Spring 4-13-2018

USING d13C, d15N, and d2H TO BETTER UNDERSTAND THE ECOLOGY OF GREEN SEA TURTLES

Laura Pagès Barceló
University of New Mexico

Laura Pagès Barceló

Candidate

Biology

Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Seth D. Newsome, Chairperson

Jeffrey A. Seminoff

Howard L. Snell

USING $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ TO BETTER UNDERSTAND THE ECOLOGY OF GREEN SEA TURTLES

by

LAURA PAGÈS BARCELÓ

B.S., Biology, University of Barcelona, 2011

THESIS

Submitted in Partial Fulfillment of the
Requirements for the Degree of

Master of Science
Biology

The University of New Mexico
Albuquerque, New Mexico

May, 2018

ACKNOWLEDGEMENTS

Numerous people have contributed both with academic and personal support to make this research possible. I would first like to thank my thesis advisor and committee chair, Seth Newsome, for his encouragement and dedication throughout this process. I would also like to thank my co-advisor, Jeffrey Seminoff, for contributing with his vast knowledge of sea turtle ecology and conservation, and for providing me with opportunities to participate in field work with his research team, and to interact with other sea turtle researchers. Lastly, I would like to thank my third committee member, Howard Snell, for encouraging me to maintain a broad ecological perspective throughout my work.

In addition to my committee members, I would also like to thank numerous people in the UNM Biology Department who have helped me throughout this process. My lab mates, Emma Elliott Smith, John Whiteman, Alexi Besser, Deborah Boro, Mauriel Rodriguez Curras, Allyson Richins, Jenny Noble and Sarah Lujan have all provided invaluable encouragement and support. I would also like to thank Carlos Alberto López Montalvo, Geraldine Busquets-Vass, Laguna San Ignacio Ecosystem Science Program team, Ecoturismo Kuyima and their pangeros for support during the field work portion of this project. Finally I would also like to thank friends from my country, Catalunya, my hometown, Banyoles, and from my new home, Albuquerque, for their emotional support.

This research would have not been possible without support from my parents, Teresa Barceló Badosa and Josep Pagès José, who encouraged me since the day I left my home country to pursue my career dreams. Finally, I would like to thank my life partner, David Van Horn, for his unconditional love, support, and continuous encouragement through the years.

USING $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ TO BETTER UNDERSTAND THE ECOLOGY OF GREEN SEA TURTLES

by

LAURA PAGÈS BARCELÓ

B.S., Biology, University of Barcelona, 2011

M.S., Biology, University of New Mexico, 2018

ABSTRACT

Many green sea turtle populations are slowly recuperating from a recent severe decline due to anthropogenic factors including human consumption and mortality related to the fishing industry. Despite being charismatic animals that have been extensively studied, there is still a limited understanding of their feeding strategies and diet plasticity. This research explores the use of hydrogen isotopes in marine ecosystems to better understand green sea turtle ecology. This study is presented in two chapters: I first examined the trophic discrimination factor ($\Delta^{2}H_{NET}$) for hydrogen isotope ($\delta^{2}H$) as a tool to correct hydrogen isotope data for wild populations, and next explored the use of hydrogen isotopes in conjunction with nitrogen and carbon isotopes to study green sea turtle diet and habitat use in Baja California Sur. Together, this research was used to (a) differentiate feeding strategies and habitat use for wild green sea turtles living in different habitats (open ocean versus lagoon) along the Pacific coast of Baja California Sur and (b) determine the utility of hydrogen isotopes to trace regional migration and trophic level patterns in wild sea turtle populations. Results from these studies provide additional evidence of wide feeding plasticity in green sea turtles as has been reported in previous

studies. Additionally, this work provides insights regarding inter-individual diet variation within lagoon habitats (specialists vs generalists) in comparison to open ocean habitats where individuals show a small dietary niche breath and feed on prey items at a higher trophic level. This work also supports the use of hydrogen isotopes as a new tool to distinguish between prey items, elucidate local migration patterns, and determine trophic level status within consumers in marine ecosystems.

INTRODUCTION

Humans are rapidly altering marine ecosystems by increasing temperatures from global warming, decreasing pH from elevated CO_2 uptake, and altering food web structure from overfishing. A challenge for marine ecologists and conservation biologists is to determine how organisms adapt their life strategies to these environmental perturbations. A group of organisms that has been severely impacted by these disturbances is sea turtles, with nearly all species classified as endangered. In addition to their vulnerability, sea turtles are also an ideal study organism because their low metabolic rates result in very long life spans and thus they preserve a record of environmental conditions in their tissues (Agland et al., 2011; Opkins, 2007; Páez-osuna et al., 2010; Segars and Kucklick, 2005). In spite of efforts to learn more about their ecology and behavior, basic information about their life history is still unknown, including information on their resource and habitat use: are sea turtle herbivores as suggested by limited observational data or are they opportunistic omnivores? What habitats are crucial for foraging and are thus important conservation priorities?

Stable isotopes are an increasingly important tool in biology used to understand animal foraging and migration strategies by tracking ecological connections between animals and their diets (Hobson et al., 1999a; Mcclellan et al., 2010). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes can estimate sources of primary production and the trophic status of an organism. Hydrogen isotope ($\delta^{2}H$) values have been used recently to characterize animal migration (Bowen et al., 2005a), but the potential of this new tool is still unknown, particularly in marine systems.

The research in this thesis focuses on using carbon ($\delta^{13}C$), nitrogen ($\delta^{15}N$), and hydrogen ($\delta^{2}H$) isotope values to better understand green sea turtle resource and habitat use. In the first chapter of my thesis I analyze the $\delta^{2}H$ values of sea turtle tissues collected during two feeding experiments (Seminoff et al., 2006; Vander Zanden et al., 2012) to understand how hydrogen isotopes are sorted by physiologically mediated processes such as excretion or the routing of dietary macromolecules (e.g., protein). Next, I apply what I learned in captive sea turtles to a study of tissues from wild populations from the Pacific coast of Baja California, Mexico. $\delta^{2}H$ data is used in conjunction with $\delta^{13}C$ and $\delta^{15}N$ values to learn about sea turtle migration, breeding and foraging strategies in the wild, and to advance a novel isotopic method ($\delta^{2}H$ analysis) that has the potential to provide valuable insights into what resources turtles use in different habitats.

Despite the fact that sea turtles are charismatic animals, much of their life history remains an enigma due to their distribution across large expanses of ocean. Thus, tools such as stable isotopes are essential to understand sea turtle habitat use, diets, and migration patterns, knowledge that is crucial for developing conservation strategies. Sea turtle populations are an essential component of healthy oceans and are sentinels of ecosystem health: they maintain sea grass beds biodiversity (Bjorndal, 1980); bring nutrients to beaches through their hatching process (Bouchard and Bjorndal, 2000); help to maintain stable jellyfish populations through predation (Houghton et al., 2015); and are a food source to other marine organisms (Frick et al., 2000a; Frick et al., 2000b). Thus, an improved understanding of sea turtle ecology will help develop conservation strategies to protect these animals and their environment.

REFERENCES

Agland, J. A. M. R., Rendt, M. I. D. A., Ucklick, J. O. H. N. R. K. and Eller, J. E. M. K. (2011). Persistent organic pollutans in blood plasma of satellite-tracked adult male Loggerhead sea turtles (*Caretta caretta*). 30, 1549–1556.

Bjorndal, K. A. (1980). Nutrition and grazing behavior of the green turtle *Chelonia mydas*. *Mar. Biol.* 56, 147–154.

Bouchard, S. S. and Bjorndal, K. A. (2000). Sea turtles as biological transporters of nutrients and energy from marine to terrestrial ecosystems. 81, 2305–2313.

Bowen, G. J., Wassenaar, L. I. and Hobson, K. A. (2005). Global application of stable hydrogen and oxygen isotopes to wildlife forensics. *Oecologia* 143, 337–348.

Frick, M. G., Williams, K. L. and Veljacic, D. (2000a). Additional evidence supporting a cleaning association between epibiotic crabs and sea turtles: how will the harvest of Sargassum seaweed impact this relationship? *Mar. Turt. Newsl.* 90, 11–13.

Frick, M. G., Williams, K. L., Veljacic, D., Pierrard, L., Jackson, J. A. and Knight, S. E. (2000b). Newly documented epibiont species from nesting loggerhead sea turtles (*Caretta caretta*) in Georgia, USA. *Mar. Turt. Newsl.* 88, 3–5.

Hobson, K. A., Atwell, L. and Wassenaar, L. I. (1999). Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proc. Natl. Acad. Sci.* 96, 8003–8006.

Houghton, J. D. R., Doyle, T. K., Wilson, M. W., Davenport, J., Hays, G. C. and Hays, C. (2015). Jellyfish Aggregations and Leatherback Turtle Foraging Patterns in a Temperate Coastal Environment Published by : Ecological Society of America Stable URL : http://www.jstor.org/stable/20069181 Your use of the JSTOR archive

indicates your acceptance of th. 87, 1967–1972.

Mcclellan, C. M., Mcneill, J. B. and Wallace, B. P. (2010). Stable isotopes confirm a foraging dichotomy in juvenile loggerhead sea turtles.

Opkins, W. I. A. H. (2007). INFLUENCE OF FEEDING ECOLOGY ON BLOOD MERCURY CONCENTRATIONS IN FOUR SPECIES OF TURTLES. 26, 1733–1741.

Páez-osuna, F., Calderón-campuzano, M. F., Soto-jiménez, M. F. and Ruelas-inzunza, J. R. (2010). Lead in blood and eggs of the sea turtle, *Lepidochelys olivacea*, from the Eastern Pacific : Concentration , isotopic composition and maternal transfer. *Mar. Pollut. Bull.* 60, 433–439.

Segars, A. L. L. and Kucklick, J. R. (2005). Perfluorinated Compounds in the Plasma of Loggerhead and Kemp ' s Ridley Sea Turtles from the Southeastern Coast of the United. 9101–9108.

Seminoff, J. A., Jones, T. T., Eguchi, T., Jones, D. R. and Dutton, P. H. (2006). Stable isotope discrimination (δ13C and δ15N) between soft tissues of the green sea turtle *Chelonia mydas* and its diet. *Mar. Ecol. Prog. Ser.* 308, 271–278.

Vander Zanden, H. B., Bjorndal, K. A., Mustin, W., Ponciano, J. M. and Bolten, A. B. (2012). Inherent variation in stable isotope values and discrimination factors in two life stages of green turtles. *Physiol. Biochem. Zool.* 85, 431–441.

TABLE OF CONTENTS

CHAPTER 1: HYDROGEN ISOTOPE ASSIMILATION AND DISCRIMINATION IN GREEN SEA TURTLES (*CHELONIA MYDAS*)

Laura Pagès Barceló[1], Jeffrey A. Seminoff[2], Hannah B. Vander Zanden[3], T. Todd Jones[4], and Seth D. Newsome[1]

[1]Department of Biology, University of New Mexico, Albuquerque, New Mexico

[2]National Oceanic and Atmospheric Administration–National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, California

[3]Department of Biology, University of Florida, Gainesville, Florida

[4]NOAA Fisheries, Pacific Islands Fisheries Science Center, Honolulu, Hawaii

ABSTRACT

Hydrogen isotopes (δ^2H) are commonly used as tracers of animal movement and migration, however, minimal research has investigated the use of δ^2H as a potential proxy to quantify resource and habitat use. In contrast to carbon and nitrogen that are ultimately derived from a single source (food), the proportion of hydrogen in consumer tissues originates from two distinct sources: water and food. Before δ^2H can be effectively used as a resource tracer, we need estimates of (net) discrimination factors (Δ^2H_{Net}) that account for the physiologically mediated differences in the δ^2H values of animal tissues relative to that of the food and water sources they use tissue synthesis. Here we estimated Δ^2H_{Net} in captive green sea turtles (*Chelonia mydas*) by measuring δ^2H values of tissues (epidermis and blood components) and diet collected after two controlled feeding

experiments. We found that tissue δ^2H values and associated Δ^2H_{Net} varied systematically among tissues, with epidermis having higher δ^2H values and Δ^2H_{Net} than whole blood, blood serum, or red blood cells. This pattern mirrors that found between keratinaceous tissues (feathers, hair) and blood components in birds and mammals. Serum of adult female green sea turtles had significantly lower δ^2H values in comparison to that collected from juveniles, likely due to lipid storage associated with egg formation and other reproductive processes. This is the first study to quantify Δ^2H_{Net} in a marine ectotherm, and we anticipate our results will further refine the use of hydrogen isotope analysis to better understand animal resource and habitat use in marine ecosystems.

INTRODUCTION

The use of stable isotope analysis (SIA) to characterize habitat and resource use by organisms has grown exponentially in the past decade (Hobson, 1999a; Peterson and Fry, 1987; Post, 2002). SIA is now routinely used to assess diet composition (Burgett et al., 2018; Connan et al., 2018; Díaz-Gamboa et al., 2017), assess energy within and among ecosystems (Doucett et al., 1996), estimate trophic level and food chain length (Boecklen et al., 2011; Cabana and Rasmussen, 1996; Post, 2002) and trace movement and migration patterns (Hobson, 1999b; Rubenstein and Hobson, 2004). In particular, studies of individual- and population-level foraging strategies have greatly benefited from SIA, which provides information about resource assimilation over a variety of timescales depending on the type of tissue analyzed (Martinez del Rio et al., 2009). This is one of the primary advantages of SIA in comparison to conventional dietary proxies, such as gut content or scat analysis, which only provide dietary ‘snapshots’ and may not accurately

identify which dietary items are assimilated by an organism (Hobson, 1999b; Hobson and Wassenaar, 1996, Newsome et al. 2010).

Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analyses have been most frequently used by animal ecologists to characterize resource and habitat use (DeNiro and Epstein, 1978; Hobson, 1999a; Marra et al., 1998; Phillips, 2012; Rubenstein and Hobson, 2004). In comparison to $\delta^{13}C$ and $\delta^{15}N$, hydrogen isotope ($\delta^{2}H$) values have been used much less frequently to study resource and habitat use, but instead have been primarily used to track the geographic origin and seasonal movement of birds (Chamberlain et al., 1996; Hobson, 2005a; Hobson, 2005b; Hobson and Wassenaar, 1996), insects (Wassenaar and Hobson, 1998) and even humans (Ehleringer et al., 2008) across regional to continental scales. Only a few studies have used $\delta^{2}H$ as a tracer of food resources, to quantify autochthonous (instream algae) and allochthonous (riparian plants) resources in freshwater ecosystems (Berggren et al., 2014; Cole et al., 2011; Doucett et al., 2007; Finlay et al., 2010; Jardine et al., 2009), or to coarsely assess trophic level (Birchall et al., 2005).

Although the use of $\delta^{2}H$ in ecological studies has increased over the last decade, a persistent challenge is to understand the ecological and physiological factors that contribute to variation in consumer tissue $\delta^{2}H$ values. As stable isotopes of these elements move up the food chain, they fractionate in predictable ways, which leads to offsets in isotope values between consumers and their diet, commonly referred to as trophic discrimination (Δdt; Martinez del Rio et al., 2009; Newsome et al., 2010). Whereas food is the only source of carbon and nitrogen available to animals to synthesize tissues, there are two distinct sources of hydrogen: food and water (Ehleringer et al.,

2008; Wolf et al., 2011; Wolf et al., 2013). To expand the use of δ^2H analysis to study animal ecology, we must better understand how animals assimilate and sort hydrogen isotopes. This requires knowledge of (1) the proportion of the hydrogen in tissues that is sourced from environmental water versus food, and (2) the offset in δ^2H values of consumers and these sources, commonly referred to as trophic discrimination factors (Δ^2H_{net}). In regard to the first challenge, controlled feeding experiments show that the majority of hydrogen in consumer tissues is derived from diet (~70–80%), whereas only ~20–30% is sourced from environmental or drinking water (Hobson et al., 1999a; Macko et al., 1983; Malej et al., 1993; Newsome et al., 2017; Rodriguez Curras et al. in review; Solomon et al., 2009; Soto et al., 2013; Wolf et al., 2011). Fewer studies have focused on quantifying trophic discrimination factors, which are mediated by physiological processes during assimilation, synthesis, and excretion. Similar to patterns observed in $\Delta^{13}C$ and $\Delta^{15}N$, variation in Δ^2H are likely influenced by tissue amino acid composition and the potential for disproportionate routing of dietary protein to tissue synthesis (Ambrose and Norr, 1993; Schwarcz and Schoeninger, 1991; Tieszen and Fagre, 1993).

Whereas δ^2H has the potential to become a powerful method to study animal ecology and eco-physiology, only a handful of studies have focused on quantifying trophic discrimination factors (Hobson et al., 1999a; Hobson et al., 1999b; Newsome et al., 2017; Rodriguez Curras et al. in review). These studies have revealed that in general consumer tissues have δ^2H values greater than potential food sources but lower δ^2H values than source of environmental water (Peters et al., 2012; Solomon et al., 2009), a pattern that results in apparent increases in δ^2H values with trophic level (Birchall et al., 2005; Hobson, 2005b). Similar to patterns observed in $\Delta^{13}C$ and $\Delta^{15}N$ (Bearhop et al.,

2002; Hobson and Clark, 1992; Kurle et al., 2014; Robbins et al., 2005); variation in Δ^2H are likely influenced by tissue amino acid composition and the potential for disproportionate routing of dietary protein to tissue synthesis (Fogel et al. 2016, Newsome et al. 2017, Curras Rodriguez et al. in review).

Even though the first study to measure δ^2H values of biological samples reported data for algae and invertebrates collected from a nearshore marine ecosystem (Estep and Dabrowski, 1980), δ^2H has rarely been used since to study marine ecology. Significant variation in δ^2H values among marine primary producers (Estep and Dabrowski, 1980; Fenton and Ritz, 1989; Schiegl and Vogel, 1970; Smith and Epstein, 1970) suggests that hydrogen isotopes may be useful for quantifying resource (Ostrom et al., 2014) and habitat use in marine settings, especially in nearshore ecosystems fueled by a combination of phytoplankton, macroalgae, seagrass, and even terrestrial plants. In addition, more recent work has identified predictable δ^2H discrimination among sources in marine ecosystems (Hondula and Pace, 2014), similar to that observed in freshwater aquatic and terrestrial food webs (Birchall et al., 2005; Hobson et al., 2004; Peters et al., 2012; Soto et al., 2013). Thus, δ^2H may complement $\delta^{13}C$ and $\delta^{15}N$ data to better quantify the relative contributions of different sources of primary production and characterize food web dynamics in marine ecosystems. But before ecologists can confidently apply this tool to assess resource and habitat use by wild populations, additional controlled feeding experiments are needed to better understand the factors that influence hydrogen isotope assimilation and discrimination in consumer tissues.

Here we investigate hydrogen isotope assimilation and discrimination in an omnivorous marine ectotherm by sampling tissues from captive green sea turtles

(*Chelonia mydas*) fed controlled diets in two separate feeding experiments. Our objective was to estimate trophic discrimination factors (Δ^2H_{net}) between the sources of hydrogen available for tissue synthesis and green sea turtle blood plasma, red blood cells, and skin (epidermis). We analyzed the isotopic composition of dietary macromolecules and used a mass balance mixing model framework to examine the factors that cause δ^2H variation among tissue types and life stages. We anticipate that our results will help refine the use of δ^2H as a tool to study resource and habitat use by marine consumers that inhabit nearshore ecosystems characterized by large variation in δ^2H values among primary producers.

MATERIALS AND METHODS

In this study we analyzed green sea turtle tissues and diet samples collected during two previous controlled feeding experiments described in Seminoff et al. (2006) and Vander Zanden et al. (2012). Both feeding experiments used green sea turtles bred at Cayman Turtle Farm (British West Indies). However, turtles from Seminoff et al. (2006) were transferred to University of British Columbia (Vancouver) as hatchlings. In this experiment, eight juvenile green sea turtles [body mass= 11.7±0.7kg, straight carapace length (SCL) = 45.2±1.2cm] were fed a constant pellet diet composed of 54% protein, 16% lipids, and 30% carbohydrates by weight (Aquamax Grower 500 5D05; PMI Nutrition International, LLC; Brentwood, Missouri) for 619 days. We analyzed samples of the pelleted diet, skin epidermis, and blood components (whole blood, plasma, red blood cells) collected at the end of this experiment. In the Vander Zanden et al. (2012) experiment, 70 green sea turtles (30 adults and 40 juveniles; body $mass_{adults}$= 125 ±

29.2kg, curved carapace length (CCL_{adults}) = 101 ± 5.2cm; body $mass_{juveniles}$= 42 ± 8.1kg, $CCL_{juveniles}$= 73 ± 5.5cm) were kept in controlled conditions for four years (~1460 days) and fed a constant pellet diet containing 42% protein, 5% lipids, and 53% carbohydrates by weight (Southfresh Feeds, Alabama, USA). Three tissue types were collected at the end of this experiment, including blood plasma, red blood cells, and epidermis. For convenience, we will refer to Seminoff et al. (2006) experiment as FE1 and Vander Zanden et al. (2012) as FE2 in the following sections.

Separation of Diet Macromolecules. δ^2H values for dietary lipids and proteins were obtained via lipid-extraction followed by hydrolysis and cation exchange separation. Lipids were extracted from homogenized diet pellets via three ~24 hour soaks in petroleum ether. After each soak, the removed petroleum ether was saved in a separate glass scintillation vial and transferred to pre-weighed silver capsules for δ^2H lipid analysis. Lipid-extracted diet samples were then rinsed with deionized water to remove any residual solvent and dried at ~45ºC for 48 hours. Approximately 6–7 mg of the lipid-extracted diet samples were then hydrolyzed in 6N HCl for 20 hours at 110 ºC, breaking proteins into constituent amino acids, then dried under a flow of N_2 gas at 110 ºC. A cation exchange resin (Dowex 50WX8, 100-200 mesh) was used to separate the amino acids portion (Amelung and Zhang, 2001). The hydrolyzed sample was added to a column containing the Dowex resin and then washed with 3–4 mL of 0.01N HCl solution. Subsequently, 4 ml of 2N NH_4OH was then added to the column and the effluent containing amino acids was collected in another pre-combusted glass vial. The amino acid fraction was dried down at 80˚C under N_2 and temporarily re-suspended in

ethanol for transfer to silver capsules, which were then dried at 45˚C to evaporate solvent prior to δ^2H analysis (Amelung et al., 1996; Andrews, 1989; Christianson et al., 1960; Wall, 1953). δ^2H values for the carbohydrate portion of diet were estimated by a mixing model using measured values for dietary lipids and protein in combination with data on the relative proportion of these macromolecules in each experimental diet (Table 1).

δ^2H Analysis. Samples of blood plasma and red blood cells were dried at 60°C for 24 hours and homogenized with a mortar and pestle. Epidermis samples were rinsed with deionized water (DI) water and then dried at 60°C for 24 to 48 hours. Skin epidermis samples were lipid-extracted in three sequential 24 h soaks with petroleum ether and then thoroughly rinsed in deionized water before being dried at 45°C for ~48 hours (Seminoff et al., 2006; Vander Zanden et al., 2012). To evaluate the influence of lipids on δ^2H values, we separately compared a subsample of FE2 epidermis (n=20; 10 juveniles and 10 adults) before and after lipid removal.

Approximately 0.1–0.3 mg of sea turtle tissue or pelleted diet samples were weighed into 3.5x5mm silver capsules sealed for δ^2H analysis. Proteins can exchange ~10–20% of their hydrogen with ambient water vapor in the atmosphere (Bowen et al., 2005b; Coplen and Qi, 2012; Meier-Augenstein et al., 2011; Sauer et al., 2009; Wassenaar and Hobson, 2000). Thus, samples and associated in-house reference materials used to correct for exchangeable hydrogen sat for at least three weeks before analysis to ensure equilibration between the exchangeable hydrogen fraction of each sample or standard and local water vapor (Bowen et al., 2005b; Sauer et al., 2009; Wassenaar and Hobson, 2000; Wassenaar and Hobson, 2003).

δ^2H values and weight percent hydrogen concentration values ([H]) were measured with a Thermo Scientific high-temperature conversion elemental analyzer connected to a Thermo Scientific Delta V isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremmen, Germany) at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM). Hydrogen isotope values are expressed in delta (δ) notation using the equation: $\delta^2H = [(R_{sample} - R_{standard}/R_{standard}) - 1] \times 1000$, where R represents the ratio of the heavy to the light isotope ($^2H/^1H$) in sample or standard respectively relative to Vienna-Standard Mean Ocean Water (V-SMOW), which is the internationally accepted standard for δ^2H analysis. Units were expressed in parts per mil (‰).

To correct the data we used a series of keratin in-house reference materials for which the hydrogen isotope composition of the non-exchangeable portion ($\delta^2H_{non\text{-}exchangeable}$) of the tissue had been previously estimated via room-temperature exchange experiments identical to those used by Bowen et al. 2005. $\delta^2H_{non\text{-}exchangeable}$ values for the three keratin internal reference materials ranged from –55‰ to –175‰. Muscle and whole blood from cows raised in Wyoming and Florida were also used to compare δ^2H in similar tissues (–70‰ and –150‰ for muscle; –82‰ and –160‰ for blood). The among-run variation in δ^2H of cow muscle and whole blood was ≤5‰ and ≤4‰, respectively. We used the oil standard NBS-22 (δ^2H: –120‰) to correct lipid samples extracted from pelleted diet because lipids do not contain exchangeable hydrogen. All standards had a within-run hydrogen isotope variation (SD) of ≤3‰.

Hydrogen Mixing Model. The hydrogen trophic discrimination factors between tissue type and diet or water sources were estimated with mass balance equation mixing model (*Equation 1*):

$$\delta^2H_{Tissue} = p_{Water}\,(\delta^2H_{Water} + \Delta^2H_{Water}) + p_{Protein}\,(\delta^2H_{Protein} + \Delta^2H_{Protein})$$

$$+ p_{Carbohydrates}\,(\delta^2H_{Carbohydrates} + \Delta^2H_{Carbohydrates}) + p_{Lipids}\,(\delta^2H_{Lipids} + \Delta^2H_{Lipids}),$$

where δ^2H_C is the hydrogen isotope value of component C, p_C is the proportion of hydrogen coming from each of the source components I, and Δ^2H_C is the discrimination factor derived from the different source components. There are two potential sources of hydrogen used by consumers to synthesize tissues (diet and water), thus (*Equation 2*):

$$p_{Water} + p_{Protein} + p_{Carbohydrates} + p_{Lipids} = 1$$

The hydrogen discrimination factor associated with each of the diet macromolecular components in Equation 1 can be isolated and combined as the net effective trophic discrimination factor (TDF) or Δ^2H_{Net} (*Equation 3*):

$$\Delta^2H_{Net} = p_{Water}(\Delta^2H_{Water}) + p_{Protein}(\Delta^2H_{Protein}) + p_{Carbohydrates}(\Delta^2H_{Carbohydrates}) +$$

$$p_{Lipids}(\Delta^2H_{Lipids})$$

Thus, Equation 1 becomes (*Equation 4*):

$$\delta^2H_{Tissue} = p_{Water}\,(\delta^2H_{Water}) + p_{Protein}\,(\delta^2H_{Protein}) + p_{Carbohydrates}\,(\delta^2H_{Carbohydrates}) + p_{Lipids}$$

$$(\delta^2H_{Lipids}) + \Delta^2H_{Net}$$

Body water and diet macromolecule proportions (p_{water}=0.2 and p_{diet}=0.8) were obtained as an average approximation from previous feeding experiments done on terrestrial and aquatic organisms (Hobson et al., 1999; Newsome et al., 2017; Solomon et al., 2009; Soto et al., 2013; Wolf et al., 2011; Rodriguez Curras, unpubl. data). Proportions proved

to be conserved among distinct taxa independent of the environment. We assessed the potential impacts of this assumption with a sensitivity analysis in which we manipulated the macromolecule proportions within a 15% range.

Data Analysis. We used one and two-way ANOVAs followed by post-hoc Tukey HSD pairwise comparisons with Bonferroni correction to evaluate differences between tissues for Δ^2H_{Net} in experiment FE1. For experiment FE2, we used a 2-way ANOVA with 2 factors: 1) Age (2 levels: Juvenile and Adult); 2) Tissue (3 levels: EPI, SER, RBC) corrected for unbalanced data (lsmeans) followed by pairwise comparison using the Bonferroni correction. When comparing between experiments, we used a 2-way ANOVA with two factors: 1) Experiment (two levels: FE1 and FE2); 2) Tissue (three levels: EPI, SER, RBC) corrected for unbalanced data (lsmeans) followed by pairwise comparison using Bonferroni correction. All models were run using Rstudio packages (e.g. car, emmeans, nortest; version 1.1.383).

RESULTS

Diet and Water. δ^2H values of bulk and lipid-extracted diets did not differ between the two feeding experiments ($F = 14.8$; $df = 8$, $P > 0.1$; Fig. 2A). However, at the macromolecular level, the δ^2H values for proteins and carbohydrates significantly differed between feeding experiments ($F = 295.1$; $df = 26$, $P \leq 0.003$) with higher values for carbohydrates and lower values for protein in the FE2 versus the FE1 diet (Fig. 2A). Dietary lipid δ^2H values from the two feeding experiments were not significantly different ($P = 0.98$; Fig. 2A).

δ^2H values for water had to be estimated since no samples were collected when the feeding experiments were conducted. We used isoscapes of seawater $\delta^{18}O$ values (LeGrande and Schmidt, 2006) and the equation for the global meteoric water line ($\delta^2H = 8\ \delta^{18}O + 10‰$; Craig and Gordon, 1965) to estimate the δ^2H of seawater in the two locations where feeding experiments were conducted to estimate seawater δ^2H values (Kendall and Coplen, 2001). This approach resulted in a δ^2H_{Water} value for the FE1 experiment in Vancouver, BC of –10‰, and a δ^2H_{Water} value for the FE2 experiment in the Caribbean Cayman Islands of +10‰.

Results of our sensitivity analysis shows that changing the proportions within a 5% range from our selected values resulted in an overall difference within tissues and feeding experiments of 5–6‰. These values are slightly higher than analytical precision (3–4‰) of δ^2H measurements on organic materials via TCEA-IRMS and are similar to the observed variation in δ^2H within tissues but much smaller than differences in δ^2H among tissues (Table 2).

Tissue δ^2H Patterns Within and Between Feeding Experiments. Epidermis differed from the other tissues in FE1 ($F = 47.2$; $df = 28$, $P < 0.001$; Fig. 2A). In contrast, all tissues differed within and between life stages ($F = 560.9$, $df = 199$, $P < 0.005$) in FE2, with the exception of red blood cells and serum for the juvenile group. Adults had lower serum δ^2H values and high weigh percent hydrogen ([H]) concentrations than juveniles in FE2 (Fig. 3). The only tissue that differed between feeding experiments was epidermis (F= 308.3, $df = 137$, $P < 0.001$; Fig. 2A). Lastly, there were no significant differences in mean (±SD) δ^2H or weigh percent hydrogen ([H]) values between bulk (δ^2H: –74 ± 8; [H]: 5.6

± 0.1) and lipid-extracted epidermis (δ^2H: –73 ± 6; [H]: 6.0 ± 0.1) (t = –0.27, df = 38, P = 0.7888).

Tissue $\Delta^2H_{Net.}$ Epidermis had a significantly higher Δ^2H_{Net} than other tissues in FE1 (F = 53.7, df = 21, P < 0.001; Fig. 2B). In FE2, multiple pairwise comparisons between life stage and between tissues show that all tissues except serum and red blood cells in juveniles had significantly different Δ^2H_{Net} (F = 572.9, df = 200, P < 0.005). Juvenile epidermis and serum had higher Δ^2H_{Net} than adults while red blood cells showed the opposite pattern. Estimates of Δ^2H_{Net} for juveniles were slightly but significantly different between FE1 and FE2 experiments for serum and RBC (F = 315.5, df = 138, P < 0.001), but similar for epidermis (F = 315.5, df = 138, P = 1; Fig. 2B).

DISCUSSION

We quantified tissue-specific hydrogen isotope discrimination factors (Δ^2H_{net}) in juvenile and adult green sea turtles using tissues collected during two previous controlled feeding experiments (Seminoff et al., 2006; Vander Zanden et al., 2012). In the following sections, we assess the factors that influence Δ^2H_{net} and discuss possible physiological mechanisms responsible for the observed differences among diet treatments and age classes. Overall, our results corroborate Δ^2H_{net} patterns observed in previous controlled feeding experiments on other taxa (Fogel et al., 2016; Newsome et al., 2017a; Nielson and Bowen, 2010; Podlesak et al., 2008; Rodriguez Curras in review; Solomon et al., 2009; Soto et al., 2013; Storm-Suke et al., 2012; Wolf et al., 2012; Wolf et al., 2013), and we anticipate that these data will enable ecologists to accurately apply δ^2H isotopes to

study diet composition, habitat use, and movement in marine ectothermic consumers, particularly marine turtles.

When comparing results from different feeding experiments, it is important to consider differences in diet macromolecular composition and the proportional contribution food and water to tissue synthesis, both of which have been shown to influence tissue δ^2H and associated Δ^2H_{net}. Our models assumed that ~80% and ~20% of the hydrogen in sea turtle tissues was synthesized from diet versus water, respectively, which is similar to contributions found in a wide variety of vertebrate taxa (Hobson et al. 1999, Solomon et al. 2009, Wolf et al. 2011, Soto et al. 2013, Newsome et al., 2017a; Rodriguez Curras in review). With respect to diet composition, the diets used in the two feeding experiments described here were sufficiently similar in terms of macromolecular content and bulk diet δ^2H values (Table 1) that green sea turtle tissues had remarkably similar δ^2H values for nearly all tissue types (Fig. 2); the only exception to this was blood serum for adult turtles in the FE2 (Vander Zanden et al. 2012; see *Tissue δ^2H* section).

In regard to the hydrogen isotope composition of ingested water, we were unable to directly measure the δ^2H value of seawater used in each experiment, so we assumed δ^2H_{water} based on regional isoscapes for ocean water from the two study locations (Fry, 2006; Gat, 1996; Xu et al., 2012a). For the experiment at the University of British Columbia (Vancouver, BC; Seminoff et al., 2006), we used seawater δ^2H values of –10‰ (Smith and Epstein, 1970), which reflects influence of ^2H-depleted river water in coastal areas in this wet temperate region. For the experiment on Grand Cayman Island in the British West Indies (Vander Zanden et al. 2012), we used seawater δ^2H values of +10‰ (Sternberg and Swart, 1987), which reflects influence of evaporation of surface seawater

in this relatively hot tropical region. Given the relatively small contribution (20%) of hydrogen from water that is used to build tissues, calculations from our sensitivity analysis show that small variation of 5–10‰ in our estimated seawater δ^2H values do not heavily impact our estimates of Δ^2H_{net}.

Tissue δ^2H: Green sea turtle epidermis had higher mean δ^2H values relative to blood components by ~20‰ in both feeding experiments (Fig. 2), which is similar to the patterns in δ^2H values of keratinaceous tissues (feathers and claws) and blood components observed in other feeding experiments (Hobson et al., 1999a; Wolf et al., 2011; Wolf et al., 2012; Wolf et al., 2013). There are two primary explanations for these patterns. First, previous feeding experiments reporting $\delta^{13}C$ and δ^2H data have suggested that such tissue-specific patterns are likely driven by differences in the amino acid ([AA]) composition among tissues (Newsome et al., 2017; Wolf et al., 2012; Curras Rodriguez et al. in review). Hydrogen isotope values of individual amino acids within a single organism can vary by up to 200–300‰ (Fogel et al. 2016), thus subtle changes in a tissues amino acid composition could drive the variation in bulk tissue δ^2H values observed here and elsewhere (Newsome et al., 2017; Curras Rodriguez et al. in review). A second potential explanation for the observed tissue-specific δ^2H patterns between epidermis and blood components is that the hydrogen in the former tissue is directly routed from dietary protein, which had higher δ^2H values than that of bulk diet or other dietary macromolecules (carbohydrates or lipids) in both experiments (Fig. 2). Here, we define protein routing as the potential mismatch between dietary protein content (weight percent) and the relative proportion of how much dietary protein is used to synthesize

tissues. Protein routing has been observed in other δ^2H-based controlled feeding experiments (Newsome et al., 2017; Curras Rodriquez et al. in review); however, the animals in those experiments were fed diets that had much lower protein contents than the diets fed to green sea turtles in our experiments, which was exceptionally high (42–56%) for an omnivore. Since dietary protein was readily available, the potential for protein routing to particular tissues (e.g., epidermis) was likely minimal in our experiments. Furthermore, tissue-specific patterns in δ^2H values are similar among a wide range of unrelated taxa (birds, mammals, and reptiles), and thus a biochemical mechanism involving [AA] is the most parsimonious explanation for variation in tissue δ^2H values in organisms that are fed (food and water) resources that do not vary in their hydrogen isotope composition.

The significantly lower serum δ^2H values observed in adult female versus juvenile turtles from the FE2 experiment (Vander Zanden et al. 2012) are likely related to elevated lipid content in this tissue type. Lipids have relatively high weight percent hydrogen ([H]) contents and lower δ^2H values in comparison to associated proteins due to a large isotopic discrimination during the formation of acetyl CoA from pyruvate (Estep and Hoering, 1980; Hayes, 2001; Schmidt et al., 2003; Sessions and Hayes, 2005; Sessions et al., 1999). Thus, the significant negative relationship between serum δ^2H value and [H] observed in serum (Fig. 3) supports the idea that adult serum had higher lipid content than that of juveniles. Because all adults in our experiment were sexually mature females, higher concentration of free lipids in the blood stream (i.e., plasma) could be due to egg production. Females mobilize lipids prior to and during the nesting season to help with vitellogenesis (also known as yolk deposition), during which lipids

and proteins are stored in the oocytes during egg formation (Milton and Lutz, 2003) and other related reproductive processes (Hamann et al., 2002). Lastly in regard to tissue lipid content, comparison of δ^2H for lipid and non-lipid extracted epidermis suggests that this tissue has sufficiently low lipid content such that lipid-extraction prior to isotope analysis is unnecessary. These results mirror those found in a previous study that showed $\delta^{13}C$ values of sea turtle epidermis were unaffected by lipid removal (Vander Zanden et al., 2012).

Tissue Δ^2H_{Net}. By definition (Equation 4), patterns in tissue-specific trophic discrimination factors are influenced by tissue hydrogen isotope values, thus Δ^2H_{Net} typically mirror the patterns in tissue δ^2H. Thus, the observed differences in Δ^2H_{Net} between epidermis and blood components could simply reflect differences in [AA] among these tissues. Δ^2H_{Net} estimates are also sensitive to the proportional contribution of dietary macromolecules (Equation 3), especially protein since it can be directly routed from diet and thus could contribute more to proteinaceous tissue synthesis relative to its dietary content. Our previous feeding experiment on tilapia showed that when fish were fed a low-protein diet (~10%), between 34–44% of the hydrogen in proteinaceous tissues (muscle and liver) derived from dietary protein (Newsome et al., 2017). In this study, turtles in both experiments were fed diets with a high protein content (40–50%, Table 1), and thus the proportion of tissue hydrogen derived from dietary protein was more similar to the proportion of protein in diet. In other words, protein routing was likely minimal in our experiment in comparison to the previous work on tilapia. Thus, if turtles had been fed a low-protein diet (≤10%) better reflecting the diets of free-ranging individuals in the

Eastern Pacific (including Baja California) or Caribbean herbivorous and omnivorous diets (Bjorndal, 1980; López-Mendilaharsu et al., 2005; Seminoff et al., 2002), and essentially decrease the degree of dietary protein routing to tissue synthesis, our estimates of Δ^2H_{Net} likely would have been different. In the following calculations, the proportional decrease in dietary protein content was added to the proportional contribution of dietary carbohydrates. Decreasing the dietary protein content from 42% to 10% in the FE2 experiment (Vander Zanden et al., 2012) increased epidermis Δ^2H_{Net} from only 8‰ to 11‰ in juveniles and 1‰ to 7‰ in adults. Interestingly, decreasing the dietary protein content from 54% to 10% in the FE1 experiment (Seminoff et al. 2006) resulted in increases in epidermis Δ^2H_{Net} from 11‰ to 45‰ in juveniles. This relatively large change in Δ^2H_{Net} is due to the larger proportion decrease in dietary protein content in the FE1 versus FE2 experiment. Since the protein content of the diets used in our feeding experiments are likely higher than those consumed by green sea turtle in nearly all their natural habitats, our estimates of Δ^2H_{Net} (~10‰) for epidermis may be on the low end of the spectrum for this species. However, our study provides a valuable first approximation of the range in Δ^2H_{Net} for an omnivorous marine consumer.

Conclusions. Overall, our study is an initial step for expanding the use of δ^2H analysis to potentially evaluate resource and habitat use in marine consumers that inhabit nearshore environments (e.g., estuaries, lagoons) in which the hydrogen isotope composition of primary producers can vary by more than 100‰ (Estep and Dabrowski, 1980). We stress that additional feeding experiments are needed to better understand hydrogen isotope assimilation and discrimination in other marine species. When possible, feeding

experiments should vary diet quality (e.g., protein content), but attempt to include diets that mimic the macromolecular composition of prey consumed by wild populations. This approach would help refine our understanding of the processes that influence discrimination of hydrogen isotopes in animals, and enable us to broaden our use of this tool to study the ecology of wild populations of marine consumers.

FIGURES

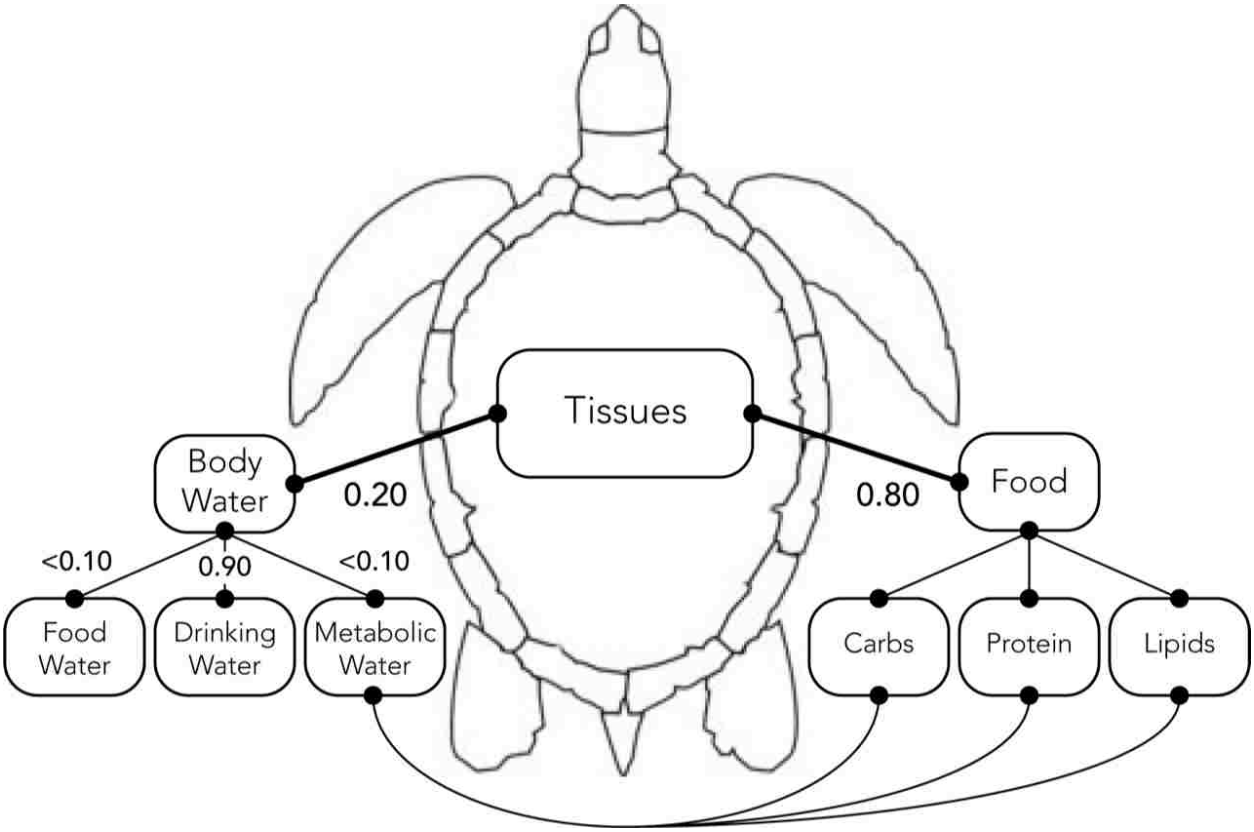

Fig. 1. Mass-balance model for δ^2H in a marine endothermic organism.

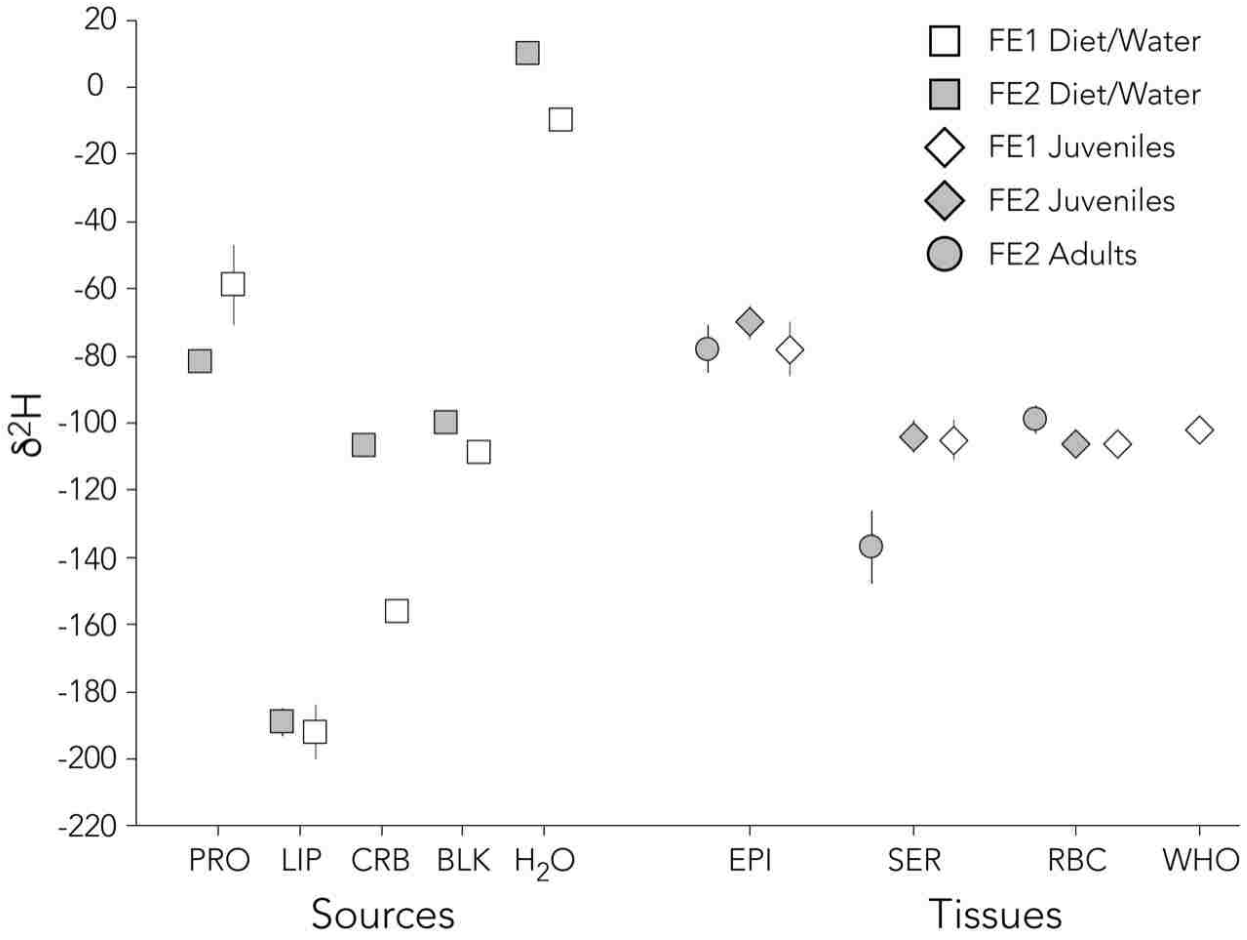

Fig. 2A. Mean δ^2H across sea turtle tissue types (Epidermis – EPI, Serum – SER, red blood cells – RBC and whole blood – WHO) and hydrogen pools (diet macromolecules: proteins – PRO, lipids – LIP, carbohydrates – CRB and bulk diet – BLK; and water – H_2O; all in square symbols) between feeding experiments (FE1: white and FE2: grey) and life stages (Juvenile: diamond and Adult: circle).

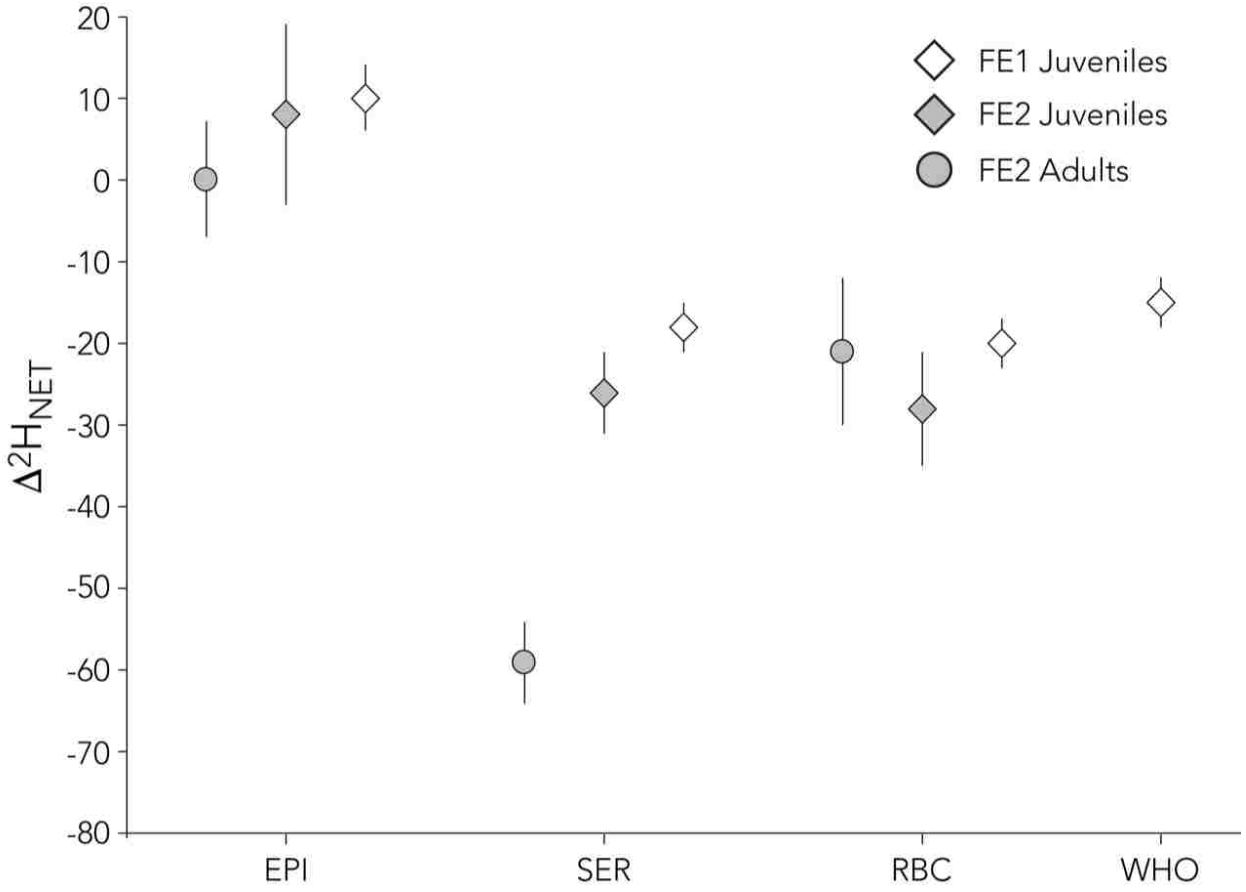

Fig. 2B. Mean δ^2H discrimination for the two different feeding experiments (FE1: white and FE2: grey); the four different tissue types (Epidermis – EPI, Serum – SER, red blood cells – RBC and whole blood – WHO); and the two life stages (Juvenile: diamonds and Adult: circles).

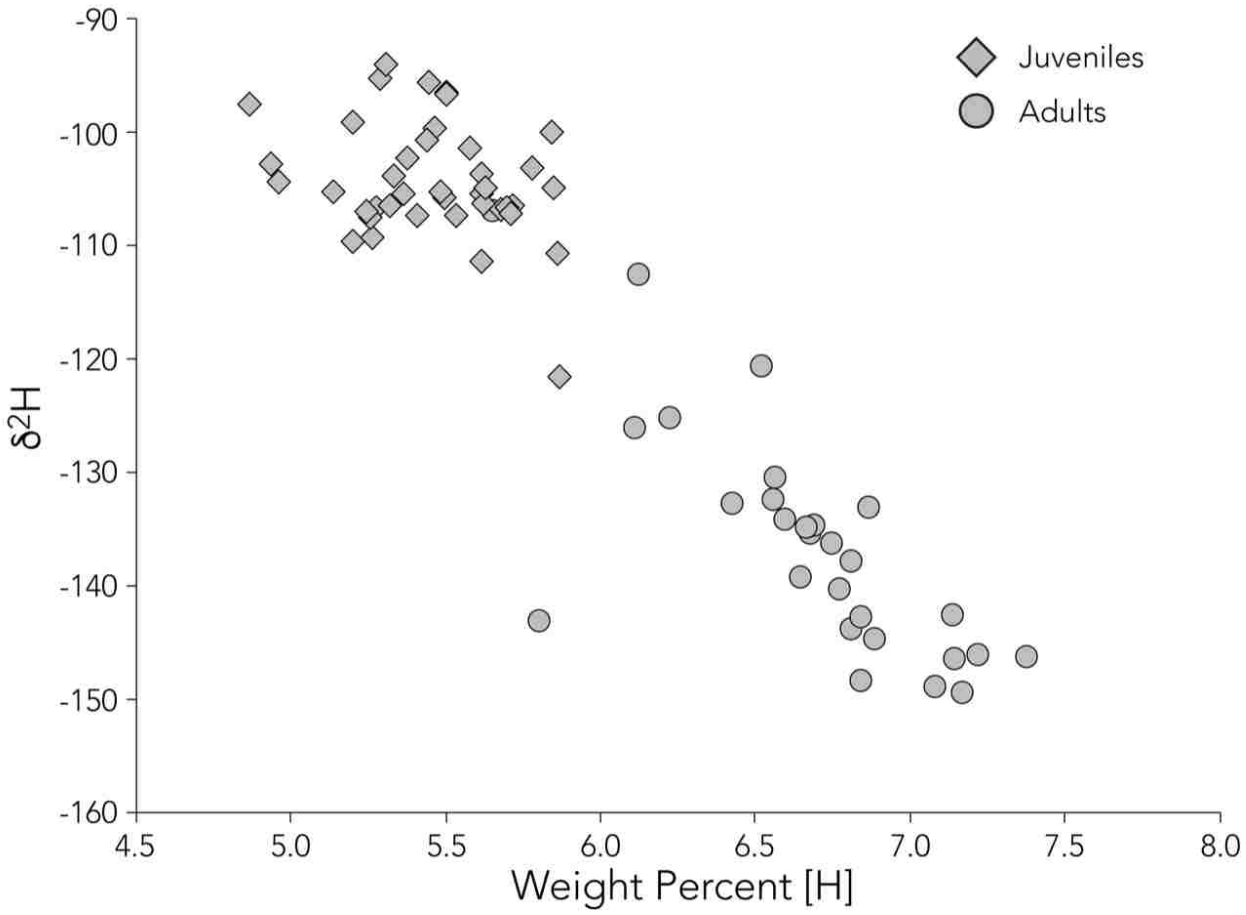

Fig. 3. Serum δ^2H distribution by weigh percent hydrogen ([H]) across life stages (Juvenile: diamonds and Adult: circles) for Vander Zanden et al. 2012 feeding experiment (FE2).

Diet Component	Vander Zanden et al. 2012		Seminoff et al. 2006	
	Value	**SD**	**Value**	**SD**
Bulk Diet δ^2H	–100	3	–109	3
Diet Bulk [H]	5.9	0.6	6.2	0.1
LE Diet δ^2H	–97	6	–86	7
LE Diet [H]	6.1	0.1	5.5	0.4
Lipids %	4.6%	–	15.9%	–
Lipids δ^2H	–189	4	–192	8
Lipids [H]	9.9	0.4	4.5	1
Carbohydrates %	53.2%	–	30.0%	–
Carbohydrates δ^2H	–107*	–	–156*	–
Carbohydrates [H]	2.9	0.2	3.0	0.3
Proteins %	42.2%	–	54.0%	–
Proteins δ^2H	–82*	3	–59*	12
Proteins [H]	5.6	0.5	8.1	0.3

Table 1. Mean (± SD) δ^2H values for the sea turtle diet and its associate macromolecular components with their respective hydrogen concentrations ([H]) and relative proportions (weigh %).

Stage	Tissue type	Vander Zanden et al. 2012		Seminoff et al. 2006	
		δ2H	**[H]**	**δ2H**	**[H]**
Juvenile	Epidermis	−70 ± 5[a]	6.0 ± 0.1	−79 ± 9[b]	4.6 ± 0.4
	Serum	−104 ± 5[c]	5.5 ± 0.3	−108 ± 7[c]	5.8 ± 0.4
	Red blood cells	−106 ± 3[c]	5.8 ± 0.2	−109 ± 3[c]	6 ± 0.1
	Whole blood	–	–	−105 ± 3	5.9 ± 0.1
Adult	Epidermis	−78 ± 7[b]	6.1 ± 0.1	–	–
	Serum	−137 ± 11[d]	6.7 ± 0.4	–	–
	Red blood cells	−99 ± 4[e]	6.1 ± 0.1	–	–
	Whole blood	–	–	–	–

Table 2. Mean (± SD) δ^2H values and hydrogen concentrations ([H]) of the different tissue types for each of the feeding experiments and life stages.

Stage	Tissue type	Vander Zanden et al. 2012		Seminoff et al. 2006	
		Δ^2H_{net}	SD	Δ^2H_{net}	SD
Juvenile	Epidermis	8[a]	5	11[a]	9
	Serum	–26[c]	5	–18[d]	7
	Red blood cells	–28[c]	3	–19[d]	3
	Whole blood	–	–	–15	3
Adult	Epidermis	1[b]	7	–	–
	Serum	–58[e]	11	–	–
	Red blood cells	–21[d]	4	–	–

Table 3. Mean (± SD) hydrogen discrimination factor values (Δ^2H_{net}) of the different tissue types for each of the feeding experiments and life stages.

REFERENCES

Ambrose, S. H. and Norr, L. (1993). Experimental evidence for the relationship of the carbon isotope ratios of whole diet and dietary protein to those of bone collagen and carbonate. In *Prehistoric human bone*, pp. 1–37. Springer.

Amelung, W. and Zhang, X. (2001). Determination of amino acid enantiomers in soils. *Soil Biol. Biochem.* 33, 553–562.

Amelung, W., Cheshire, M. V and Guggenberger, G. (1996). Determination of neutral and acidic sugars in soil by capillary gas-liquid chromatography after trifluoroacetic acid hydrolysis. *Soil Biol. Biochem.* 28, 1631–1639.

Andrews, M. A. (1989). Capillary gas-chromatographic analysis of monosaccharides: improvements and comparisons using trifluoroacetylation and trimethylsilylation of sugar O-benzyl-and O-methyl-oximes. *Carbohydr. Res.* 194, 1–19.

Bearhop, S., Waldron, S., Votier, S. C. and Furness, R. W. (2002). Factors that influence assimilation rates and fractionation of nitrogen and carbon stable isotopes in avian blood and feathers. *Physiol. Biochem. Zool.* 75, 451–458.

Berggren, M., Ziegler, S. E., St-Gelais, N. F., Beisner, B. E. and del Giorgio, P. A. (2014). Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes. *Ecology* 95, 1947–1959.

Birchall, J., O'CONNELL, T. C., Heaton, T. H. E. and Hedges, R. E. M. (2005). Hydrogen isotope ratios in animal body protein reflect trophic level. *J. Anim. Ecol.* 74, 877–881.

Bjorndal, K. A. (1980). Nutrition and grazing behavior of the green turtle *Chelonia mydas*. *Mar. Biol.* 56, 147–154.

Boecklen, W. J., Yarnes, C. T., Cook, B. A. and James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annu. Rev. Ecol. Evol. Syst.* 42, 411–440.

Bowen, G. J., Chesson, L., Nielson, K., Cerling, T. E. and Ehleringer, J. R. (2005). Treatment methods for the determination of δ2H and δ18O of hair keratin by continuous-flow isotope-ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* 19, 2371–2378.

Burgett, C. M., Burkholder, D. A., Coates, K. A., Fourqurean, V. L., Kenworthy, W. J., Manuel, S. A., Outerbridge, M. E. and Fourqurean, J. W. (2018). Ontogenetic diet shifts of green sea turtles (*Chelonia mydas*) in a mid-ocean developmental habitat. *Mar. Biol.* 165, 33.

Cabana, G. and Rasmussen, J. B. (1996). Comparison of aquatic food chains using nitrogen isotopes. *Proc. Natl. Acad. Sci.* 93, 10844–10847.

Chamberlain, C. P., Blum, J. D., Holmes, R. T., Feng, X., Sherry, T. W. and Graves, G. R. (1996). The use of isotope tracers for identifying populations of migratory birds. *Oecologia* 109, 132–141.

Christianson, D. D., Wall, J. S., Dimler, R. J. and Senti, F. R. (1960). Separation and determination of quaternary nitrogen compounds and other nitrogenous substances by ion exchange chromatography. Application to analysis of corn extracts. *Anal. Chem.* 32, 874–878.

Cole, J. J., Carpenter, S. R., Kitchell, J., Pace, M. L., Solomon, C. T. and Weidel, B. (2011). Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proc. Natl. Acad. Sci.* 108, 1975–1980.

Connan, M., Bonnevie, B. and McQuaid, C. (2018). Ontogeny, tissue, and species but not sex influence stable isotopic values of three albatross species. *Polar Biol*. 1–12.

Coplen, T. B. and Qi, H. (2012). USGS42 and USGS43: Human-hair stable hydrogen and oxygen isotopic reference materials and analytical methods for forensic science and implications for published measurement results. *Forensic Sci. Int*. 214, 135–141.

Craig, H. and Gordon, L. I. (1965). Deuterium and oxygen 18 variations in the ocean and the marine atmosphere.

DeNiro, M. J. and Epstein, S. (1978). Influence of diet on the distribution of carbon isotopes in animals. *Geochim. Cosmochim. Acta* 42, 495–506.

Díaz-Gamboa, R. E., Gendron, D. and Busquets-Vass, G. (2017). Isotopic niche width differentiation between common bottlenose dolphin ecotypes and sperm whales in the Gulf of California. *Mar. Mammal Sci*.

Doucett, R. R., Power, G., Barton, D. R., Drimmie, R. J. and Cunjak, R. A. (1996). Stable isotope analysis of nutrient pathways leading to Atlantic salmon. *Can. J. Fish. Aquat. Sci*. 53, 2058–2066.

Doucett, R. R., Marks, J. C., Blinn, D. W., Caron, M. and Hungate, B. A. (2007). Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology* 88, 1587–1592.

Ehleringer, J. R., Bowen, G. J., Chesson, L. A., West, A. G., Podlesak, D. W. and Cerling, T. E. (2008). Hydrogen and oxygen isotope ratios in human hair are related to geography. *Proc. Natl. Acad. Sci*. 105, 2788–2793.

Estep, M. F. and Dabrowski, H. (1980). Tracing food webs with stable hydrogen isotopes. *Science (80-.)*. 209, 1537–1538.

Estep, M. F. and Hoering, T. C. (1980). Biogeochemistry of the stable hydrogen isotopes. *Geochim. Cosmochim. Acta* 44, 1197–1206.

Fenton, G. E. and Ritz, D. A. (1989). Spatial variability of 13C: 12C and D: H in *Ecklonia radiata* (C. Ag.) J. Agardh (Laminariales). *Estuar. Coast. Shelf Sci.* 28, 95–101.

Finlay, J. C., Doucett, R. R. and McNEELY, C. (2010). Tracing energy flow in stream food webs using stable isotopes of hydrogen. *Freshw. Biol.* 55, 941–951.

Fogel, M. L., Griffin, P. L. and Newsome, S. D. (2016). Hydrogen isotopes in individual amino acids reflect differentiated pools of hydrogen from food and water in *Escherichia coli*. *Proc. Natl. Acad. Sci.* 201525703.

Fry, B. (2006). *Stable isotope ecology*. Springer.

Gat, J. R. (1996). Oxygen and hydrogen isotopes in the hydrologic cycle. *Annu. Rev. Earth Planet. Sci.* 24, 225–262.

Hamann, M., Limpus, C. and Whittier, J. (2002). Patterns of lipid storage and mobilisation in the female green sea turtle (*Chelonia mydas*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* 172, 485–493.

Hayes, J. M. (2001). Fractionation of carbon and hydrogen isotopes in biosynthetic processes. *Rev. Mineral. geochemistry* 43, 225–277.

Hobson, K. a (1999a). Tracing origins and migration of widlife using stable isotopes: a review. *Oecologia* 120, 314–326.

Hobson, K. A. (1999b). Tracing origins and migration of wildlife using stable isotopes: a review. *Oecologia* 120, 314–326.

Hobson, K. A. (2005a). Stable isotopes and the determination of avian migratory

connectivity and seasonal interactions. *Auk* 122, 1037–1048.

Hobson, K. A. (2005b). Using stable isotopes to trace long-distance dispersal in birds and other taxa. *Divers. Distrib.* 11, 157–164.

Hobson, K. A. and Clark, R. G. (1992). Assessing avian diets using stable isotopes II: factors influencing diet-tissue fractionation. *Condor* 189–197.

Hobson, K. A. and Wassenaar, L. I. (1996). Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. *Oecologia* 109, 142–148.

Hobson, K. A., Atwell, L. and Wassenaar, L. I. (1999a). Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proc. Natl. Acad. Sci.* 96, 8003–8006.

Hobson, K. A., Wassenaar, L. I. and Taylor, O. R. (1999b). Stable isotopes (δD and δ13C) are geographic indicators of natal origins of monarch butterflies in eastern North America. *Oecologia* 120, 397–404.

Hobson, K. A., Bowen, G. J., Wassenaar, L. I., Ferrand, Y. and Lormee, H. (2004). Using stable hydrogen and oxygen isotope measurements of feathers to infer geographical origins of migrating European birds. *Oecologia* 141, 477–488.

Hondula, K. L. and Pace, M. L. (2014). Macroalgal support of cultured hard clams in a low nitrogen coastal lagoon. *Mar. Ecol. Prog. Ser.* 498, 187–201.

Jardine, T. D., Kidd, K. A. and Cunjak, R. A. (2009). An evaluation of deuterium as a food source tracer in temperate streams of eastern Canada. *J. North Am. Benthol. Soc.* 28, 885–893.

Kendall, C. and Coplen, T. B. (2001). Distribution of oxygen-18 and deuterium in river

waters across the United States. *Hydrol. Process.* 15, 1363–1393.

Kurle, C. M., Koch, P. L., Tershy, B. R. and Croll, D. A. (2014). The effects of sex, tissue type, and dietary components on stable isotope discrimination factors (Δ13C and Δ15N) in mammalian omnivores. *Isotopes Environ. Health Stud.* 50, 307–321.

LeGrande, A. N. and Schmidt, G. A. (2006). Global gridded data set of the oxygen isotopic composition in seawater. *Geophys. Res. Lett.* 33,.

López-Mendilaharsu, M., Gardner, S. C., Seminoff, J. A. and Riosmena-Rodriguez, R. (2005). Identifying critical foraging habitats of the green turtle (*Chelonia mydas*) along the Pacific coast of the Baja California peninsula, Mexico. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 15, 259–269.

Macko, S. A., Estep, M. L. F. and Lee, W. Y. (1983). Stable hydrogen isotope analysis of foodwebs on laboratory and field populations of marine amphipods. *J. Exp. Mar. Bio. Ecol.* 72, 243–249.

Malej, A., Faganeli, J. and Pezdič, J. (1993). Stable isotope and biochemical fractionation in the marine pelagic food chain: the jellyfish *Pelagia noctiluca* and net zooplankton. *Mar. Biol.* 116, 565–570.

Marra, P. P., Hobson, K. A. and Holmes, R. T. (1998). Linking winter and summer events in a migratory bird by using stable-carbon isotopes. *Science (80-.).* 282, 1884–1886.

Martinez del Rio, C., Wolf, N., Carleton, S. A. and Gannes, L. Z. (2009). Isotopic ecology ten years after a call for more laboratory experiments. *Biol. Rev.* 84, 91–111.

Meier-Augenstein, W., Chartrand, M. M. G., Kemp, H. F. and St-Jean, G. (2011). An

inter-laboratory comparative study into sample preparation for both reproducible and repeatable forensic 2H isotope analysis of human hair by continuous flow isotope ratio mass spectrometry. *Rapid Commun. Mass Spectrom.* 25, 3331–3338.

Milton, S. and Lutz, P. (2003). Physiological and genetic responses to environmental stress. *Biol. sea turtles, CRC Press. Boca Raton, Florida.*

Newsome, S. D., Bentall, G. B., Tinker, M. T., Oftedal, O. T., Ralls, K., Estes, J. A. and Fogel, M. L. (2010). Variation in δ13C and δ15N diet-vibrissae trophic discrimination factors in a wild population of California sea otters. *Ecol. Appl.* 20, 1744–1752.

Newsome, S. D., Wolf, N., Bradley, C. J. and Fogel, M. L. (2017). Assimilation and isotopic discrimination of hydrogen in tilapia: implications for studying animal diet with δ2H. *Ecosphere* 8,.

Nielson, K. E. and Bowen, G. J. (2010). Hydrogen and oxygen in brine shrimp chitin reflect environmental water and dietary isotopic composition. *Geochim. Cosmochim. Acta* 74, 1812–1822.

Ostrom, P. H., Wiley, A. E., Rossman, S., Stricker, C. A. and James, H. F. (2014). Unexpected hydrogen isotope variation in oceanic pelagic seabirds. *Oecologia* 175, 1227–1235.

Peters, J. M., Wolf, N., Stricker, C. A., Collier, T. R. and del Rio, C. M. (2012). Effects of trophic level and metamorphosis on discrimination of hydrogen isotopes in a plant-herbivore system. *PLoS One* 7, e32744.

Peterson, B. J. and Fry, B. (1987). Stable isotopes in ecosystem studies. *Annu. Rev. Ecol. Sytematics* 18, 293–320.

Phillips, D. L. (2012). Converting isotope values to diet composition: the use of mixing models. *J. Mammal*. 93, 342–352.

Podlesak, D. W., Torregrossa, A.-M., Ehleringer, J. R., Dearing, M. D., Passey, B. H. and Cerling, T. E. (2008). Turnover of oxygen and hydrogen isotopes in the body water, CO 2, hair, and enamel of a small mammal. *Geochim. Cosmochim. Acta* 72, 19–35.

Post, D. M. (2002). Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83, 703–718.

Robbins, C. T., Felicetti, L. A. and Sponheimer, M. (2005). The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds. *Oecologia* 144, 534–540.

Rubenstein, D. R. and Hobson, K. A. (2004). From birds to butterflies: animal movement patterns and stable isotopes. *Trends Ecol. Evol*. 19, 256–263.

Sauer, P. E., Schimmelmann, A., Sessions, A. L. and Topalov, K. (2009). Simplified batch equilibration for D/H determination of non-exchangeable hydrogen in solid organic material. *Rapid Commun. mass Spectrom*. 23, 949–956.

Schiegl, W. E. and Vogel, J. C. (1970). Deuterium content of organic matter. *Earth Planet. Sci. Lett*. 7, 307–313.

Schmidt, H.-L., Werner, R. A. and Eisenreich, W. (2003). Systematics of 2 H patterns in natural compounds and its importance for the elucidation of biosynthetic pathways. *Phytochem. Rev*. 2, 61–85.

Schwarcz, H. P. and Schoeninger, M. J. (1991). Stable isotope analyses in human nutritional ecology. *Am. J. Phys. Anthropol*. 34, 283–321.

Seminoff, J. A., Resendiz, A. and Nichols, W. J. (2002). Home range of green turtles

Chelonia mydas at a coastal foraging area in the Gulf of California, Mexico. *Mar. Ecol. Prog. Ser.* 242, 253–265.

Seminoff, J. A., Jones, T. T., Eguchi, T., Jones, D. R. and Dutton, P. H. (2006). Stable isotope discrimination (δ13C and δ15N) between soft tissues of the green sea turtle *Chelonia mydas* and its diet. *Mar. Ecol. Prog. Ser.* 308, 271–278.

Sessions, A. L. and Hayes, J. M. (2005). Calculation of hydrogen isotopic fractionations in biogeochemical systems. *Geochim. Cosmochim. Acta* 69, 593–597.

Sessions, A. L., Burgoyne, T. W., Schimmelmann, A. and Hayes, J. M. (1999). Fractionation of hydrogen isotopes in lipid biosynthesis. *Org. Geochem.* 30, 1193–1200.

Smith, B. N. and Epstein, S. (1970). Biogeochemistry of the stable isotopes of hydrogen and carbon in salt marsh biota. *Plant Physiol.* 46, 738–742.

Solomon, C. T., Cole, J. J., Doucett, R. R., Pace, M. L., Preston, N. D., Smith, L. E. and Weidel, B. C. (2009). The influence of environmental water on the hydrogen stable isotope ratio in aquatic consumers. *Oecologia* 161, 313–324.

Soto, D. X., Wassenaar, L. I. and Hobson, K. A. (2013). Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. *Funct. Ecol.* 27, 535–543.

Sternberg, L. da S. L. and Swart, P. K. (1987). Utilization of freshwater and ocean water by coastal plants of southern Florida. *Ecology* 68, 1898–1905.

Storm-Suke, A., Wassenaar, L. I., Nol, E. and Norris, D. R. (2012). The influence of metabolic rate on the contribution of stable-hydrogen and oxygen isotopes in drinking water to quail blood plasma and feathers. *Funct. Ecol.* 26, 1111–1119.

Tieszen, L. L. and Fagre, T. (1993). Effect of diet quality and composition on the isotopic composition of respiratory CO 2, bone collagen, bioapatite, and soft tissues. In *Prehistoric human bone*, pp. 121–155. Springer.

Vander Zanden, H. B., Bjorndal, K. A., Mustin, W., Ponciano, J. M. and Bolten, A. B. (2012). Inherent variation in stable isotope values and discrimination factors in two life stages of green turtles. *Physiol. Biochem. Zool.* 85, 431–441.

Wall, J. S. (1953). Simultaneous separation of purines, pyrimidines, amino acids, and other nitrogenous compounds. *Anal. Chem.* 25, 950–953.

Wassenaar, L. I. and Hobson, K. A. (1998). Natal origins of migratory monarch butterflies at wintering colonies in Mexico: new isotopic evidence. *Proc. Natl. Acad. Sci.* 95, 15436–15439.

Wassenaar, L. I. and Hobson, K. A. (2000). Improved method for determining the stable-hydrogen isotopic composition (δD) of complex organic materials of environmental interest. *Environ. Sci. Technol.* 34, 2354–2360.

Wassenaar, L. I. and Hobson, K. A. (2003). Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. *Isotopes Environ. Health Stud.* 39, 211–217.

Wolf, N., Bowen, G. J. and Del Rio, C. M. (2011). The influence of drinking water on the δD and δ18O values of house sparrow plasma, blood and feathers. *J. Exp. Biol.* 214, 98–103.

Wolf, N., Newsome, S. D., Fogel, M. L. and del Rio, C. M. (2012). An experimental exploration of the incorporation of hydrogen isotopes from dietary sources into avian tissues. *J. Exp. Biol.* 215, 1915–1922.

Wolf, N., Newsome, S. D., Fogel, M. L. and Del Rio, C. M. (2013). The relationship between drinking water and the hydrogen and oxygen stable isotope values of tissues in Japanese Quail (*Cortunix japonica*). *Auk* 130, 323–330.

Xu, X., Werner, M., Butzin, M. and Lohmann, G. (2012). Water isotope variations in the global ocean model MPI-OM. *Geosci. Model Dev.* 5, 809–818.

CHAPTER 2: VARIABILITY IN GREEN SEA TURTLE DIET AND HABITAT USE AT OFFSHORE AND LAGOON SITES IN BAJA CALIFORNIA PENINSULA

Laura Pagès Barceló[1], Jeffrey A. Seminoff[2], Calandra Turner-Tomaszewicz[2,3], David Aurioles[4], Seth D. Newsome[1]

[1]Department of Biology, University of New Mexico, Albuquerque, New Mexico

[2]National Oceanic and Atmospheric Administration–National Marine Fisheries Service, Southwest Fisheries Science Center, La Jolla, California

[3]Division of Biological Sciences, Ecology, Behavior and Evolution Section, University of California, San Diego, La Jolla, California

[4]Centro Interdisciplinario de Ciencias Marinas, Instituto Politécnico Nacional, La Paz Baja California Sur, 23060 Mexico

ABSTRACT

Sea turtles have been severely impacted by numerous anthropogenic activities and yet we know little about their ecology due to their elusive nature which makes them difficult to study. Two aspects we still know little about are their resource and habitat preferences, and how these vary among individuals. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope analysis has proven to be a useful tool to study sea turtle diet composition and habitat use. Here we compare $\delta^{13}C$ and $\delta^{15}N$ data with hydrogen isotopes ($\delta^{2}H$) to determine if $\delta^{2}H$ can be used to characterize resource and habitat use in green sea turtles living along the Pacific coast of Baja California Peninsula, Mexico. We measured epidermis $\delta^{13}C$,

$\delta^{15}N$ and $\delta^{2}H$ values from 119 green sea turtles collected from one open ocean and four lagoon sites. Our results show inter-individual variation in feeding strategies (specialist vs. generalists) within lagoon turtles. However, open ocean turtles, consistently feed at a higher trophic level suggesting possible interactions with fisheries. Furthermore, we also found that hydrogen isotopes can be used to distinguish between basal energy sources in marine ecosystems. Finally, a weak positive correlation between $\delta^{2}H$ and $\delta^{15}N$ values suggests that this pattern may be driven by differences in trophic level. These findings suggest that the use of hydrogen stable isotope as a third variable may be a valuable tool for understanding sea turtle ecology in coastal settings.

INTRODUCTION

Green sea turtles are one of the most widely distributed sea turtle species in spite of having suffered significant (historical) population declines in the past. For example, Eastern Pacific green sea turtles (*Chelonia mydas agassizii*) experienced an extreme decline from the 1940s to 1980s (Cliffton et al., 1982; Groombridge and Luxmoore, 1989; Jackson, 1997; Márquez, 1990). Despite conservation efforts, direct hunting of turtles for meat and eggs as well as incidental mortality from fisheries (bycatch) are the two main causes of mortality for many green sea turtle populations (Gardner and Nichols, 2001; Koch et al., 2006; Koch et al., 2013; Nichols, 2003; Peckham et al., 2007; Peckham et al., 2008; Senko et al., 2014).

Efforts to manage sea turtle populations have been hampered by a limited understanding of their complex foraging ecology. Green sea turtles demonstrate high dietary plasticity depending on habitat and availability of local food resources and prey

(Amorocho and Reina, 2008; Bjorndal, 1997; Carrión-Cortez et al., 2010; Hatase et al., 2006; Seminoff et al., 2002). For example, turtlegrass (*Thalassia testudinum*) is the dominant food consumed by green sea turtle populations in the Caribbean, whereas eastern Pacific populations forage on a combination of eelgrass (*Zostera marina*), marine macroalgae, and benthic invertebrates (Bjorndal, 1997; López-Mendilaharsu et al., 2005; Seminoff et al., 2002; Williams, 1988). Whereas green sea turtles were originally considered to be herbivories, Bjorndal (1997) described the eastern Pacific sea turtle population as carnivorous. Later, Hatase et al. (2006) documented a facultative ontogenetic shift from omnivory to herbivory in female green sea turtles living off of Japan. While this studies suggest that green sea turtles express substantial dietary plasticity, we still know little about green turtle foraging ecology throughout their life stages and across populations in specific locations, which complicates conservation and recovery efforts (Seminoff et al., 2002; Seminoff et al., 2003).

The Pacific coast of Baja California Peninsula (and its lagoons systems) with its diverse coastline containing lagoons and estuaries adjacent to productive upwelling zones provides diverse prey items and foraging habitats for green turtles (Koch et al., 2006; Koch et al., 2007; López-Castro et al., 2010; Nichols, 2003; Senko et al., 2010a) and offers an ideal study system for understanding the factors that influence turtle diet and habitat use. This region is also home to several of the most productive commercial fisheries along the eastern Pacific coast of North America (Alverson, 1963; Etnoyer et al., 2006; Squire and Suzuki, 1991; Wingfield et al., 2011). Both industrial and artisanal fisheries efforts overlap with sea turtle foraging grounds, causing thousands of incidental mortalities annually (Gardner and Nichols, 2001; Koch et al., 2006; Parker et al., 2011;

Peckham et al., 2007). Consequently, this region is known to have one of the highest sea turtle mortality rates in the world (Gardner and Nichols, 2001; Hays et al., 2003; Koch et al., 2006; Mancini and Koch, 2009; Mancini et al., 2011; Nichols and Safina, 2004; Peckham et al., 2007; Peckham et al., 2008). A thorough understanding of green sea turtle diet is thus key to understanding possible interactions between this species and local fisheries and assess the importance of lagoonal ecosystems as green sea turtle habitat in the eastern Pacific Ocean.

Green turtles along the Pacific coast of BCP occur in two main habitats: lagoons where primary production comes from a combination of phytoplankton, macroalgae, seagrass and mangroves and open ocean pelagic ecosystems dominated by phytoplankton production. Juveniles are more common inside the shallow protected lagoons (Koch et al., 2006; Koch et al., 2007; López-Castro et al., 2010), whereas adults are more often found offshore in pelagic habitats (Koch et al., 2007; López-Mendilaharsu et al., 2005; Seminoff et al., 2003). These two habitats contain different food resources and it is currently unknown to what extent sea turtles, with their high dietary plasticity, are habitat, and by extension, dietary generalists or specialists in this region. For example, green turtles residing in lagoon systems may have a primarily herbivorous diet due to mangrove and seagrass availability, while oceanic turtles may display a more carnivorous diet, like loggerhead turtles, focused on surface-dwelling invertebrates, jellyfish, pelagic red crabs, and even fisheries discards. This hypothesis would prove that habitat determines foraging strategy regardless of species. Alternatively, some individuals may be omnivorous because they move between lagoon and open ocean habitats (López-Mendilaharsu et al., 2005; Senko et al., 2010b). This ecological plasticity may have

historically played a role in buffering this species against extinction, and a further, more detailed understanding of this capacity (unravelling their trophic ecology) will likely provide vital information to grasp how they will react to future environmental changes.

The study of elusive species such as sea turtles that are distributed across vast ocean habitats is difficult with conventional techniques such as biotelemetry or gut content analysis, which only provides dietary information for a small period of time (or snapshot). Thus, stable isotopes are increasingly used to understand animal resource use and movement among habitats because they offer information about nutrients assimilated over a variety of timelines (Hobson et al., 1999a; McClellan et al., 2010). Carbon isotopes ($\delta^{13}C$) can be used to trace different physiological pathways between sources of primary production, for example, to differentiate between mangrove and eelgrass in shallow lagoon habitats or phytoplankton in open ocean habitats (Arthur et al., 2009; Fry et al., 1977; Kharlamenko et al., 2001; Maberly et al., 1992; McClelland and Valiela, 1998; McConnaughey and McRoy, 1979). Nitrogen isotopes ($\delta^{15}N$) are used to estimate trophic position since their values increase between 2–5‰ for each trophic level (Cabana and Rasmussen, 1996; Minagawa and Wada, 1984; Post, 2002) thus informing of the different feeding strategies (herbivorous vs. carnivorous) for organisms living in unlike habitats (e.g., lagoon vs open ocean). Even though $\delta^{13}C$ and $\delta^{15}N$ are the most widely used isotopes by ecologist, their resolution level when using mixing models is still broad. Mixing models are a useful tool to quantify the contribution of each diet source into the sample, thus, the analysis of a third isotope ($\delta^{2}H$) may increase the overall resolution for this approach.

Hydrogen isotopes ($\delta^{2}H$) have been widely used in terrestrial ecosystems to

characterize animal movement across continental and regional scales (Bowen et al., 2005a; Ehleringer et al., 2008; Hobson and Wassenaar, 1996; Wassenaar and Hobson, 1998). At the watershed and ecosystem scale, δ^2H has been used to quantify riparian (allochthonous) or instream (autochthonous) resources into freshwater ecosystems (Berggren et al., 2014; Cole et al., 2011; Doucett et al., 2007; Finlay et al., 2010; Jardine et al., 2009). Estep and Dabrowski (1980) first reported significant (~100‰) variation in δ^2H values of primary producers in nearshore marine food webs (Estep and Dabrowski, 1980), but only a few studies have used hydrogen isotopes to quantify resource and habitat use in marine ecosystems (Hondula and Pace, 2014; Macko et al., 1983; Ostrom et al., 2014). δ^2H values display a wide range of variation due to a differential fractionation in physiological processes within distinct primary producers (DeNiro and Epstein, 1981; Doucett et al., 2007; Hondula and Pace, 2014; Roden and Ehleringer, 1999). Carvalho et al. (2017), for example, showed that the group of green algae Ulvophyceae had significantly depleted δ^2H (–94‰ to –130‰) in comparison to red, brown and other green algae groups (Bryopsidophyceae: –54‰ to –72‰) (Carvalho et al., 2017). Moreover, hydrogen isotope values may also be useful for quantifying trophic level; however, the magnitude of trophic discrimination in δ^2H may systematically vary with trophic level (Solomon et al. 2009) and no study has rigorously examined these patterns with controlled mesocosm experiments. Hydrogen isotopes have been used in conjunction with carbon and nitrogen isotopes to evaluate the proportion of primary producers in marine food webs (Hondula and Pace, 2014). In this framework, the number of sources and types of isotopes used in a mixing model affect the final outcome, thus the addition of a third element (hydrogen) could improve the general resolution of the model

(Fry, 2006; Peterson et al., 1985).

Here we used a combination of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values to better understand resource and habitat use in green sea turtles living on the Pacific coast of Baja California Peninsula, Mexico. Our primary goal was to assess the utility of $\delta^{2}H$ measurements as a complementary tool to $\delta^{13}C$ and $\delta^{15}N$ analyses to understand green sea turtle ecology. Our specific objectives were to assess whether these three isotope systems could (1) differentiate between feeding habitats (lagoon versus oceanic); (2) evaluate individual feeding strategies (specialists versus generalists) in lagoon habitats and (3) trace regional migration patterns. We used loggerhead epidermis samples as a proxy to detect green turtles feeding in a higher trophic level. Overall, these findings will help characterize green sea turtle resource and habitat use, which will inform conservation and management efforts that conserve this species. More generally, this research will also demonstrate the utility of using hydrogen isotopes in coastal marine ecosystems to characterize resource and habitat use by marine consumers.

MATERIALS & METHODS

Study Area and Sample Collection. Sea turtle tissue and potential prey items were collected at five sites along the Pacific coast of Baja California Peninsula (BCP), Mexico (Fig. 1). Locations were classified as open ocean (offshore) or lagoon (inshore). Green sea turtle epidermis was primarily sampled from stranded animals. Epidermis samples from the three lagoon sites were collected during several field seasons from 2005 to 2009 in Laguna Ojo de Liebre (LOL, n=19), Laguna San Ignacio (LSI, n=14) and Bahía Magdalena (BMG, n=13). Samples from Punta Abreojos (PAO, n=13), were collected

during the same field seasons in a relatively small and shallow coastal lagoon (Estero Coyote) just north of the mouth of Laguna San Ignacio (Tomaszewicz et al., 2018). Green and loggerhead turtle epidermis samples from San Lázaro (SLZ-*Chelonia mydas*, n=26; SLZ-*Caretta caretta*, n=60) were collected in 2008–2010 (CC) and 2012 (CM) along a ~44km stretch of beach on the west coast of Isla Magdalena (Turner-Tomaszewicz et al. 2017). Samples collected from San Lazaro on the western shore of Isla Magdalena were assumed to be from green turtles living in offshore (open ocean) habitats. Overall, turtle curved carapace length (CCL) ranged from 38.2 to 102 cm (mean ± SD: 60.4 ± 12 cm). For full site descriptions see López-Castro et al. 2010.

Eelgrass (*Zostera marina*), red mangrove (*Rhizophora mangle*), as well as red (Rhodophyta), green (Chlorophyta), and brown (Ochrophyta) macroalgae are the major sources of primary production in lagoons on the Pacific coast of Baja California (López-Mendilaharsu et al., 2005). *Zostera marina* has been found in LOL, BMG and LSI, but in BMG is currently almost nonexistent (Riosmena-Rodriguez et al., 2013). Red mangrove is found in two of the three lagoons we sampled (BMG and LSI; Whitmore et al., 2005). In contrast, phytoplankton are the main primary producers at the open-ocean sites, followed by benthic algal groups in sites less than 5m deep (Arreguín-Sànchez et al., 2004). These open-ocean sites are highly productive because they are situated at an important upwelling zone (Zaytsev et al., 2003) and thus support a wide variety of organisms, including sea turtles.

Sample Preparation. Sea turtle epidermal skin was collected with a 6-mm biopsy punch or razorblade from dorsal neck or upper shoulder region and stored in the field with salt

or 70% ethanol solution (Barrow et al., 2008). Samples were rinsed with DI water in the lab and permanently stored at –20°C until they were prepared for isotope analysis. Epidermis was freeze-dried, homogenized, and lipids where extracted with petroleum ether using a Soxhlet apparatus for two 10 hour cycles, or an accelerated solvent extractor (ASE Model 200 or 300, Dionex) for three 5 min cycles at 100°C and 1500 PSI of pressure. Samples were then freeze-dried prior to stable isotope analysis.

Macroalgae, seagrasses, and red mangroves were collected in March 2017 at several sites within each of the 3 lagoons (LOL, LSI and BMG) when present. Macroalgae were collected by snorkeling or by hand at low tide. We collected at least four samples for each primary producer per site when available (see Table 1). After collection, samples were rinsed with DI water and freeze-dried (Labconco, FreeZone 4.5 plus, 73860 series, Kansas City, MO, USA) before being weighed for stable isotope analysis.

Fish muscle and invertebrate samples were collected in 2013 from Puerto Alcatraz (PA) located between Bahía Magdalena and Bahía Almejas (Fig. 1). Samples were stored frozen in the field, rinsed with DI water and dried at 60 °C. Finally, samples were homogenized with mortar and pestle (Rosas-Hernández et al. in press). Lipid extraction was done afterwards in 3 consecutives 24 hour baths with petroleum ether. Each bath was followed by deionized water rinse. Samples were finally dried at 45°C for ~48 hours.

Stable Isotope Analysis. Approximately 0.5-0.6 mg of sea turtle epidermis and proteinaceous prey items (fish and invertebrates) and 5-6 mg of algae or plant matter prey

were loaded into tin capsules for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope analysis. Pelagic red crab $\delta^{13}C$ and $\delta^{15}N$ values were obtain from a previous work done in the same study area (Madigan et al., 2012). Only 0.1–0.2 mg of sample material was sealed into a silver capsule for hydrogen ($\delta^{2}H$) analysis. $\delta^{2}H$ were measured at the University of New Mexico Center for Stable Isotopes (Albuquerque, NM) with a Thermo Scientific Delta V isotope ratio mass spectrometer connected to a Thermo Scientific high-temperature conversion elemental analyzer (TCEA). $\delta^{13}C$, $\delta^{15}N$, and elemental concentrations (%C, N%) were measured at University of New Mexico Center for Stable Isotopes (Albuquerque, NM) with a Costech 4010 elemental analyzer coupled to Thermo Scientific Delta V isotope ratio mass spectrometer or at the Stable Isotope Geochemistry Lab at the University of Florida (Gainesville, FL) with a Carlo Erba NA 1500 CNS elemental analyzer interfaced to a Thermo Electron DeltaV Advantage isotope ratio mass spectrometer (Finnigan MAT) using a ConFlo II device (Finnigan MAT, Bremen, Germany). Isotope values are expressed in delta (δ) values where $\delta^{13}C$; $\delta^{15}N$ or $\delta^{2}H$ = $[(R_{sample} - R_{standard}/R_{standard}) - 1] \times 1000$, where R represents $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{2}H/^{1}H$ of the sample and the standard. Units are expressed in per mil (‰). The internationally accepted standards are: Vienna Pee Dee Belemnite (VPDB) for $\delta^{13}C$, atmospheric N_2 for $\delta^{15}N$, and Vienna Standard Mean Ocean Water (VSMOW) for $\delta^{2}H$. Within-run analytical precision for $\delta^{13}C$ and $\delta^{15}N$ was measured with a suite of protein –and carbohydrate– rich internal reference materials; within-run SD was ≤0.2‰ for both $\delta^{13}C$ and $\delta^{15}N$.

Samples analyzed for hydrogen stable isotopes were corrected for the

exchangeable proportion of hydrogen using several internal reference materials for which the non-exchangeable δ^2H values had been measured in previous equilibration experiments (Bowen et al., 2005a) and sat for at least three weeks before analysis to ensure equilibration with ambient water vapor δ^2H (Bowen et al., 2005b; Sauer et al., 2009; Wassenaar and Hobson, 2000; Wassenaar and Hobson, 2003). The internal reference materials used to correct our samples for the non-exchangeable hydrogen ranged from –55‰ to –175‰ (keratin: –54‰, –93‰, and –174‰ and peach leaves: –82‰). Keratin material was used to correct epidermis samples since this tissue in sea turtle, like other reptiles, is composed of keratin (Fox, 2015) and thus they have approximately the same proportion of exchangeable hydrogen (15-20%). Peach leaf material was used to correct algae samples. Other prey samples were compared to cow (*Bos taurus*) muscle internal reference materials from different localities (–71‰ and –149‰) due to their similar tissue composition.

Data Analysis. We used ANOVA followed by Tukey *post-hoc* test to detect differences between prey items as well as between sampling years. Before mixing model analysis, potential prey items were grouped *a priori* (Phillips, 2012; Phillips et al., 2005) into nine functional groups based on similar life history: Seagrass (*Zostera marina*); brown algae; red algae; green algae; mangrove fruit; crustaceans; sponges; pelagic red crab and fish. The Tukey HSD pairwise comparison test clumped some of the functional groups depending on each of the isotope elements analyzed. We then used a Bayesian mixing model in R (SIMMR) (Parnell et al., 2013) using a combination of $\delta^{13}C$, $\delta^{15}N$ and δ^2H epidermis tissue values to obtain the relative proportions from each putative prey source that contribute to sea turtle epidermis composition. Tissue samples where corrected for

trophic discrimination factors previous to mixing model generation: $\delta^{13}C$ and $\delta^{15}N$ were corrected using Vander Zanden et al., (2012) values and $\delta^{2}H$ using Pagès Barceló et al. (in preparation).

RESULTS

Prey Items. Mean (±SD) $\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$ values, weight percent [C]:[N] ratios, weight percent [H], associated sample sizes, and pairwise comparisons are reported in Tables 1 and 2; comparisons account for unequal sample size (emmeans) via ANOVA with a post-hoc Tukey HSD test and Bonferroni correction. We measured samples from a total of nine *a priori* prey types (Ben-David et al., 1997a; Ben-David et al., 1997b; Phillips, 2012; Phillips et al., 2005; Rosing et al., 1998) that were grouped based on taxonomy, ecological function, and habitat type (Table 1). Mean $\delta^{13}C$ values ranged from –26.5‰ for mangrove fruit to –12.1‰ for seagrass. The *post-hoc* Tukey HSD test identified six groups that had significant $\delta^{13}C$ values ($F_{8,148} = 67.62$, $P < 0.0001$): (1) (green/brown/red) macroalgae; (2) seagrass; (3) mangrove fruit; (4) sponges, (5) crustaceans, and (6) fish. Among primary producers, seagrass had the highest $\delta^{13}C$ values (–15.8 to –9.9‰), macroalgae had intermediate $\delta^{13}C$ values (–23.9 to –8.9‰), and mangrove fruit had the lowest $\delta^{13}C$ values (–28.6 to –23.6‰). Among invertebrate and vertebrate putative prey groups, sponges had significantly lower $\delta^{13}C$ values than crustaceans or fish; the latter two groups had similar $\delta^{13}C$ values. Mean $\delta^{15}N$ values ranged between 0.9‰ for mangrove fruit to 18.0‰ for fish. The HSD test identified five groupings for nitrogen ($F_{8,148} = 133$, $P < 0.0001$): (1) (green/brown/red) macroalgae; (2) seagrass; (3) mangrove fruit; (4) sponges/crustaceans, and (5) fish. Within primary

producers prey type, macroalgae had the highest $\delta^{15}N$ values, seagrass had intermediate values, and mangrove fruit had the lowest $\delta^{15}N$ values. All primary producer groups had significantly lower $\delta^{15}N$ values than fish, sponges/crustaceans, and pelagic red crabs. Among the invertebrate and vertebrate prey, fish had the highest $\delta^{15}N$ (9.2 to 18.0‰) and the sponges/crustaceans group had the lowest $\delta^{15}N$ values (7.3 to 16.8‰). Pelagic red crabs did not have significantly different $\delta^{13}C$ or $\delta^{15}N$ values than fish or sponges/crustaceans, but red crabs are shown as a separate food source in Figure 2A because they are a potentially important food source for the San Lazaro green turtles that inhabited offshore pelagic habitats.

Mean $\delta^{2}H$ values ranged from –190‰ for green algae to +10‰ for mangrove fruit. The Tukey *post-hoc* comparison identified five groupings for $\delta^{2}H$ ($F_{7,107} = 28.89$, $P < 0.0001$): (1) (green/brown/red) macroalgae; (2) seagrass; (3) mangrove fruit; (4) sponges/crustaceans, and (5) fish. Mangrove fruit had the highest $\delta^{2}H$ values among producers, followed by seagrass with intermediate values, and macroalgae have the lowest values. Among the invertebrate and vertebrate prey type, fish had significantly lower $\delta^{2}H$ values than the sponges/crustacean group. Red mangrove leaves were also analyzed for stable isotopes but were not included in our mixing models because previous work has shown that mangrove leaves are not an important food source for green sea turtles at our study sites; mean (±SD) isotope values for red mangrove leaves were –26.2‰ ± 1.6 for $\delta^{13}C$, 8.8‰ ± 5.3 for $\delta^{15}N$ and –68‰ ± 26 for $\delta^{2}H$. We only included red mangrove leaves $\delta^{2}H$ values to increase the terrestrial prey group sample size when assessing differences in hydrogen isotope values between mangroves and fully marine primary producers (macroalgae and seagrass). Mangrove (seeds and leaves) had

significantly higher δ^2H values than all other (marine) primary producers ($t = -7.42$, $df = 73$, $P < 0.0001$; Figures 2B and 2C). Finally, we did not have a good representation of δ^2H values for some high-trophic level prey consumers (e.g. pelagic red crab) representing open ocean sources, thus lagoon turtles were the only group with a isotopically well-represented prey selection.

Sea Turtle Epidermis. Sea turtle epidermis had a wide range of variation in stable isotope values within and between locations (Table 2). Overall, epidermis $\delta^{13}C$ ranged from –21.4‰ to –8.1‰ (BMG) whereas $\delta^{15}N$ oscillated between 5.9‰ (BMG) to 19.1‰ (SLZ-CC) and δ^2H between –101‰ (BMG) to +5‰ for (SLZ-CM). In general, BMG turtles had the highest degree of within-individual variation whereas LOL had the lowest for all tree isotope elements analyzed (Figure 2A, B and C). We did not find any significant differences in epidermis isotopic composition over time at sites where samples were collected over several years (BMG) (Tukey's HSD test: $\delta^{13}C_{year}$ ($F = 0.17$, $df = 10$, $P = 0.84$); $\delta^{15}N_{year}$ ($F = 1.23$, $df = 10$, $P = 0.33$); $\delta^{13}C_{month}$ ($F = 2.07$, $df = 7$, $P = 0.19$); $\delta^{15}N_{month}$ ($F = 0.32$, $df = 10$, $P = 0.88$). Lastly, we did not find a relationship between curved carapace length (CCL) and epidermis values ($\delta^{13}C$: $R^2 = 0.07$, $F = 5.66$, $df = 79$; $\delta^{15}N$: $R^2 = 0.07$, $F = 5.49$, $df = 79$; δ^2H: $R^2 = 0.0008$, $F = 0.06$, $df = 79$;); however, δ^2H and $\delta^{15}N$ values were positively correlated ($R^2 = 0.20$, Figure 2C).

Prey Proportions via Mixing Model. Mean (±SD) prey proportions estimated with Bayesian mixing models (SIMMR) are reported in Table 3. For open ocean green (SLZ-CM) and loggerhead turtles (SLZ-CC), mixing models using $\delta^{13}C$ and $\delta^{15}N$ data show

that pelagic red crab was the most important prey item with a relative contribution of 55.3 ± 9.8% and 61.7 ± 6.5%, respectively. For lagoon habitats, models using $\delta^{13}C$ and $\delta^{15}N$ data showed different proportion combinations of prey items among locations. For BMG green turtles, the relative contribution listed in order of importance was macroalgae (28.5 ± 20.1%), seagrass (22.2 ± 12.6%), mangrove fruit (16.1 ± 9.7%), sponges (16.7 ± 11.4%), crustaceans (16.5 ± 11.0%). For LOL green turtles, the relative contribution listed in order of importance was seagrass (41.5± 4.7%), crustaceans (29.8 ± 7.2%), sponges (11 ± 6.1%), macroalgae (10.6 ± 6.2%), and mangrove fruit (7.2 ± 3.0%). For LSI green turtles, the relative contribution listed in order of importance was crustaceans (26.8 ± 13.4%), seagrass (26 ± 11.1%), macroalgae (20.5 ± 15.3%), sponges (17.9 ± 12.0%), and mangrove fruit (8.7 ± 5.6%). Finally, the relative contribution for PAO green turtles listed in order of importance was crustaceans (73.5 ± 9.0%), sponges (8.9 ± 6.7%), macroalgae (7.0 ± 5.2%), seagrass (6.1 ± 4.0%), mangrove fruit (4.4 ± 2.6%). When using a 3-isotope model ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{2}H$), the order of importance for the different prey types did not change in comparison to the 2-isotope model ($\delta^{13}C$ and $\delta^{15}N$). However, the precision of mixing model results did not significantly improve when using the 3-isotope versus the 2-isotope approach in lagoon habitats where we had triple isotope data for all prey types (Table 3).

DISCUSSION

Here we present a detailed analysis of green sea turtles foraging ecology for the Baja California Peninsula population. We report the intra-individual variation in feeding strategies (specialists vs. generalists) within and between habitat types that are closely

related to presence of various food types. This study also corroborates that oceanic turtles forage on different food sources than lagoonal turtles. We also establish the use of hydrogen isotopes as a third variable to explore marine ecosystems and suggest that this additional isotope provides a means to distinguish between origin sources (marine vs. terrestrial) and trophic levels (trophic effect).

Potential Prey. By analyzing a diverse set of potential food items, we characterized green sea turtle diets within and between lagoons in Baja California. As expected, potential prey had a large degree of variation in $\delta^{13}C$, $\delta^{15}N$, and even $\delta^{2}H$ isotope composition (Fig. 2A, 2B, 2C and 3). First, the lagoons on the western coast of Baja California Peninsula contain a variety of primary producers that are known to have very different $\delta^{13}C$ values. Further, seagrass, an aquatic angiosperm with a rhizome structure, had relatively high mean $\delta^{13}C$ values, in comparison to macroalgae and mangroves, a pattern that results from CO_2 limitation and use of bicarbonate (HCO_3^-) as a inorganic source of carbon for photosynthesis (Andrews and Abel, 1979; Hemminga and Mateo, 1996). In general, macroalgae had intermediate $\delta^{13}C$ values, but there was general trend of green algae (*Codium* sp. or *Ulva* sp.) having more positive values and red algae (*Spyridia* sp. or *Gracilaria* sp.) with lower values, likely reflecting ability of some groups (green and brown) to alternate between the use of CO_2 ($\delta^{13}C$: –8‰) and HCO_3^- ($\delta^{13}C$: 0‰) as a substrate for photosynthesis (Maberly, 1990; Maberly et al., 1992; Raven et al., 2002); note some green algae may also use the C_4 photosynthetic pathway (Xu et al., 2012b). These algal $\delta^{13}C$ values fall within the range of values reported for other benthic marine algal species (Raven et al., 2002). By far the most depleted $\delta^{13}C$ values we observed were

in red mangroves—a terrestrial C_3 plant (Andrews et al., 1984; Ball, 1988; Craig, 1953)—that is common in Bahia Magdalena and San Ignacio lagoon; mangroves do not occur in Ojo de Liebre. In contrast to patterns in $\delta^{13}C$, seagrass and mangroves had similar $\delta^{15}N$ values, possibly because these groups use similar nitrogen pools in sediments (Raven, 1981), whereas macroalgae had slightly higher $\delta^{15}N$ values and rely on uptake of dissolved nitrogen species from the water column.

The carbon and nitrogen isotopic values from other consumer prey groups were within the respective ranges previously seen in other studies within the same area (Aurioles-Gamboa et al., 2013; Hernández-Aguilar et al. 2015). Sponges, as water column filter feeders, displayed the highest amount of variation, potentially due to the heterogeneous sources of particulate organic matter (Botto et al., 2006; Davenport and Bax, 2002). The $\delta^{15}N$ values of the crustacean group were similar to those of the sponges, indicating that these organisms operate at the same trophic level; however, enriched $\delta^{13}C$ values suggest a reliance on macroalgae sources. Furthermore, the crustacean values could also suggest a reliance on other food sources, because juveniles crabs likely selectively consume microbes living in detritus as opposed to bulk detritus (Fantle et al., 1999). $\delta^{15}N$ values for fish were the highest, indicating an elevated trophic level as compared to the invertebrate groups. The $\delta^{13}C$ values for fish (and invertebrates) where obtained from a lagoon location (BMG) and are thus likely enriched in comparison to oceanic fish (Aurioles-Gamboa et al., 2013; Clementz and Koch, 2001; Clementz et al., 2006).

Sea Turtles. Patterns in green sea turtle epidermis $\delta^{13}C$ and $\delta^{15}N$ values within and among sampling locations suggest that (1) this species has a high degree of dietary plasticity and forages across at least two trophic levels in these habitats; (2) the diversity of foraging strategies used by green sea turtles varies among lagoons; (3) the dispersion of individuals in the 3-dimensional prey space suggests that some individuals are dietary generalist, while others are diet specialists that likely have high fidelity to particular habitats types (mangroves or seagrass beds) (Figures 2A and 3). With respect to the breadth of feeding strategies of green turtles at different sites, our finding of wide variation in tissue isotopic values for turtles collected from lagoons suggests that these populations have access to an extensive variety of food sources, a situation that may enhance dietary specialization (López-Mendilaharsu et al., 2005, 2008) on particular food sources (e.g., mangroves). For example, Bahia Magdalena (BMG) is the largest lagoon on the west coast of Baja California and is a hotspot of coastal diversity (Koch et al., 2006; Koch et al., 2007; Nichols, 2003; Rodriguez-Salinas et al., 2010). Thus, we would expect the greatest degree of green sea turtle dietary diversification in this area. We identified five distinct foraging strategies for this lagoon based on $\delta^{13}C$ and $\delta^{15}N$ data: seagrass specialists; invertebrate (sponge) specialists; macroalgae specialist; and two groups that displayed generalist feeding patterns: those that ate a combination of macroalgae and mangrove fruit; and a population that fed on macroalgae and invertebrate (sponge) sources. From these possible combinations, the SIMMR model results suggest macroalgae and seagrass were the most common items foraged; however, the strong negative correlation between these two food sources indicates that the model cannot clearly determine their relative contributions (Parnell et al., 2013). Similarly, turtles from

Laguna San Ignacio (LSI), the third largest lagoon on the west coast, had four different distinct foraging strategies: the three same specialist strategies observed in BMG and one generalist population that ate a combination of seagrass and invertebrate prey. This difference in feeding strategies and consequent decrease in isotopic niche between BMG and LSI is likely due to higher macroalgae abundance (mostly red) in LSI (Núñez-López and Valdez, 1998). In comparison to the other large lagoons, green sea turtles in Laguna Ojo de Liebre (LOL), which is the second largest lagoon on the peninsular, had a relatively low degree of isotopic variation consisting of two major foraging strategies: macroalgae specialists or generalist diets of seagrass, macroalgae. and invertebrates. Note that mangroves do not occur in LOL. This pattern has been reported in previous studies (citation), but it hasn't been studied in detail since previous studies have centered their attention on the overall population patterns instead of the intra-individual differences within each lagoon location (Rodriguez Barón, 2010; Santos Baca, 2008; Tomaszewicz et al., 2018). Lastly, green sea turtles captured in the small lagoon (km) at Punta Abreojos ate a mixed diet composed of mostly invertebrates and some seagrass. This difference may be due to the physical and geomorphological characteristics of this lagoon, which is the smallest and shallowest of any included in our study, likely reducing the number of available microhabitats and overall prey availability. The isotopic values of PAO greens turtles were also similar to the open ocean green and loggerhead turtles suggesting potential movement of individuals between this small lagoon and adjacent nearshore pelagic habitats (Senko et al., 2010a).

It should also be noted that mixed diet (or generalist) values in lagoons could represent a either a combination of only local primary producers and food items (e.g.

seagrass or mangrove), or may be generated from turtles feeding on a mixture of local producers and pelagic prey (e.g. fish). Thus, these feeding strategies (generalists patterns) may indicate movement among locations with different prey availability within the lagoon or between lagoon sites and open ocean which is directly connected to food availability/abundance in each area. Future research should include gathering and analyzing a variety of open ocean food sources.

In contrast to the lagoon populations, open ocean green and loggerhead sea turtles from San Lazaro (SLZ) displayed the smallest degree of variation in $\delta^{13}C$ and $\delta^{15}N$ values with overlapping isotopic signatures, suggesting that these species consume similar types of pelagic prey. In addition, the relatively high $\delta^{15}N$ values of San Lazaro green sea turtles indicate that they generally consume higher trophic prey (invertebrates and fish) than their counterparts that inhabit lagoon habitats (Tomaszewicz et al., 2018). Consumption of high trophic level prey likely bring pelagic turtles into potential conflict with artisanal and commercial fisheries (Peckham et al., 2007; 2008; Tomaszewicz et al., 2018).

Added Value of δ^2H? Previous studies suggest that the addition of a third isotope (δ^2H) increases the resolution of source (prey) proportions in mixing models (Hondula and Pace, 2014). Our study shows that while δ^2H did not appreciably increase the resolution of mixing model output as measured by the relative error of prey proportion estimates, the order of importance and relative contribution of the different prey items did not change when using a 3-isotope versus 2-isotope model (Table 3). This suggests that the Δ^2H_{net} we used to correct epidermis δ^2H for physiologically mediated isotope

discrimination that occurs during resource assimilation and tissue synthesis was within an appropriate range (Pagès Barceló et al. in preparation). Tissue-specific isotopic discrimination factors for all three isotope system were obtained from two controlled feeding experiments in which green sea turtles were fed diets that contained high protein contents (42–54%), and thus did likely not mimic the diet composition of wild herbivorous and omnivorous sea turtles (Arthur et al., 2008; McDermid et al., 2007).

Our results also show that δ^2H is effective for distinguishing between mangrove and marine primary producers in lagoon systems (Fig. 2B and 2C) as mangroves have significantly higher δ^2H values than macroalgae or seagrass (Table 1). Increased δ^2H in mangroves relative to other (aquatic) primary producers is likely driven by (1) ^2H-enrichment of leaf water during evapotranspiration (Craig and Gordon, 1965; Thorburn et al., 1993; Wershaw et al., 1966) and/or (2) hydrogen isotope fractionation that occurs during saltwater uptake (Ellsworth and Williams, 2007), which subsequently gets assimilated into carbohydrates during photosynthesis. In contrast, cell water in macroalgae and seagrass are more in equilibrium with environmental water (seawater), and thus are expected to have lower δ^2H values (Becker, 1994; Estep and Hoering, 1980; Nelson et al., 2002; Sessions et al., 1999; Yakir, 1992). Note that the patterns and magnitude of ^2H-enrichment in terrestrial versus marine primary producers observed here is similar to that observed in riparian (allochthonous) versus aquatic (autochthonous) producers in arid environments (Doucett et al., 2007; Finlay et al., 2010).

The weak but significant positive correlation in epidermis $\delta^{15}N$ and δ^2H values (Fig. 2C) suggests that some of the variation in hydrogen isotope values among green sea turtle populations may be driven by trophic level. Similar relationships have been

reported in terrestrial (Birchall et al., 2005) as well as marine (Malej et al., 1993) and freshwater (Soto et al., 2011) aquatic food webs. On average, SLZ green sea turtles had significantly higher $\delta^{15}N$ and $\delta^{2}H$ values than turtles stranded in the lagoons, suggesting that green turtles that inhabit in pelagic food webs feed at a higher trophic level than their lagoonal counterparts. This finding is supported by mixing models that show lagoon turtles (e.g., BMG) were more herbivorous than those from SLZ (Table 2).

Conclusions. Green sea turtles from Baja California Peninsula showed a wide range of feeding plasticity in comparison to other sea turtle populations (Bjorndal, 2017; Tomaszewicz et al., 2017). Population dietary breadth, particularly within lagoons, was correlated with diversity in the number of primary producers and resource availability. Further, the presence of primary producers in lagoon habitats allowed for individual specialization on a particular resource (e.g. mangrove or seagrass) or a more generalized mixed diet. Green sea turtles from oceanic habitats appeared to be feeding consistently at a higher trophic level. These patterns further complicated by the potential movement of turtles between lagoon and offshore habitats. Together, these results provide valuable information regarding turtle feeding patterns with strong implications for conservation of this and other sea turtle species.

FIGURES

Figure 1. Map of Baja California Peninsula (BCP) study sites. Red dots represent lagoon sites: Laguna Ojo de Liebre (LOL), Punta Abreojos (PAO), Laguna San Ignacio (LSI) and Bahía Magdalena (BMG). Blue dot represents the oceanic site of San Làzaro beach (SL). Red star represents the prey sampling area of Puerto Alcatraz (PA)

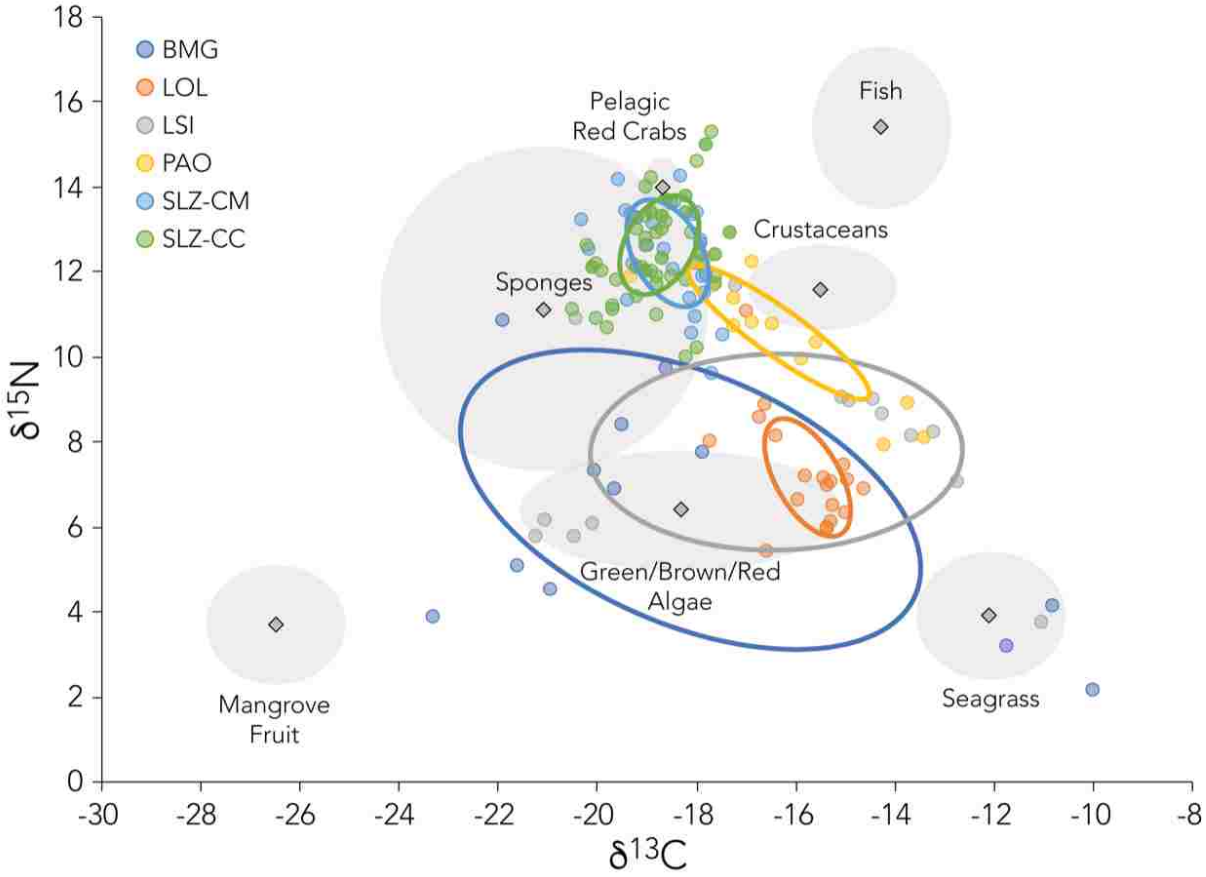

Figure 2. A. $\delta^{13}C$ and $\delta^{15}N$ green sea turtle epidermis (circles) and prey items (grey diamonds) values on a bivariate plot showing the isotopic niche space with standard ellipse areas across all locations in Baja California Peninsula. Colors represent locations (BMG: dark blue; LOL: orange; LSI: grey; PAO: yellow; SLZ-CM: light blue; SLZ-CC: green). Solid lines represent standard ellipse areas (SEA).

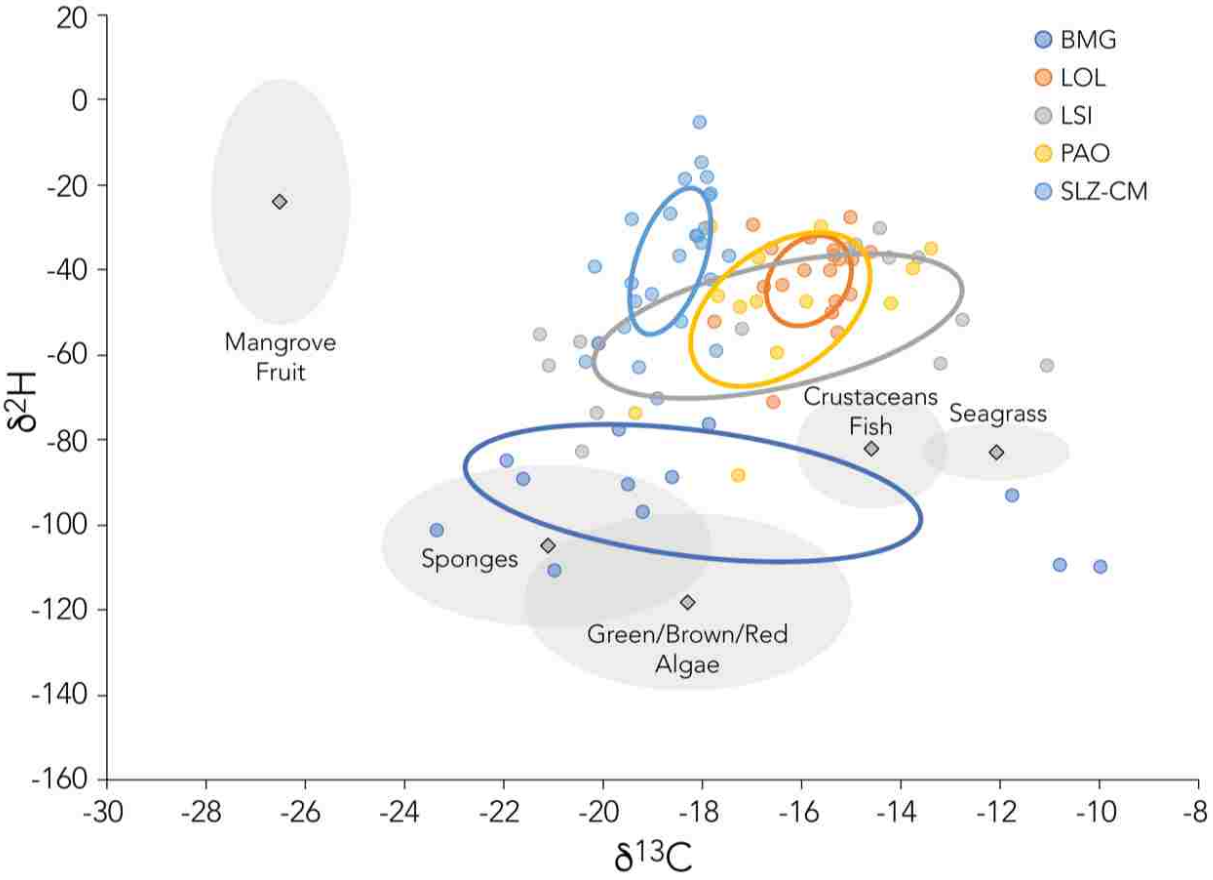

Figure 2. B. $\delta^{13}C$ and $\delta^{2}H$ green sea turtle epidermis (circles) and prey items (grey diamonds) values on a bivariate plot showing the isotopic niche space with standard ellipse areas across all locations in Baja California Peninsula. Colors represent locations (BMG: dark blue; LOL: orange; LSI: grey; PAO: yellow; SLZ-CM: light blue). Solid lines represent standard ellipse areas (SEA).

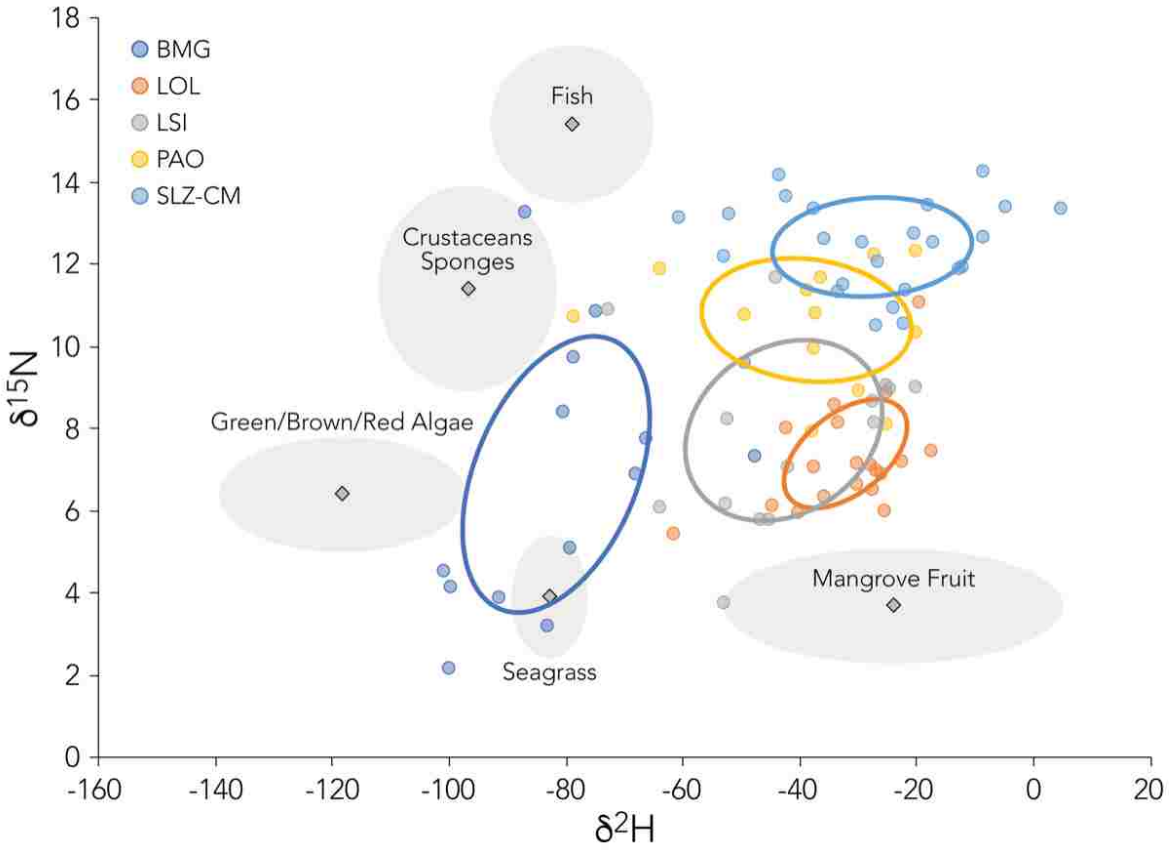

Figure 2. C. δ^2H and $\delta^{15}N$ green sea turtle epidermis (circles) and prey items (grey diamonds) values on a bivariate plot showing the isotopic niche space with standard ellipse areas across all locations in Baja California Peninsula. Colors represent locations (BMG: dark blue; LOL: orange; LSI: grey; PAO: yellow; SLZ-CM: light blue). Solid lines represent standard ellipse areas (SEA).

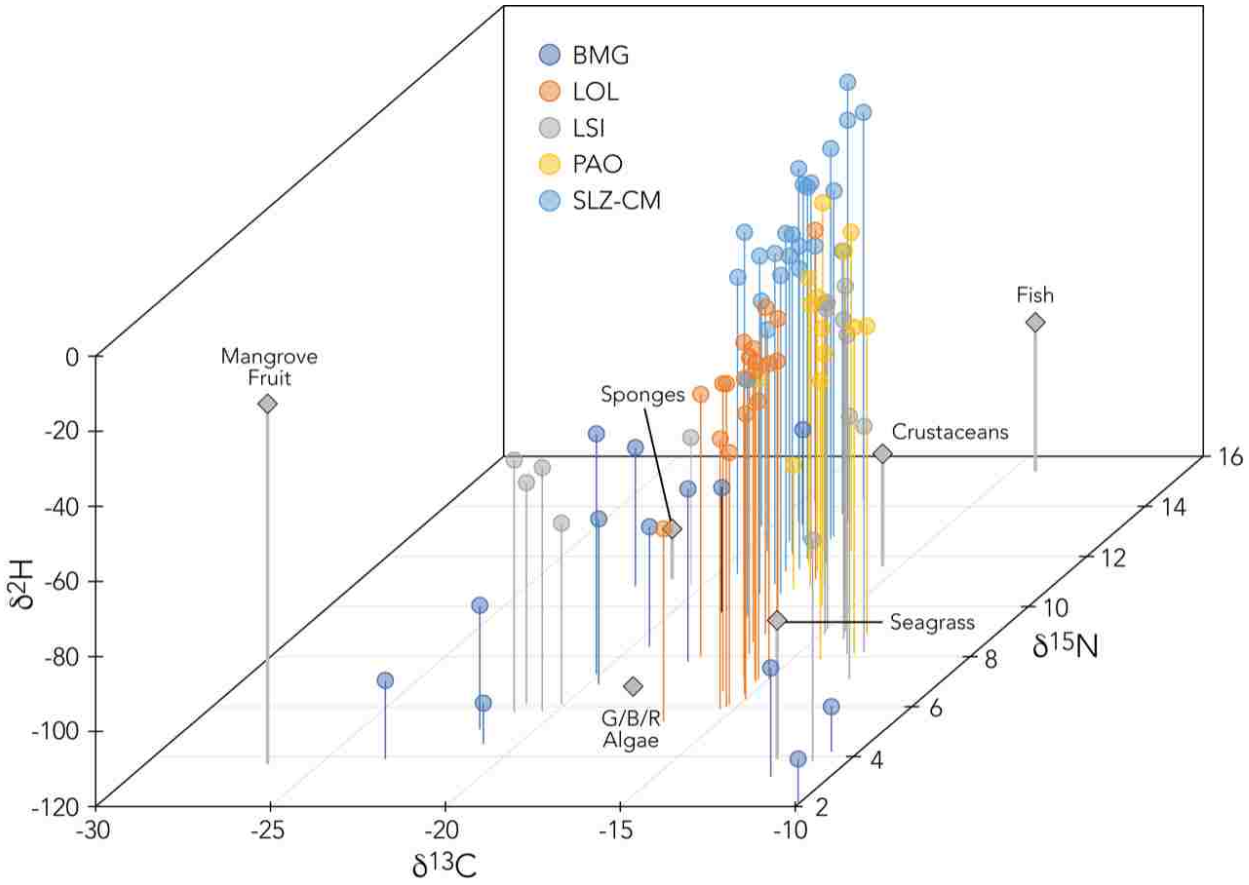

Figure 3. $\delta^{13}C$, $\delta^{2}H$ and $\delta^{15}N$ green sea turtle epidermis (circles) and prey items (grey diamonds) values on a 3-dimentional plot showing the isotopic niche space across all locations in Baja California Peninsula. Colors represent locations (BMG: dark blue; LOL: orange; LSI: grey; PAO: yellow; SLZ-CM: light blue)

Prey Type	Group	n	Mean $\delta^{15}N$	Mean $\delta^{13}C$	[C]/[N]	n	Mean $\delta^{2}H$	[H]
Producers	Brown	24	6.2 ± 1.5^{a}	-17.0 ± 3.8^{a}	21.5 ± 9.4	20	-121 ± 17^{a}	3.4 ± 1.0
	Green	20	6.8 ± 1.5^{a}	$-18.3 \pm 2.0^{a,b}$	15.4 ± 9.5	19	-125 ± 26^{a}	3.4 ± 1.3
	Red	15	6.1 ± 0.8^{a}	-20.5 ± 2.9^{b}	13.8 ± 7.9	15	-106.3 ± 15^{a}	3.3 ± 1.3
	Mangrove Fruit	16	3.7 ± 1.4^{b}	-26.5 ± 1.4^{c}	72.2 ± 25.6	4	-24 ± 29^{b}	5.3 ± 0.3
	Seagrass	35	3.9 ± 1.5^{c}	-12.1 ± 1.5^{d}	25.7 ± 10.6	10	-82.8 ± 7^{c}	4.3 ± 0.5
Invertebrates	Crustaceans	9	11.6 ± 1.0^{d}	-15.5 ± 1.5^{e}	3.9 ± 0.5	9	$-90 \pm 7^{d,e}$	5.7 ± 0.6
	Sponges	7	11.1 ± 3.8^{d}	-21.1 ± 3.3^{f}	4.5 ± 0.5	7	-105 ± 19^{d}	3 ± 1.2
	Pelagic Red Crab	5	$14.0 \pm 0.7^{d,e}$	$-18.7 \pm 0.2^{e,f}$	–	–	–	–
Vertebrates	Fish	30	15.4 ± 1.9^{e}	-14.3 ± 1.4^{e}	3.3 ± 0.3	30	-79 ± 14^{e}	5.1 ± 0.8

Table 1. Mean (± SD) $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values, isotope concentrations ([H]) and isotope ratios ([C]/[N]) of potential prey sources group for green sea turtles in Baja California Peninsula (Mexico) with their correspondent sample size (n).

Habitat type	Location	n	Mean $\delta^{15}N$	Mean $\delta^{13}C$	[C]/[N]	Mean $\delta^{2}H$	[H]
Lagoon	BMG	13	10.5 ± 3.3	-16.2 ± 4.4	3.9 ± 0.6	-81 ± 15	4.2 ± 1.2
	LOL	19	11.0 ± 1.3	-13.9 ± 0.8	2.9 ± 0.1	-32 ± 10	5.9 ± 0.2
	LSI	14	11.6 ± 2.2	-14.5 ± 3.6	3.1 ± 0.2	-43 ± 16	5.1 ± 0.8
	PAO	13	14.3 ± 1.5	-14.4 ± 1.7	2.9 ± 0.1	-39 ± 17	5.7 ± 0.4
Open ocean	SLZ - CM	25	16.2 ± 1.2	-16.7 ± 0.8	–	-28 ± 17	5.1 ± 0.4
	SLZ - CC	60	16.3 ± 1.1	-16.8 ± 0.8	3.1 ± 0.2	–	–

Table 2. Mean (± SD) $\delta^{13}C$, $\delta^{15}N$ and $\delta^{2}H$ values, isotope concentrations ([H]) and isotope ratios ([C]/[N]) for green sea turtles from different locations and habitat types within Baja California Peninsula (Mexico) with their correspondent sample size (n).

Prey	BMG			LOL			LSI			PAO		
	CN	CNH - TDF8	CNH - TDF 20	CN	CNH - TDF8	CNH - TDF 20	CN	CNH - TDF8	CNH - TDF 20	CN	CNH - TDF8	CNH - TDF 20
Green/Brown/Red	0.285 ± 0.201	0.292 ± 0.201	0.29 ± 0.204	0.106 ± 0.062	0.106 ± 0.064	0.106 ± 0.063	0.205 ± 0.153	0.21 ± 0.155	0.204 ± 0.155	0.07 ± 0.052	0.069 ± 0.05	0.068 ± 0.049
Seagrass	0.222 ± 0.126	0.221 ± 0.125	0.222 ± 0.125	0.415 ± 0.047	0.415 ± 0.047	0.415 ± 0.047	0.26 ± 0.111	0.26 ± 0.112	0.264 ± 0.112	0.061 ± 0.04	0.062 ± 0.039	0.061 ± 0.038
Mangrove Fruit	0.161 ± 0.097	0.157 ± 0.095	0.16 ± 0.096	0.072 ±0.03	0.073 ± 0.031	0.072 ± 0.031	0.087 ± 0.056	0.087 ± 0.055	0.088 ± 0.056	0.044 ± 0.026	0.044 ± 0.026	0.044 ± 0.026
Sponges	0.167 ± 0.114	0.168 ± 0.116	0.165 ± 0.112	0.11 ± 0.061	0.107 ± 0.06	0.107 ±0.06	0.179 ± 0.12	0.179 ± 0.121	0.178 ± 0.119	0.089 ± 0.067	0.089 ± 0.067	0.089 ± 0.066
Crustaceans	0.165 ± 0.112	0.163 ± 0.11	0.163 ± 0.109	0.298 ± 0.072	0.299 ± 0.072	0.299 ± 0.072	0.268 ± 0.134	0.263 ± 0.131	0.267 ± 0.133	0.735 ± 0.09	0.736 ± 0.088	0.738 ± 0.086

Table 3. SIMMR output for the proportional prey contribution in the 2-isotope mixing model (CN) and in the 3-isotope mixing model (CNH) using different hydrogen trophic discrimination factors (TDF8 and TDF20).

REFERENCES

Alverson, F.G., 1963. The food of yellowfin and skipjack tunas in the eastern tropical Pacific Ocean. Inter-American Trop. Tuna Comm. Bull. 7, 293–396.

Amorocho, D.F., Reina, R.D., 2008. Intake passage time, digesta composition and digestibility in East Pacific green turtles (*Chelonia mydas agassizii*) at Gorgona National Park, Colombian Pacific. J. Exp. Mar. Bio. Ecol. 360, 117–124.

Andrews, T.J., Abel, K.M., 1979. Photosynthetic carbon metabolism in seagrasses 14C-labeling evidence for the C3 pathway. Plant Physiol. 63, 650–656.

Andrews, T.J., Clough, B.F., Muller, G.J., 1984. Photosynthetic gas exchange properties and carbon isotope ratios of some mangroves in North Queensland, in: Physiology and Management of Mangroves. Springer, pp. 15–23.

Arreguín-Sànchez, F., Hernàndez-Herrera, A., Ramírez-Rodríguez, M., Pérez-España, H., 2004. Optimal management scenarios for the artisanal fisheries in the ecosystem of La Paz Bay, Baja California Sur, Mexico. Ecol. Modell. 172, 373–382.

Arthur, K.E., Boyle, M.C., Limpus, C.J., 2008. Ontogenetic changes in diet and habitat use in green sea turtle (*Chelonia mydas*) life history. Mar. Ecol. Prog. Ser. 362, 303–311.

Arthur, K.E., McMahon, K.M., Limpus, C.J., Dennison, W.C., 2009. Feeding ecology of green turtles (*Chelonia mydas*) from Shoalwater Bay, Australia. Mar. Turt. Newsl. 6.

Aurioles-Gamboa, D., Rodriguez-Pérez, M.Y., Sánchez-Velasco, L., Lavin, M.F., 2013. Habitat, trophic level, and residence of marine mammals in the Gulf of California assessed by stable isotope analysis. Mar. Ecol. Prog. Ser. 488, 275–290.

Ball, M.C., 1988. Ecophysiology of mangroves. Trees 2, 129–142.

Barrow, L.M., Bjorndal, K.A., Reich, K.J., 2008. Effects of preservation method on stable carbon and nitrogen isotope values. Physiol. Biochem. Zool. 81, 688–693.

Becker, E.W., 1994. Microalgae: biotechnology and microbiology. Cambridge University Press.

Ben-David, M., Flynn, R.W., Schell, D.M., 1997a. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia 111, 280–291.

Ben-David, M., Hanley, T.A., Klein, D.R., Schell, D.M., 1997b. Seasonal changes in diets of coastal and riverine mink: the role of spawning Pacific salmon. Can. J. Zool. 75, 803–811.

Berggren, M., Ziegler, S.E., St-Gelais, N.F., Beisner, B.E., del Giorgio, P.A., 2014. Contrasting patterns of allochthony among three major groups of crustacean zooplankton in boreal and temperate lakes. Ecology 95, 1947–1959.

Birchall, J., O'CONNELL, T.C., Heaton, T.H.E., Hedges, R.E.M., 2005. Hydrogen isotope ratios in animal body protein reflect trophic level. J. Anim. Ecol. 74, 877–881.

Bjorndal, K.A., 2017. Foraging ecology and nutrition of sea turtles, in: The Biology of Sea Turtles, Volume I. CRC press, pp. 213–246.

Bjorndal, K.A., 1997. Foraging ecology and nutrition of sea turtles. Biol. sea turtles 1, 199–231.

Botto, F., Bremec, C., Marecos, A., Schejter, L., Lasta, M., Iribarne, O., 2006. Identifying predators of the SW Atlantic Patagonian scallop *Zygochlamys patagonica* using stable isotopes. Fish. Res. 81, 45–50.

Bowen, G.J., Chesson, L., Nielson, K., Cerling, T.E., Ehleringer, J.R., 2005a. Treatment methods for the determination of δ2H and δ18O of hair keratin by continuous-flow isotope-ratio mass spectrometry. Rapid Commun. Mass Spectrom. 19, 2371–2378.

Bowen, G.J., Wassenaar, L.I., Hobson, K.A., 2005b. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143, 337–348.

Cabana, G., Rasmussen, J.B., 1996. Comparison of aquatic food chains using nitrogen isotopes. Proc. Natl. Acad. Sci. 93, 10844–10847.

Carrión-Cortez, J.A., Zárate, P., Seminoff, J.A., 2010. Feeding ecology of the green sea turtle (*Chelonia mydas*) in the Galapagos Islands. J. Mar. Biol. Assoc. United Kingdom 90, 1005–1013.

Carvalho, M.C., Macedo Carneiro, P.B., Dellatorre, F.G., Gibilisco, P.E., Sachs, J., Eyre, B.D., 2017. Bulk hydrogen stable isotope composition of seaweeds: Clear separation between Ulvophyceae and other classes. J. Phycol.

Clementz, M.T., Goswami, A., Gingerich, P.D., Koch, P.L., 2006. Isotopic records from early whales and sea cows: contrasting patterns of ecological transition. J. Vertebr. Paleontol. 26, 355–370.

Clementz, M.T., Koch, P.L., 2001. Differentiating aquatic mammal habitat and foraging ecology with stable isotopes in tooth enamel. Oecologia 129, 461–472.

Cliffton, K., Cornejo, D.O., Felger, R.S., 1982. Sea turtles of the Pacific coast of Mexico. Biol. Conserv. sea turtles 2, 199–209.

Cole, J.J., Carpenter, S.R., Kitchell, J., Pace, M.L., Solomon, C.T., Weidel, B., 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. Proc. Natl. Acad. Sci. 108, 1975–1980.

Craig, H., 1953. The geochemistry of the stable carbon isotopes. Geochim. Cosmochim. Acta 3, 53–92.

Craig, H., Gordon, L.I., 1965. Deuterium and oxygen 18 variations in the ocean and the marine atmosphere.

Davenport, S.R., Bax, N.J., 2002. A trophic study of a marine ecosystem off southeastern Australia using stable isotopes of carbon and nitrogen. Can. J. Fish. Aquat. Sci. 59, 514–530.

DeNiro, M.J., Epstein, S., 1981. Isotopic composition of cellulose from aquatic organisms. Geochim. Cosmochim. Acta 45, 1885–1894.

Doucett, R.R., Marks, J.C., Blinn, D.W., Caron, M., Hungate, B.A., 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. Ecology 88, 1587–1592.

Ehleringer, J.R., Bowen, G.J., Chesson, L.A., West, A.G., Podlesak, D.W., Cerling, T.E., 2008. Hydrogen and oxygen isotope ratios in human hair are related to geography. Proc. Natl. Acad. Sci. 105, 2788–2793.

Ellsworth, P.Z., Williams, D.G., 2007. Hydrogen isotope fractionation during water uptake by woody xerophytes. Plant Soil 291, 93–107.

Estep, M.F., Dabrowski, H., 1980. Tracing food webs with stable hydrogen isotopes. Science (80-.). 209, 1537–1538.

Estep, M.F., Hoering, T.C., 1980. Biogeochemistry of the stable hydrogen isotopes. Geochim. Cosmochim. Acta 44, 1197–1206.

Etnoyer, P., Canny, D., Mate, B.R., Morgan, L.E., Ortega-Ortiz, J.G., Nichols, W.J., 2006. Sea-surface temperature gradients across blue whale and sea turtle foraging

trajectories off the Baja California Peninsula, Mexico. Deep Sea Res. Part II Top. Stud. Oceanogr. 53, 340–358.

Fantle, M.S., Dittel, A.I., Schwalm, S.M., Epifanio, C.E., Fogel, M.L., 1999. A food web analysis of the juvenile blue crab, *Callinectes sapidus*, using stable isotopes in whole animals and individual amino acids. Oecologia 120, 416–426.

Finlay, J.C., Doucett, R.R., McNEELY, C., 2010. Tracing energy flow in stream food webs using stable isotopes of hydrogen. Freshw. Biol. 55, 941–951.

Fox, J.G., 2015. Laboratory animal medicine. Elsevier.

Fry, B., 2006. Stable isotope ecology. Springer.

Fry, B., Scalan, R.S., Parker, P.L., 1977. Stable carbon isotope evidence for two sources of organic matter in coastal sediments: seagrasses and plankton. Geochim. Cosmochim. Acta 41, 1875–1877.

Gardner, S.C., Nichols, W.J., 2001. Assessment of sea turtle mortality rates in the Bahía Magdalena region, Baja California Sur, México. Chelonian Conserv. Biol. 4, 197–199.

Groombridge, B., Luxmoore, R., 1989. The green turtle and hawksbill (Reptilia: Cheloniidae): world status, exploitation and trade. CITES.

Hatase, H., Sato, K., Yamaguchi, M., Takahashi, K., Tsukamoto, K., 2006. Individual variation in feeding habitat use by adult female green sea turtles (*Chelonia mydas*): Are they obligately neritic herbivores? Oecologia 149, 52–64. doi:10.1007/s00442-006-0431-2

Hays, G.C., Broderick, A.C., Godley, B.J., Luschi, P., Nichols, W.J., 2003. Satellite telemetry suggests high levels of fishing-induced mortality in marine turtles. Mar.

Ecol. Prog. Ser. 262, 305–309.

Hemminga, M.A., Mateo, M.A., 1996. Stable carbon isotopes in seagrasses: variability in ratios and use in ecological studies. Mar. Ecol. Prog. Ser. 285–298.

Hernández-Aguilar, S.B., Escobar-Sánchez, O., Galván-Magaña, F., Abitia-Cárdenas, L.A., n.d. Trophic ecology of the blue shark (*Prionace glauca*) based on stable isotopes (δ13 C and δ15 N) and stomach content. J. Mar. Biol. Assoc. United Kingdom 96, 1403–1410.

Hobson, K.A., Atwell, L., Wassenaar, L.I., 1999. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. Proc. Natl. Acad. Sci. 96, 8003–8006.

Hobson, K.A., Wassenaar, L.I., 1996. Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. Oecologia 109, 142–148.

Hondula, K.L., Pace, M.L., 2014. Macroalgal support of cultured hard clams in a low nitrogen coastal lagoon. Mar. Ecol. Prog. Ser. 498, 187–201.

Jackson, J.B.C., 1997. Reefs since columbus. Coral reefs 16, S23--S32.

Jardine, T.D., Kidd, K.A., Cunjak, R.A., 2009. An evaluation of deuterium as a food source tracer in temperate streams of eastern Canada. J. North Am. Benthol. Soc. 28, 885–893.

Kharlamenko, V.I., Kiyashko, S.I., Imbs, A.B., Vyshkvartzev, D.I., 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. Mar. Ecol. Prog. Ser. 220, 103–117.

Koch, V., Brooks, L.B., Nichols, W.J., 2007. Population ecology of the green/black turtle (*Chelonia mydas*) in Bahía Magdalena, Mexico. Mar. Biol. 153, 35–46.

Koch, V., Nichols, W.J., Peckham, H., de la Toba, V., 2006. Estimates of sea turtle mortality from poaching and bycatch in Bahia Magdalena, Baja California Sur, Mexico. Biol. Conserv. 128, 327–334.

Koch, V., Peckham, H., Mancini, A., Eguchi, T., 2013. Estimating at-sea mortality of marine turtles from stranding frequencies and drifter experiments. PLoS One 8, e56776.

López-Castro, M.C., Koch, V., Mariscal-Loza, A., Nichols, W.J., 2010. Long-term monitoring of black turtles *Chelonia mydas* at coastal foraging areas off the Baja California Peninsula. Endanger. Species Res. 11, 35–45.

López-Mendilaharsu, M., Gardner, S.C., Riosmena-Rodriguez, R., Seminoff, J.A., 2008. Diet selection by immature green turtles (C*helonia mydas*) at Bahía Magdalena foraging ground in the Pacific Coast of the Baja California Peninsula, México. J. Mar. Biol. Assoc. United Kingdom 88, 641–647.

López-Mendilaharsu, M., Gardner, S.C., Seminoff, J.A., Riosmena-Rodriguez, R., 2005. Identifying critical foraging habitats of the green turtle (*Chelonia mydas*) along the Pacific coast of the Baja California peninsula, Mexico. Aquat. Conserv. Mar. Freshw. Ecosyst. 15, 259–269.

Maberly, S.C., 1990. Exogenous sources of inorganic carbon for photosynthesis by marine macroalgae. J. Phycol. 26, 439–449.

Maberly, S.C., Raven, J.A., Johnston, A.M., 1992. Discrimination between 12 C and 13 C by marine plants. Oecologia 91, 481–492.

Macko, S.A., Estep, M.L.F., Lee, W.Y., 1983. Stable hydrogen isotope analysis of foodwebs on laboratory and field populations of marine amphipods. J. Exp. Mar. Bio. Ecol. 72, 243–249.

Madigan, D.J., Litvin, S.Y., Popp, B.N., Carlisle, A.B., Farwell, C.J., Block, B.A., 2012. Tissue Turnover Rates and Isotopic Trophic Discrimination Factors in the Endothermic Teleost, Pacific Bluefin Tuna (*Thunnus orientalis*). PLoS One 7, 1–13. doi:10.1371/journal.pone.0049220

Malej, A., Faganeli, J., Pezdič, J., 1993. Stable isotope and biochemical fractionation in the marine pelagic food chain: the jellyfish *Pelagia noctiluca* and net zooplankton. Mar. Biol. 116, 565–570. doi:10.1007/BF00355475

Mancini, A., Koch, V., 2009. Sea turtle consumption and black market trade in Baja California Sur, Mexico. Endanger. Species Res. 7, 1–10.

Mancini, A., Koch, V., Seminoff, J.A., Madon, B., 2011. Small-scale gill-net fisheries cause massive green turtle *Chelonia mydas* mortality in Baja California Sur, Mexico. Fauna Flora Int. Oryx 46, 69–77.

Márquez, M.R., 1990. FAO species catalogue. v. 11: Sea turtles of the world. FAO.

McClellan, C.M., Braun-McNeill, J., Avens, L., Wallace, B.P., Read, A.J., 2010. Stable isotopes confirm a foraging dichotomy in juvenile loggerhead sea turtles. J. Exp. Mar. Bio. Ecol. 387, 44–51. doi:10.1016/j.jembe.2010.02.020

McClelland, J.W., Valiela, I., 1998. Changes in food web structure under the influence of increased anthropogenic nitrogen inputs to estuaries. Mar. Ecol. Prog. Ser. 259–271.

McConnaughey, T., McRoy, C.P., 1979. 13 C label identifies eelgrass (*Zostera marina*) carbon in an Alaskan estuarine food web. Mar. Biol. 53, 263–269.

McDermid, K.J., Stuercke, B., Balazs, G.H., 2007. Nutritional composition of marine plants in the diet of the green sea turtle (*Chelonia mydas*) in the Hawaiian Islands. Bull. Mar. Sci. 81, 55–71.

Minagawa, M., Wada, E., 1984. Stepwise enrichment of ^{15}N along food chains: Further evidence and the relation between $\delta^{15}N$ and animal age. Geochim. Cosmochim. Acta 48, 1135–1140. doi:10.1016/0016-7037(84)90204-7

Nelson, M.M., Phleger, C.F., Nichols, P.D., 2002. Seasonal lipid composition in macroalgae of the northeastern Pacific Ocean. Bot. Mar. 45, 58–65.

Nichols, W.J., 2003. Biology and conservation of sea turtles in Baja California, Mexico.

Nichols, W.J., Safina, C., 2004. Lunch with a turtle poacher. Conservation 5, 30–34.

Núñez-López, R.A., Valdez, M.C., 1998. Seasonal variation of seaweed biomass in San Ignacio Lagoon, Baja California Sur, Mexico. Bot. Mar. 41, 421–426.

Ostrom, P.H., Wiley, A.E., Rossman, S., Stricker, C.A., James, H.F., 2014. Unexpected hydrogen isotope variation in oceanic pelagic seabirds. Oecologia 175, 1227–1235. doi:10.1007/s00442-014-2985-8

Parker, D.M., Dutton, P.H., Balazs, G.H., 2011. Oceanic diet and distribution of haplotypes for the green turtle, *Chelonia mydas*, in the Central North Pacific. Pacific Sci. 65, 419–431.

Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J., Inger, R., 2013. Bayesian stable isotope mixing models. Environmetrics 24, 387–399.

Peckham, S.H., Diaz, D.M., Walli, A., Ruiz, G., Crowder, L.B., Nichols, W.J., 2007. Small-scale fisheries bycatch jeopardizes endangered Pacific loggerhead turtles.

PLoS One 2, e1041.

Peckham, S.H., Maldonado-Diaz, D., Koch, V., Mancini, A., Gaos, A., Tinker, M.T., Nichols, W.J., 2008. High mortality of loggerhead turtles due to bycatch, human consumption and strandings at Baja California Sur, Mexico, 2003 to 2007. Endanger. Species Res. 5, 171–183.

Peterson, B.J., Howarth, R.W., Garritt, R.H., 1985. Multiple stable isotopes used to trace the flow of organic matter in estuarine food webs. Science (80-.). 227, 1361–1363.

Phillips, D.L., 2012. Converting isotope values to diet composition: the use of mixing models. J. Mammal. 93, 342–352.

Phillips, D.L., Newsome, S.D., Gregg, J.W., 2005. Combining sources in stable isotope mixing models: alternative methods. Oecologia 144, 520–527.

Post, D.M., 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. Ecology 83, 703–718.

Raven, J.A., 1981. Nutritional strategies of submerged benthic plants: the acquisition of C, N and P by rhizophytes and haptophytes. New Phytol. 88, 1–30.

Raven, J.A., Johnston, A.M., Kübler, J.E., Korb, R., McInroy, S.G., Handley, L.L., Scrimgeour, C.M., Walker, D.I., Beardall, J., Vanderklift, M., others, 2002. Mechanistic interpretation of carbon isotope discrimination by marine macroalgae and seagrasses. Funct. Plant Biol. 29, 355–378.

Riosmena-Rodriguez, R., Muñiz-Salazar, R., López-Calderón, J., Torre-Cosio, J., Meling, A., Talbo, S.L., Sage, G.K., Ward, D.H., Cabello-Pasini, A., 2013. Conservation status of *Zostera marina* populations at Mexican Pacific. Adv. Environ. Res. 27, 35–63.

Roden, J.S., Ehleringer, J.R., 1999. Observations of hydrogen and oxygen isotopes in leaf water confirm the Craig-Gordon model under wide-ranging environmental conditions. Plant Physiol. 120, 1165–1174.

Rodriguez-Salinas, P., Riosmena-Rodriguez, R., Hinojosa-Arango, G., Muñiz-Salazar, R., 2010. Restoration experiment of *Zostera marina* L. in a subtropical coastal lagoon. Ecol. Eng. 36, 12–18.

Rodriguez Barón, J.M., 2010. Afinidad trófica a zonas de alimentación de la tortuga verde (*Chelonia mydas*) en la costa occidental de Baja California Sur, México. Instituto Politécnico Nacional. Centro Interdisciplinario de Ciencias Marinas.

Rosing, M.N., Ben-David, M., Barry, R.P., 1998. Analysis of stable isotope data: AK nearest-neighbors randomization test. J. Wildl. Manage. 380–388.

Santos Baca, L., 2008. Evaluación de los hábitos de alimentación de la tortuga verde *Chelonia mydas*, en Bahia Magdalena, BCS, México, utilizando la técnica de isótopos estables (δ13C y δ15N).

Sauer, P.E., Schimmelmann, A., Sessions, A.L., Topalov, K., 2009. Simplified batch equilibration for D/H determination of non-exchangeable hydrogen in solid organic material. Rapid Commun. mass Spectrom. 23, 949–956.

Seminoff, J.A., Jones, T.T., Resendiz, A., Nichols, W.J., Chaloupka, M.Y., 2003. Monitoring green turtles (*Chelonia mydas*) at a coastal foraging area in Baja California, Mexico: multiple indices to describe population status. J. Mar. Biol. Assoc. United Kingdom 83, 1355–1362.

Seminoff, J.A., Resendiz, A., Nichols, W.J., 2002. Home range of green turtles *Chelonia mydas* at a coastal foraging area in the Gulf of California, Mexico. Mar. Ecol. Prog.

Ser. 242, 253–265.

Senko, J., Koch, V., Megill, W.M., Carthy, R.R., Templeton, R.P., Nichols, W.J., 2010a. Fine scale daily movements and habitat use of East Pacific green turtles at a shallow coastal lagoon in Baja California Sur, Mexico. J. Exp. Mar. Bio. Ecol. 391, 92–100.

Senko, J., López-Castro, M.C., Koch, V., Nichols, W.J., 2010b. Immature East Pacific Green Turtles (*Chelonia mydas*) Use Multiple Foraging Areas off the Pacific Coast of Baja California Sur, Mexico: First Evidence from Mark-Recapture Data1. Pacific Sci. 64, 125–130.

Senko, J., Mancini, A., Seminoff, J.A., Koch, V., 2014. Bycatch and directed harvest drive high green turtle mortality at Baja California Sur, Mexico. Biol. Conserv. 169, 24–30.

Sessions, A.L., Burgoyne, T.W., Schimmelmann, A., Hayes, J.M., 1999. Fractionation of hydrogen isotopes in lipid biosynthesis. Org. Geochem. 30, 1193–1200.

Soto, D.X., Wassenaar, L.I., Hobson, K.A., Catalan, J., 2011. Effects of size and diet on stable hydrogen isotope values (δD) in fish: implications for tracing origins of individuals and their food sources. Can. J. Fish. Aquat. Sci. 68, 2011–2019.

Squire, J.L., Suzuki, Z., 1991. Migration trends of Striped Marlin. Tetrapturus audax.

Thorburn, P.J., Walker, G.R., Brunel, J.-P., 1993. Extraction of water from Eucalyptus trees for analysis of deuterium and oxygen-18: laboratory and field techniques. Plant. Cell Environ. 16, 269–277.

Tomaszewicz, C.N.T., Seminoff, J.A., Avens, L., Goshe, L.R., Rguez-Baron, J.M., Peckham, S.H., Kurle, C.M., 2018. Expanding the coastal forager paradigm: long-term pelagic habitat use by green turtles *Chelonia mydas* in the eastern Pacific

Ocean. Mar. Ecol. Prog. Ser. 587, 217–234.

Tomaszewicz, T., Calandra, N., Seminoff, J.A., Price, M., Kurle, C.M., 2017. Stable isotope discrimination factors and between-tissue isotope comparisons for bone and skin from captive and wild green sea turtles (*Chelonia mydas*). Rapid Commun. Mass Spectrom.

Vander Zanden, H.B., Bjorndal, K.A., Mustin, W., Ponciano, J.M., Bolten, A.B., 2012. Inherent variation in stable isotope values and discrimination factors in two life stages of green turtles. Physiol. Biochem. Zool. 85, 431–441.

Wassenaar, L.I., Hobson, K.A., 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isotopes Environ. Health Stud. 39, 211–217.

Wassenaar, L.I., Hobson, K.A., 2000. Improved method for determining the stable-hydrogen isotopic composition (δD) of complex organic materials of environmental interest. Environ. Sci. Technol. 34, 2354–2360.

Wassenaar, L.I., Hobson, K.A., 1998. Natal origins of migratory monarch butterflies at wintering colonies in Mexico: new isotopic evidence. Proc. Natl. Acad. Sci. 95, 15436–15439.

Wershaw, R.L., Friedman, I., Heller, S.J., Frank, P.A., 1966. Hydrogen isotopic fractionation of water passing through trees. Adv. Org. geochemistry 55.

Whitmore, R.C., Brusca, R.C., de la Luz, J.L., González-Zamorano, P., Mendoza-Salgado, R., Amador-Silva, E.S., Holguin, G., Galván-Magaña, F., Hastings, P.A., Cartron, J.L.E., others, 2005. The ecological importance of mangroves in Baja California Sur: conservation implications for an endangered ecosystem.

Biodiversity, Ecosyst. Conserv. North. Mex. 298–333.

Williams, S.L., 1988. Thalassia testudinum productivity and grazing by green turtles in a highly disturbed seagrass bed. Mar. Biol. 98, 447–455. doi:10.1007/BF00391121

Wingfield, D.K., Peckham, S.H., Foley, D.G., Palacios, D.M., Lavaniegos, B.E., Durazo, R., Nichols, W.J., Croll, D.A., Bograd, S.J., 2011. The making of a productivity hotspot in the coastal ocean. PLoS One 6, e27874.

Xu, J., Fan, X., Zhang, X., Xu, D., Mou, S., Cao, S., Zheng, Z., Miao, J., Ye, N., 2012. Evidence of coexistence of C3 and C4 photosynthetic pathways in a green-tide-forming alga, *Ulva prolifera*. PLoS One 7, e37438.

Yakir, D., 1992. Variations in the natural abundance of oxygen-18 and deuterium in plant carbohydrates. Plant. Cell Environ. 15, 1005–1020.

Zaytsev, O., Cervantes-Duarte, R., Montante, O., Gallegos-Garcia, A., 2003. Coastal upwelling activity on the Pacific shelf of the Baja California Peninsula. J. Oceanogr. 59, 489–502.