Arthur and Balazs 2008; Russell and Balazs 2009; Russell and Balazs 2015). Accordingly, we sampled the macroalgae *A. spicifera*, *G. salicornia,* and *H. musciformis*, the seagrass *H. hawaiiana*, and turf algae from East Oahu. 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 and Vermeij 2016). Samples were collected at the end of the dry September 2018) and rainy seasons (February 2019), and the red mangrove leaves (*Rhizophora mangle*) were collected in March 2021. Samples were cleaned of sand and debris, rinsed with deionized water, dried at 50ºC for 24 h, and ground to a fine powder.

Stable Isotope Analysis

Following Velasquez-Vacca et al. (under review), bone samples were dried at 50ºC and processed for stable isotope analysis of $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$, without further treatment. Then, and equation (Eq. 2) was used to correct for lipid content, based on their C:N ratio.

$$\delta^{13}C_{dml} = \delta^{13}C_{unp} - 3.658 + (3.372 \times \ln (C:N)_{unp}) \ (r^2 = 0.805, p<0.001) \ (Eq. 2)$$

Where $\delta^{13}C_{dml}$ denotes the $\delta^{13}C$ value after acidification and lipid extraction, $\delta^{13}C_{unp}$ denotes the $\delta^{13}C$ value in unprocessed samples, and $(C:N)_{unp}$ is the atomic ratio of unprocessed samples. Accordingly, this equation was used to calibrate the $\delta^{13}C_{unp}$ values of all bone samples.

For all samples, carbon, and sulfur isotopic results are reported in parts per thousand (‰) relative to VPDB (Vienna Peedee Belemnite), and N isotope ratios are reported ‰ relative to N_2 in air.

Statistical analysis

Turtles were grouped into three periods for analysis: 1901-1951 (n = 12), 1992-2008 (n = 13), and 2018-2020 (n = 14). General linear models were run in R version 4.1.1 (2021-08-10) (R Core Team 2018) to assess whether stable isotope ratios changed throughout time, including CCL as a covariate. SIBER in R was used to calculate the size of the standard ellipses in the $\delta^{13}C$ - $\delta^{15}N$, $\delta^{13}C$ - $\delta^{34}S$, and $\delta^{15}N$ - $\delta^{34}S$ isospaces. $\delta^{13}C$ values were corrected for the Suess effect using SuessR (Clark et al. 2022) with 1850 as the reference year. SuessR is a package for R used for calculating and applying mathematical corrections for the Suess and Laws effects. These corrections are region-specific, accounting for the spatial variability in seawater circulation, surface residence time, water temperature, and biological production that impacts CO_2 uptake by the oceans (Eide et al. 2017), as compared with the relatively homogeneous and well-mixed global atmosphere (Clark et al. 2017). The size of the ellipses was measured as TA (convex hull area) and SEA (standard ellipse area).

Results

Red mangrove leaves were characterized by very low $\delta^{13}C$ and $\delta^{34}S$ values and very high $\delta^{15}N$ values compared to any other potential prey (Table 1). The seagrass *H. hawaiiana* was characterized by remarkably high $\delta^{13}C$ values, very low $\delta^{15}N$ values, and intermediate $\delta^{34}S$ values. Macroalgae (*A. spicifera*, *G. salicornia,* and *H. musciformis*) and turf had $\delta^{13}C$ and $\delta^{15}N$ values intermediate between red mangrove leaves and *H. hawaiiana* and were highly enriched in ^{34}S compared to both. However, turf was more enriched in ^{15}N than macroalgae.

Table 1 Stable Isotope ratios of the Hawaiian green turtle prey

Sample	$\delta^{13}C$	$\delta^{13}C$ stdv	$\delta^{15}N$	$\delta^{15}N$ stdv	$\delta^{34}S$	$d^{34}S$ stdv	n
Acanthophora	-14.4	0.2	3.0	0.2	22	1.6	10
Gracilaria	-16.3	0.7	4.7	1.2	21	1.9	10
Hypnea	-14.5	0.8	3.7	0.3	22	1.7	10
Halophila	-8.1	0.6	2.1	0.3	18	2.3	10
Turf	-22	6.6	4.9	0.5	20	2.2	10
Red mangrove	-30.3	0.3	6.2	0.3	13	1.1	10
Mahi-Mahi	-18.5	0.2	13.7	0.2	17	1.0	5

The size of the turtles (CCL) ranged from 62 - 131 cm (mean ± SE = 80 ± 31 cm) in 1901-1951, 47-127 cm (mean ± SE = 86 ± 28 cm) in 1992-2008 and 46-103 cm (mean ± SE = 66 ± 19 cm) in 2018-2020. There were no differences in CCL of the turtles from the three study periods (ANOVA, $F_2 = 22.062$, $p = 0.141$).

When analyzing the museum turtles and the contemporary dead-stranded ones (Table 2), values for $\delta^{13}C$ ranged from -16 to -8 ‰ (mean ± SE = -12 ± 2.5 ‰) in 1901-1951, -17 to -6 ‰ (mean ± SE = -12 ± 3.3 ‰) in 1992-2008 and -13 to -9 ‰ (mean ± SE = -10 ± 1.1 ‰) in 2018-2020. For $\delta^{15}N$, values ranged from 5 to 11 ‰ (mean ± SE = 8 ± 2 ‰) in 1901-1951, 4 to 14 ‰ (mean ± SE = 8 ± 2.8 ‰) in 1992-2008 and 6 to 11 ‰ (mean ± SE = 14 ± 1.3 ‰) in 2018-2020 and for $\delta^{34}S$, values ranged from 10 to 17 ‰ (mean ± SE = 13 ± 2.4 ‰) in 1901-1951, 9 to 18 ‰ (mean ± SE = 14 ± 2.1 ‰) in 1992-2008 and 10 to 15 ‰ (mean ± SE = 14 ± 1.3 ‰) in 2018-2020. No differences were found between periods (Figure 2) in any of the three stable isotope ratios ($\delta^{13}C$:

ANOVA, F_2 = 1.46, p = 0.246, δ^{15}N: ANOVA, F_2 = 0.10, p = 0.897 and δ^{34}S: ANOVA, F_2 = 0.98, p = 0.383).

Table 2 Carbon, nitrogen, and sulfur stable isotope ratios in Hawaiian green turtles from 1901-2020 in the green turtle skulls from museums and dead-stranded turtles. Values are corrected for Suess and Laws effect with SuessR with 1950 as the reference year (Clark et al. 2022). An asterisk denotes that the CCL was calculated with the equation (Eq. 1) instead of measured directly on the turtle. Bold type indicates turtles with δ^{34}S values lower than expected. Two asterisk indicate turtles that had the lowest δ^{15}N values and high δ^{13}C values, consistent with a seagrass-dominated diet and turtles with the highest δ^{15}N values and moderately low δ^{13}C values, consistent with those observed in fish muscle (Velasquez-Vacca et al. under review). NWHI = Northwestern Hawaiian Islands, FFS = French Frigate Shoals, SNMNH = Smithsonian National Museum of Natural History, SDNHM = San Diego Natural History Museum

Location	**Collection**	**Year**	**δ^{13}C (‰)**	**δ^{15}N (‰)**	**δ^{34}S (‰)**	**CCL(cm)**
			1901-1951			
Oahu	SNMNH	1901	-16.3	10.1	15.4	46*
Oahu	SNMNH	1901	**-8.7****	**5.7****	**11.8****	47*
NWHI	SNMNH	1902	-12.1	7.3	17.6	46*
FFS	SNMNH	1923	-8.7	9.3	17.3	128*
NWHI	SNMNH	1924	-12.3	6.5	11.6	131*
NWHI	SNMNH	1925	-11.8	n.a	10.2	121*
NWHI	SNMNH	1926	-16.56	n.a	11.7	75*
Oahu	SDNHM	1948	-13.9+	10.4+	15.0	62*
Oahu	SDNHM	1948	-10.3	8.4	13.8	71*
Oahu	SDNHM	1948	-12.4**	6.0**	12.8**	95*
FFS	SDNHM	1951	-13.0**	11.8**	13.2	70*
FFS	SDNHM	1951	-12.2	9.8	11.4	70*
			1992-2008			
Oahu	Bishop	1992	-10.6	7.6	12.9	98*
Oahu	Bishop	1992	-14.0**	14.7**	13.5	114*
Oahu	Bishop	1993	-9.8**	11.3**	14.8	65*
Oahu	Bishop	1994	-13.7	9.8	13.9	127*

Oahu	Bishop	1996	-12.1	8.0	14.8	101*
Oahu	Bishop	1998	-8.4**	5.3**	14.2**	61*
Oahu	Bishop	1998	-13.3	9.9	13.9	86*
Oahu	Bishop	1998	-9.8	8.0	9.5	88*
Oahu	Bishop	2001	-13.3	9.2	14.1	89*
Oahu	Bishop	2001	-6.1**	4.0**	15.5**	131*
Oahu	Bishop	2005	-17.3	n.a	16.0	54*
Oahu	Bishop	2005	-16.0	10.3	18.6	61*
Oahu	Bishop	2008	-15.9	6.7	16.3	47*
2018-2020						
Oahu	dead-stranded	2018	-13.4	11.3	14.2	50
Oahu	dead-stranded	2018	-11.6	8.3	15.3	52
Oahu	dead-stranded	2018	-11.1	7.3	13.8	64
Oahu	dead-stranded	2018	-11.6	9.7	15.1	64
Oahu	dead-stranded	2018	-10.9	8.3	15.4	65
Oahu	dead-stranded	2019	-10.5	7.6	15.2	46
Oahu	dead-stranded	2019	-9.8	6.8	13.9	70
Oahu	dead-stranded	2019	-10.7	8.1	15.6	81
Oahu	dead-stranded	2019	-10.6	8.5	**10.9**	87
Oahu	dead-stranded	2020	-11.4	8.8	15.2	45
Oahu	dead-stranded	2020	-9.8	8.8	15.3	50
Oahu	dead-stranded	2020	-9.3	6.8	13.4	58
Oahu	dead-stranded	2020	-12.5	8.3	15.2	99
Oahu	dead-stranded	2020	-10.1	8.3	13.4	103

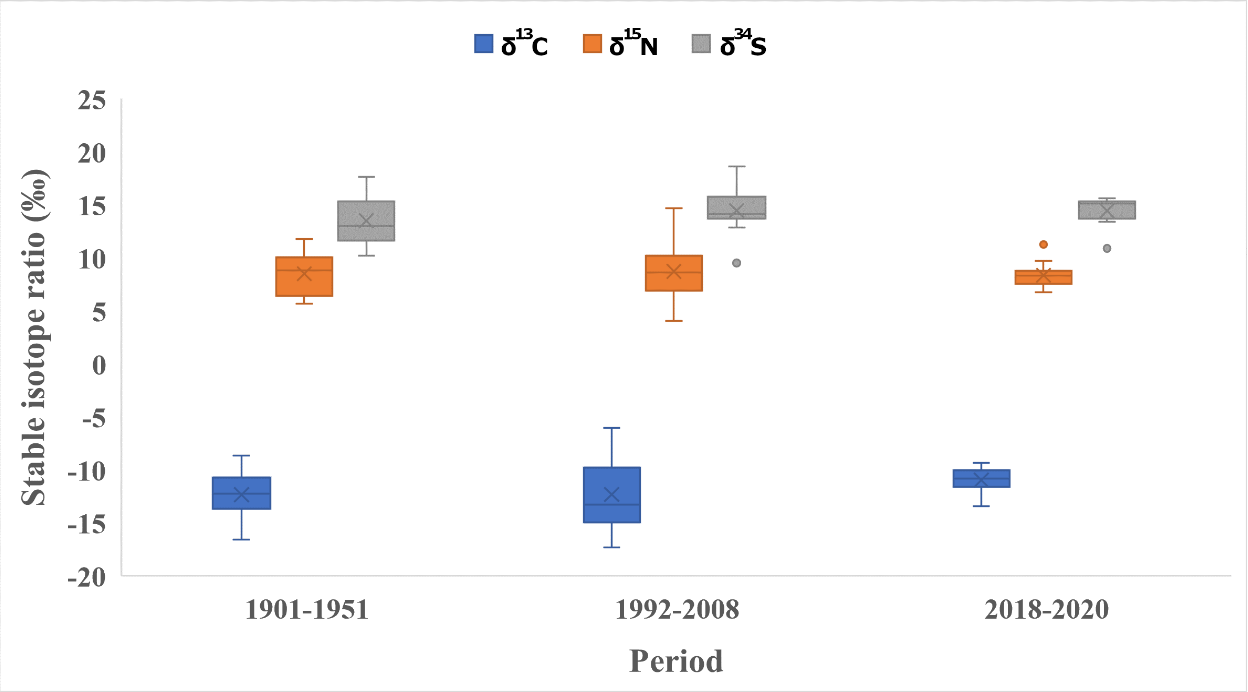

Figure 2 $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$ in green turtle bone samples from Hawaii for 1901 to 2020. The whiskers represent the minimum and maximum values, and the dots are the outliers. The mean is shown as an x, and the median as a line. The box represents the interquartile range (the bottom line of the box represents the median of the bottom half or 1st quartile, and the top line of the box represents the median of the top half or 3rd quartile).

The CCL of the green turtles (Figure 3) and year of stranding (Figure 4) were uncorrelated to $\delta^{13}C$ (Pearson, CCL: r = 0.19, p = 0.236, n = 39; year: r = 0.17, p = 0.24, n = 51), $\delta^{15}N$ (Pearson, CCL: r = -0.01, p = 0.918, n = 36; year: r = -0.03, p = 0.82, n = 47) and $\delta^{34}S$ (Pearson, CCL: r = -0.29, p = 0.063, n = 36; year: r = 0.2, p = 0.14, n = 52) when considering all periods.

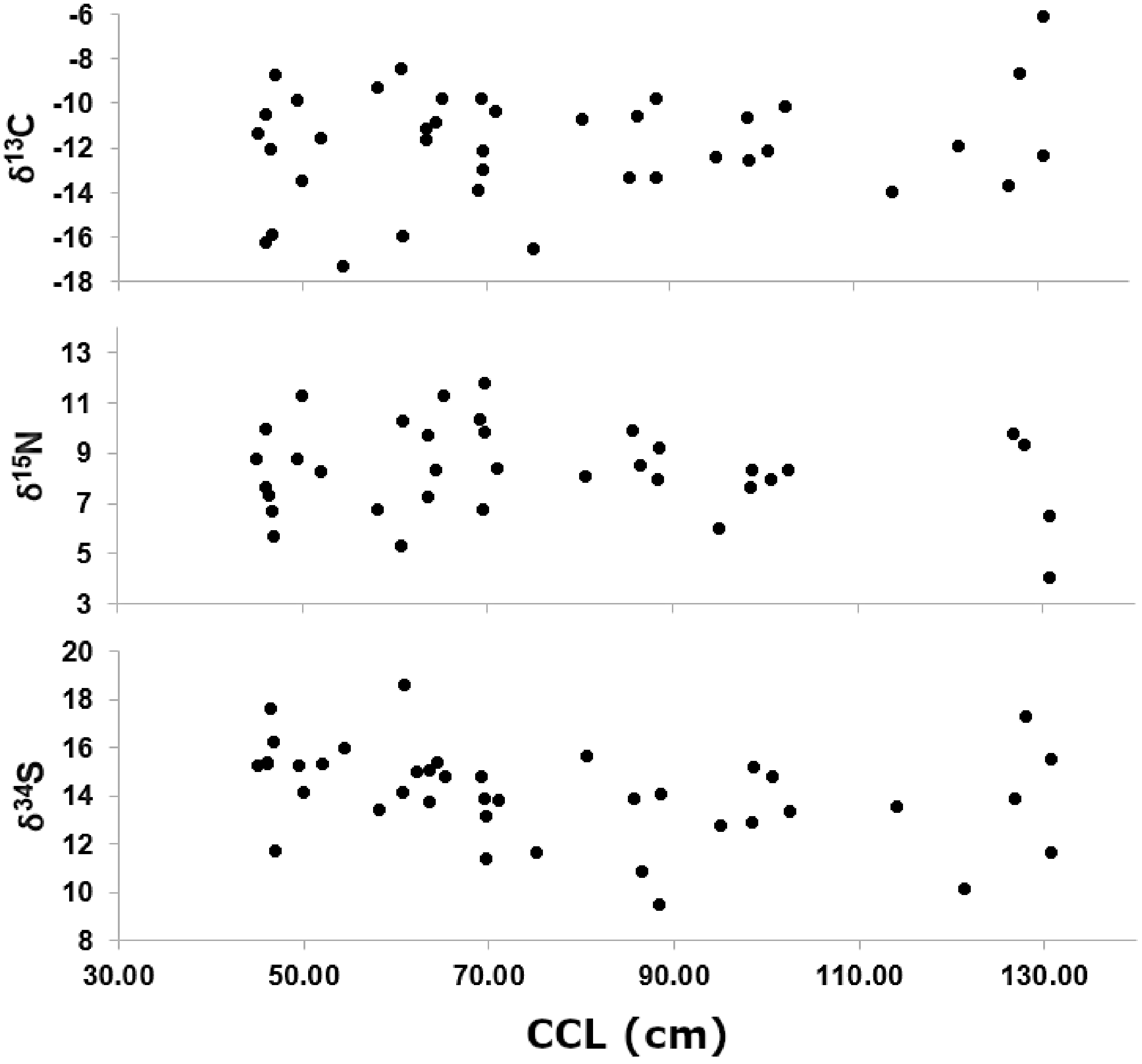

Figure 3 Stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) in the bone tissue of green turtles from Hawaii versus CCL (cm). No stable isotope ratio uncorrelated with carapace length. $\delta^{13}C$ values were corrected for the Suess and Laws effect.

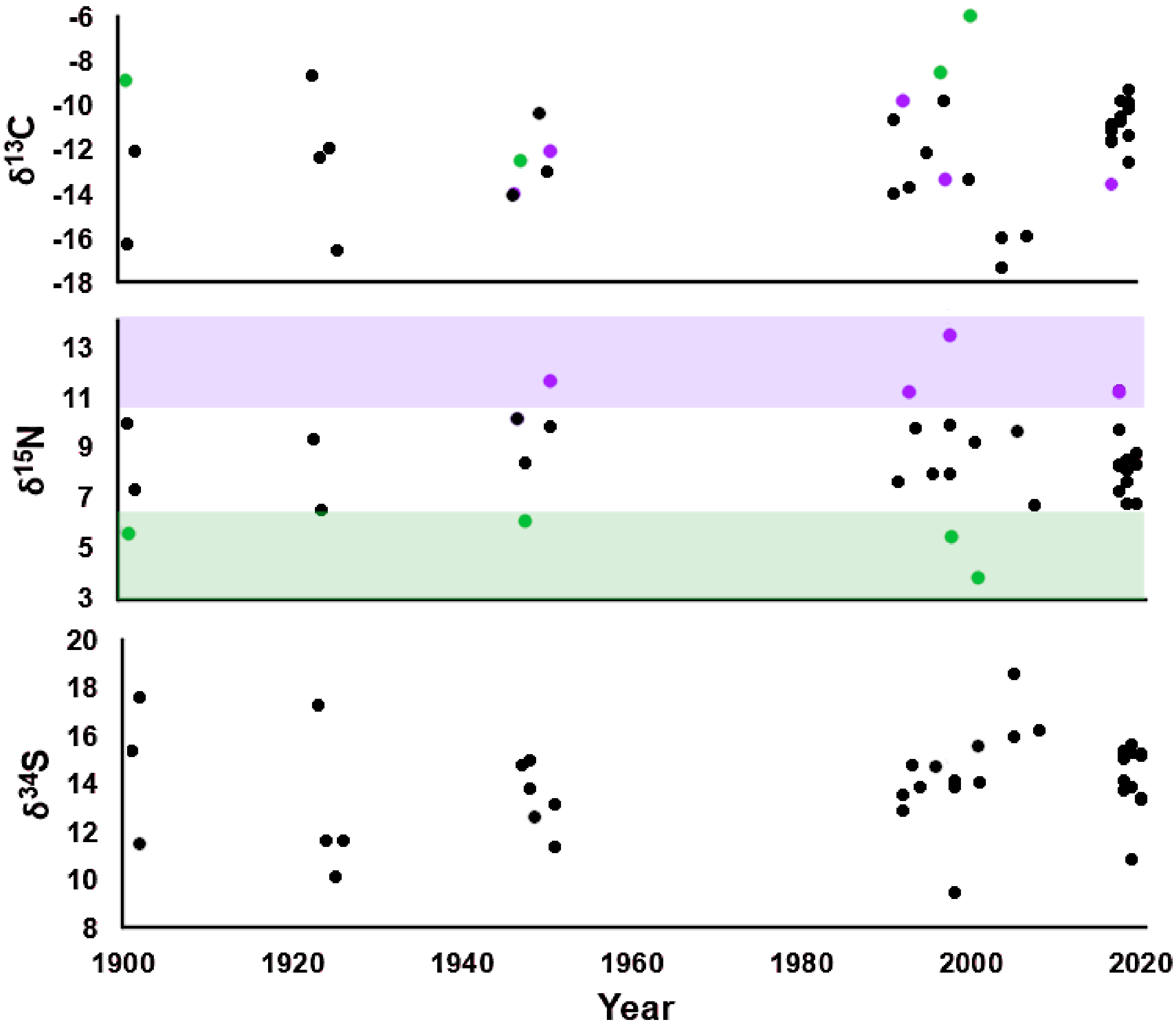

Figure 4 Stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) in the bone tissue of green turtles from Hawaii versus collection or stranding year. $\delta^{13}C$ values were corrected for the Suess and Laws effects. Turtles in green had the lowest $\delta^{15}N$ values and high $\delta^{13}C$ values, consistent with a seagrass-dominated diet. Turtles in purple had the highest $\delta^{15}N$ values and moderately low $\delta^{13}C$ values, consistent with those observed in fish muscle (Velasquez-Vacca et al, under review).

When using the GLM to account for CCL and year, neither of them had a significant effect on $\delta^{13}C$ (GLM, F (36,35) = -33.33, p = 0.110), $\delta^{15}N$ (GLM, F (33,32) = -3.84, p = 0.840) or $\delta^{34}S$ (GLM, F (37,36) = 0.81, p = 0.960). Furthermore, the standard ellipses of the three periods overlapped broadly (Figure 5 and Figure 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 (1901-1951 vs. 2018-2020: $p < 0.001$ and 1992-2008 vs. 2018-2020: $p < 0.001$). There was no difference between the two first periods (1901-1951 vs. 1992-2008: $p = 0.645$). The sizes of the ellipses calculated with SIBER are shown in (Table 3).

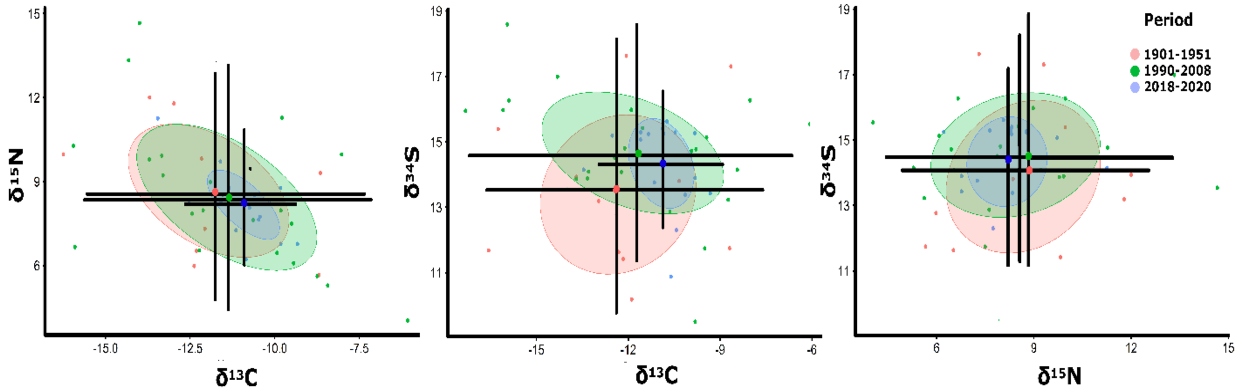

Figure 5 Values of individual green turtles in the isospace and standard ellipses of the population in the three periods: 1901-1951, 1992-2008 and 2018-2020. Carbon values were corrected for the Suess and Laws effect with SuessR with 1950 as the reference year.

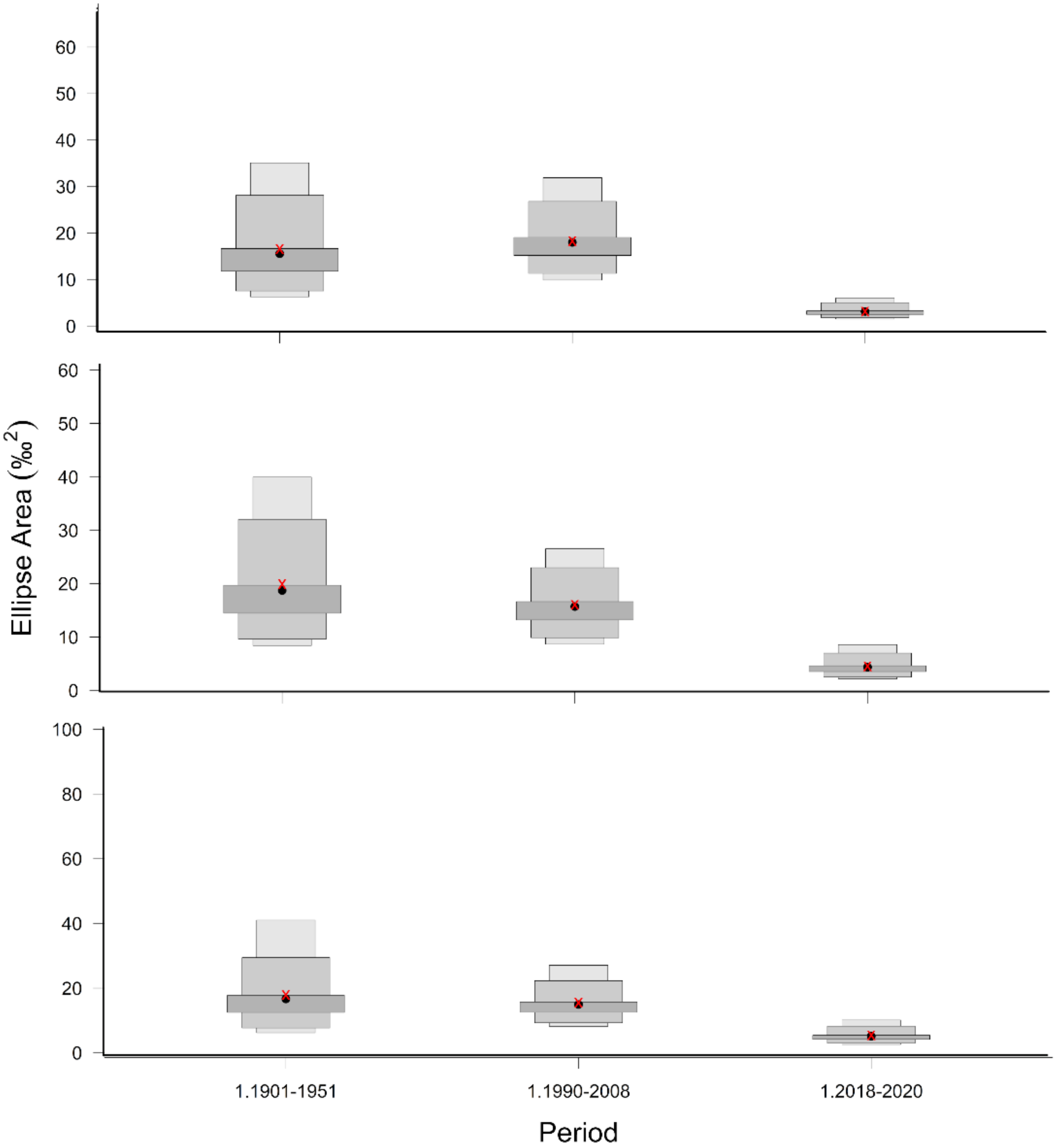

Figure 6 Bayesian estimates of the area of the standard ellipses (SEAB) in the $\delta^{13}C/\delta^{15}N$ (top), $\delta^{13}C/\delta^{34}S$ (middle), and $\delta^{15}N/\delta^{34}S$ isospaces (bottom) by period. Black dots represent the mode; red crosses the mean, and the shaded boxes represent the 40%, 95%, and 99% credible intervals from dark to light grey.

Table 3 Ellipse sizes for periods 1901-1951, 1992-2008, and 2018-2020 calculated with SIBER in R (Jackson et al. 2019). TA = area of the convex hull, SEA = Standard ellipse area, SEAc = Standard ellipse area corrected for small sample size

	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	29.6	58.6	9.0
SEA	14.7	17.4	2.9
SEAc	16.5	18.3	3.1
		$\delta^{15}N$ - $\delta^{34}S$	
TA	29.6	58.6	9.0
SEA	14.7	17.4	2.9
SEAc	16.5	18.3	3.1

Discussion

The results reported here revealed no significant change in the average $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values in the bone tissue of Hawaiian green turtles from 1901 to 2020, but a significant reduction of the isotopic niche breadth in the most recent study period (2018-2020). Accordingly, the diet of green turtles has been isotopically stable for 120 years, but the diversity of the trophic resources consumed, and the degree of individual specialization have decreased recently.

The accurate interpretation of temporal changes in stable isotope ratios relies on identifying temporal shifts in the stable isotope baselines (Casey and Post 2011). The massive release of ^{13}C-depleted CO_2 to the atmosphere since the onset of the Industrial Revolution is the most significant driver of temporal shifts in the C baseline and resulted in a steady decrease of the $\delta^{13}C$ values of marine primary producers worldwide (Eide et al. 2017). This phenomenon can be modelled accurately for each ocean basin, which allows correcting the δ^{13}C values of green turtle bone tissue from specimens collected at different times (Clark et al. 2021) and compare the corrected values to avoid any temporal bias. Currently, green turtles from Oahu rely largely on macroalgae, with seagrasses as a secondary diet source and terrestrial vegetation or mangrove leaves as minor sources (Russell et al. 2003; Arthur and Balazs 2008; Russell and Balazs 2009; Balazs et al. 2015). On this ground, the absence of significant temporal changes in the mean δ^{13}C values of green turtle bone during the past 120 years suggests a consistent macroalgae-based diet for the population during the whole study period, although nothing is known about possible changes in the topology of food sources in the isospace. Nevertheless, some of the green turtles from the first (1901-1051) and second (1980-2008) study periods were extremely enriched in ^{13}C, which in the current isospace of Oahu would suggest a seagrass-based diet. Such seagrass specialists are currently missing from Oahu, according to the stable isotope ratios reported here.

The N baseline depends on the balance between nitrification and denitrification rates, which in turn can be influenced by several factors (Somes et al. 2010). Sewage and farm runoff are two major anthropogenic drivers of the N baseline in the coastal areas of the Hawaii Islands (Dailer et al. 2010; Cox et al. 2013; Murray et al. 2019) and the long history of nutrient enrichment experienced by Kāneʻohe Bay (Conklin and Stimson 2004; Drupp et al. 2011; Jouffray et al. 2015; Murray et al. 2019) suggest potential major changes in the local N baseline throughout time. However, no consistent increase in the δ^{15}N values of green turtle bone was observed during the second study period (1980-2008), when eutrophication at Kāneʻohe Bay peaked. Actually, the highest and the lowest δ^{15}N values were recorded simultaneous at that period. Such variability suggests that individual differences in the diet of green turtles at that time were much larger than any temporal shift in the N baseline. According to the current isospace, those green turtles with very high δ^{15}N values might be consuming relevant amounts of animal food sources (Cardona et al. 2009; Monzón-Argüello et al. 2018)Velasquez-Vacca et al., under review) and those with the lowest values might be consuming large amounts of seagrasses. It is worth noting that the green turtles from the first and second study periods with the lowest δ^{15}N values had also high δ^{13}C values, consistent with a seagrass-dominated diet. Likewise, the turtles with the highest δ^{15}N values had moderately low δ^{13}C values, consistent with those observed in fish muscle (Velasquez-Vacca et al, under review).

Eutrophication resulting from nutrient enrichment may also modify the S baseline, because a higher organic matter load results in reduced sediments and hence the depletion in ^{34}S of the seagrasses growing there (Fry and Smith 2002; Connolly et al. 2004). On this ground, a decrease in the δ^{34}S values of green turtles would be expected during the second study period, when eutrophication peaked in Kāneʻohe Bay. However, and concurrently with the pattern reported above for δ^{15}N, this was the period when the highest variability was observed. This may be the result of the uneven distribution of reduced sediments within the bay but should be noted that seagrasses are not the only ^{34}S-depleted food source for green turtles in Oahu. Terrestrial vegetation, which green turtles sometimes consume in Oahu (Russell et al. 2011) and elsewhere in the Hawaiian Islands (McDermid et al. 2015; 2018), is also depleted in ^{34}S (Rodrigues et al. 2011; Bern et al. 2015), and the same is true for red mangroves, an exotic species introduced to Oahu at the beginning of the 20th century (Allen 1998). Thus, green turtles from Oahu had access to a diversity of ^{34}S-depleted food sources during the whole study period, which might have masked changes in the δ^{34}S values of seagrasses resulting from any drop in the redox potential of sediments. It is worth noting that some of the turtles from the Northern Hawaiian Islands and French Frigate Shoal from the first study period had relatively low values, consistent with the consumption of some seagrass, but never as low as the most ^{34}S-depleted individuals from Oahu.

In summary, the stable isotope ratios reported here suggest that macroalgae have been the stable food of green turtles in Oahu during the past 120 years, independently of changes in the stable isotope baselines, although their isotopic niche is currently much narrower than in the past. This is not an artifact resulting from the inclusion of some green turtles from French Frigate Shoal and the North-Western Hawaiian Islands in the first study period, because differences also exist between the second and the third periods, when only turtles from Oahu were analyzed. Differences in the body size of green turtles sampled in the three study periods do not explain either the observed reduction in the breadth of the isotopic niche, because there are no differences in the average carapace length (CCL) of the turtles from the three study periods. Hence, the narrower isotopic niche observed in the most recent study reveals a lower variability in individual stable isotope ratios in green turtles.

The individual variability in the stable isotope ratios of tissues with a high turnover rate is a good indicator of the diversity of resources consumed by the population and repetitive measures of the stable isotope ratios of such tissues offer a good approach to assess the degree of individual specialization (Araújo et al. 2011).

Stable isotope analysis of different sections of metabolically inter tissues offers a valid alternative in species than can be captured only once (Araújo et al. 2011) and the analysis of thin sections of green turtle carapace scutes has demonstrated that adult populations are composed of specialized individuals foraging consistently on the same resources throughout time (Vander Zanden et al. 2013). The same approach has revealed a much lower temporal consistency in the stable isotopic niche of oceanic juveniles and recently settled neritic juveniles and increased consistently as they grow older (Cardona et al. 2009; Vander Zanden et al. 2013). Unfortunately, most of the green turtle specimens in museum collections are skulls (e.g., https://vertnet.org). It should be noted that stable isotope ratios in tissues with a low turnover rate, such as bone, integrate dietary information over months to years and hence individual variability values also reflect the degree of individual specialization (Drago et al. 2017). If so, the reduced individual variability reported here suggests the absence of specialists in the population and the convergence on an macroalgae-based diet. The most obvious group of specialists missing from the most recent study period are seagrass consumers, characterized high $\delta^{13}C$ and low $\delta^{15}N$ values. Another missing group are those 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 missing from the most recent study period.

Optimal foraging theory predicts that increased intraspecific competition will result into a broader trophic niche when all the individuals have the same initial preferences but may also reduce trophic niche width when individuals initially differ in their preferences and converge in the use of less preferred prey when the abundance of their preferred food sources decrease due to competition (Araújo et al. 2011). Population density is often considered a proxy of intraspecific competition (Araújo et al. 2011), but this approach ignores changes in resource availability. The population of green turtles at the Hawaiian Islands has been growing steadily since they were legally protected in 1978 (Balazs and Chaloupka 2006; Kittinger et al. 2011; Van Houtan and Kittinger 2014; Balazs et al. 2015; Piacenza et al. 2016) and hence intraspecific competition might have increased. However, the coastal ecosystems of the Hawaiian Islands have also been intensely modified by human activity because of the introduction of several species of macroalgae and mangroves (Russo 1977; Allen 1998), the decline of roving herbivorous fishes except at marine protected or remote areas (Friedlander et al. 2003; Williams et al. 2008; Jouffray et al. 2015; Helyer and Samhouri 2017) and the nutrient enrichment resulting from population growth and overfertilization of farmland

(Conklin and Stimson 2004; Drupp et al. 2011; Jouffray et al. 2015; Laws and Taguchi 2018; Murray et al. 2019; Lesser 2021). All those changes are probably favorable for green turtles and the population is probably still below carrying capacity (Balazs and Chaloupka 2006; Kittinger et al. 2013; Piacenza et al. 2016), except at a few sites (Balazs and Chaloupka 2006), so any inference about the intensity of intraspecific competition is highly speculative.

Green turtles inhabiting tropical regions acquire a fermenting microbiome shortly after settling in neritic habitats (Campos et al. 2018), which allows them to digest macrophytes efficiently, although the apparent digestibility of red macroalgae is higher than that of seagrasses and brown macroalgae (Fenchel and Blackburn 1979; Bjorndal 1980; Campos and Cardona 2020). The two species of seagrasses occurring in the Hawaiian Islands have lower energy density, soluble carbohydrates and total protein that local red macroalgae (McDermid et al. 2007), thus suggesting a lower palatability for green turtles compared to red macroalgae, although electivity data are missing. Campos and Cardona (2020) suggested that seagrasses where the staple food of green turtles only where they formed extensive meadows and Seminoff et al. (2021) have reported an increase in the breadth of isotopic niche of green turtles in the Americas as seagrass availability declines and other sources are consumed. Currently, seagrasses do not form extensive meadows in the Hawaiian Islands but occur in small patches scattered mostly in shallow and sheltered area (McDermid et al. 2002; 2003). In this scenario, the convergence of green turtles on a macroalgae-based diet is hardly surprising and the former existence of seagrass specialists can be explained only if more extensive seagrass meadows existed. If so, changes in the isotopic niche of green turtles would not be density dependent but reflect decreased ecological opportunity in the increasingly simplified coastal ecosystems of the Hawaiian Islands (Layman et al. 2007).

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7. GENERAL DISCUSSION

The green turtle is a circumglobally distributed species that became a conservation icon worldwide, since past human exploitation decimated their populations globally, but conservation measures have been highly effective for some populations, particularly in the Western South Atlantic Ocean and the Hawaiian Archipelago (Chaloupka et al. 2008; Kittinger et al. 2013; Silva et al. 2017; Broderick and Patricio 2019; Chaloupka and Pilcher 2019). However, the coastal ecosystems of the Hawaiian Islands have also been intensely modified by human activity because of the introduction of several species of macroalgae and mangroves (Russo 1977; Allen 1998), the decline of roving herbivorous fishes except at marine protected or remote areas (Friedlander et al. 2003; Williams et al. 2008; Jouffray et al. 2015; Helyer and Samhouri 2017) and the nutrient enrichment resulting from population growth and overfertilization of farmland (Conklin and Stimson 2004; Drupp et al. 2011; Jouffray et al. 2015; Laws and Taguchi 2018; Murray et al. 2019; Lesser 2021). All those changes are probably favorable for green turtles, since they increase the amount of macroalgae available (Cheroske et al. 2000; Drupp et al. 2011; Lesser 2021) and decrease competition. The population is probably still below carrying capacity (Balazs and Chaloupka 2006; Kittinger et al. 2013; Piacenza et al. 2016), except at a few sites (Balazs and Chaloupka 2006).

The stable isotope analysis conducted in this thesis suggests that macroalgae have been the staple diet of Hawaiian green turtles during the past 120 years and that the long and narrow skull of Hawaiian green turtles is an adaptation for selective browsing of the most nutritious species of red macroalga in species rich algal pastures and in coral reefs, opposite to the short, broad and high skulls of green turtle populations relying mostly on monospecific seagrass meadows. If so, the current reliance of Hawaiian green turtles on red macroalgae is not a consequence of the introduction of exotic read algae during the second half of the 20th century, but a long-standing characteristic of the population. Similarly, the preference of green turtles for shallow, flat areas with dense algal pastures was also probably true in the past, because total algal cover is usually low in well preserved, subtidal habitats in the Hawaiian Archipelago, likely because of intense sea urchin grazing (Wabnitz et al. 2010).

Hawaiian green turtles settle in neritic habitats when approximately 40 cm CCL and shift to a mostly plant-based diet (Russell et al. 2003; Arthur and Balazs 2008;

Russell and Balazs 2009; Russell and Balazs 2015). The stable isotope analysis conducted in the present thesis have revealed an abrupt diet change after the settlement of green turtles in eastern Oahu, but not in the Kona coast. Previous research has suggested that the pace and timing of the ontogenic diet shift in green turtles are highly dependent on the relative availability of animal and plant food sources (Cardona et al. 2009; González Carman et al. 2014), which probably explain the differences between east Oahu and the Kona/Kohala coast. East Oahu supports meadows of *H. hawaiiana* and *H. decipens* (McDermid et al. 2002; McDermid et al. 2003; McDermid and Stuercke 2003), extensive red mangrove areas (Allen 1998), and areas of coral rubble with high algal coverage (Cheroske et al. 2000). Algal availability was even higher in the recent past when the area suffered a high nutrient load (Cheroske et al. 2000; Drupp et al. 2011; Lesser 2021). Conversely, subtidal algal coverage is low along most of the Kona/Kohala coast, as shown in the present thesis, and green turtles forage mostly on intertidal turf and macroalgae (Wabnitz et al. 2010). The Kona/Kohala coast has been less impacted by human activities than east Oahu and is probably more representative of the original ecosystem structure in in the main Hawaiian Islands. However, current green turtles inhabiting the Kona/Kohala coast also have access to the fish discarded by anglers at Honokōhau Small Boat Harbor (G. Balazs, personal observation) and fish flesh has also been reported in the gut contents of some green turtles dead-stranded along the Kona/Kohala coast (Russell et al. 2011). The combination of limited access to macroalgae and a moderate availability of animal resources experienced by green turtles in the Kona/Kohala coast probably results into the delayed ontogenic shift reported here and could have been also true in the past.

The results reported here support this interpretation, as no significant change in the average $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values in the bone tissue of Hawaiian green turtles was observed from 1901 to 2020, but some turtles from Oahu had very high $\delta^{15}N$ values, consistent with a high contribution of animal prey to their diets. Such diet is currently non-existing off eastern Oahu, where seagrass specialists are also gone. This has resulted into a significant reduction of the isotopic niche breadth in the most recent study period (2018-2020). Accordingly, the diet of green turtles has been isotopically stable for 120 years, but the diversity of the trophic resources consumed, and the degree of individual specialization has decreased recently. This is not an artifact resulting from the inclusion of some green turtles from French Frigate Shoal and the North-Western Hawaiian Islands in the first study period, because differences also exist between the second and the third periods, when only turtles from Oahu were analyzed.

Differences in the body size of green turtles sampled in the three study periods do not explain either the observed reduction in the breadth of the isotopic niche, because there are no differences in the average carapace length (CCL) of the turtles from the three study periods. Hence, the narrower isotopic niche observed in the most recent study reveals a lower variability in individual stable isotope ratios in green turtles.

Optimal foraging theory predicts that increased intraspecific competition will result into a broader trophic niche when all the individuals have the same initial preferences but may also reduce trophic niche width when individuals initially differ in their preferences and converge in the use of less preferred prey when the abundance of their preferred food sources decrease due to competition (Araújo et al. 2011). Population density is often considered a proxy of intraspecific competition (Araújo et al. 2011), although this approach ignores changes in resource availability. The population of green turtles at the Hawaiian Islands has been growing steadily since they were legally protected in 1978 (Balazs and Chaloupka 2006; Kittinger et al. 2011; Van Houtan and Kittinger 2014; Balazs et al. 2015; Piacenza et al. 2016) and hence intraspecific competition might have increased. In this scenario, the convergence of green turtles on a macroalgae-based diet is hardly surprising and the former existence of seagrass specialists can be explained only if more extensive seagrass meadows existed. If so, changes in the isotopic niche of green turtles would not be density dependent but reflect decreased ecological opportunity in the increasingly simplified coastal ecosystems of the Hawaiian Islands (Layman et al. 2007). In other words, the decrease in the diversity of foraging strategies observed currently in Hawaiian green turtles in eastern Oahu is probably a consequence of habitat simplification resulting from the anthropogenization of eastern Oahu and the high availability of macroalgal resources.

8. CONCLUSIONS

1. Hawaiian green turtles are the dominant herbivores in shallow and flat areas with a dense macroalgal cover but are scarcer than herbivorous fishes and sea urchins in corals reefs.
2. Macroalgae and algal turfs dominate the diet of Hawaiian green turtles, although seagrasses and mangroves in eastern Oahu and fish in the Kona coast are also relevant food sources.
3. Populations of green turtles relying heavily on macroalgae have longer and narrower skulls compared to populations relying heavily on seagrasses, which allow them to browse selectively the most nutritious species in mixed algal pastures. Conversely, the skull morphology of green turtles foraging on seagrasses is better suited for unselective grazing on monospecific meadows.
4. Green turtles shift quickly to an herbivorous diet after settlement in east Oahu, but the change is slower in the Kona coast, probably because of the higher availability of plant resources in the former.
5. The squamosal bone and the ribs of green turtles did not differ in their $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ values and hence the identity of the skeletal elements analyzed is not a significant source of variability in retrospective isotopic studies.
6. Stable isotope ratios of C, N, and S from skeletal elements with a thick core of trabecular bone (skull and rib) integrate dietary information similarly to the epidermis in green turtles, although probably over more extended periods, and hence are informative about diet immediately before dead.
7. The trophic discrimination factor of trabecular bone cannot be used in mixing models using stable isotope ratios of cortical bone, whose trophic discrimination factors needs to be assessed independently to reconstruct the diet of green turtles.
8. The isotopic niche of Hawaiian green turtles has not changed during the past 120 years, despite major ecological and demographic changes, thus suggesting that macroalgae have represented the unprocessed of their diet even before the

introduction of the exotic species of red macroalgae that currently dominate their diet.

9. Current Hawaiian green turtles have a narrower isotopic niche that their ancient conspecifics, which revealed a lower variability in individual trophic strategies.
10. Historic changes in the breadth of the isotopic niche of Hawaiian green turtles are probably not driven by competition but reflect a decreased ecological opportunity in the increasingly simplified coastal ecosystems of the Hawaiian Islands.

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