

## RESEARCH PAPER

# Ancient DNA Analysis and Stable Isotope Ecology of Sea Turtles (Cheloniidae) from the Gold Rush-era (1850s) Eastern Pacific Ocean

Cyler Conrad\*, Laura Pagès Barceló\*, Jeffrey A. Seminoff†, Calandra Turner Tomaszewicz‡, Marie Labonte‡, Brian M. Kemp‡, Emily Lena Jones\*, Michael Stoyka§, Kale Bruner|| and Allen Pastron¶

Historical and archaeological evidence documents the importation of sea turtles from the eastern Pacific Ocean (Baja California) to California during the Gold Rush (1848–1855) and through the end of 19<sup>th</sup> century, but it is unknown whether these 19<sup>th</sup> century sea turtles foraged in similar ways to their modern counterparts. To identify the species of two Gold Rush-era sea turtle specimens recovered from archaeological deposits in San Francisco, California, we first analyze ancient DNA (aDNA). We then analyze carbon ( $\delta^{13}\text{C}_{\text{col}}$ ), nitrogen ( $\delta^{15}\text{N}$ ), and hydrogen ( $\delta\text{D}$ ) stable isotopes of bone collagen and carbon ( $\delta^{13}\text{C}_{\text{ap}}$ ) and oxygen ( $\delta^{18}\text{O}_{\text{ap}}$ ) stable isotopes of bone apatite to test if eastern Pacific sea turtle diets have changed over the past 160 years. Ancient DNA confirms that both archaeological specimens are green sea turtles (*Chelonia mydas*). The stable isotope values from the 19<sup>th</sup>-century specimens are statistically indistinguishable from the modern comparatives in both  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}$ , suggesting that green sea turtle dietary intake has remained relatively unchanged since the 1850s. However, the values are unclear for  $\delta\text{D}$  and  $\delta^{18}\text{O}_{\text{ap}}$  and require additional research.

**Social Media:** Ancient DNA and isotopic analysis of 1850s sea turtles suggest stability in sea turtle foraging through time despite environmental changes.

**Keywords:** ancient DNA; stable isotopes; sea turtle; Cheloniidae; Gold Rush; San Francisco

## 1. Introduction

From the 1960s through the 1980s sea turtle (Cheloniidae) populations in the Eastern Pacific, especially those of green sea turtles (*Chelonia mydas*), declined in abundance to the point of near extinction (Cliffton et al. 1982; Delgado-Trejo and Alvarado-Diaz 2012; Early-Capistrán et al. 2017; Plotkin et al. 2012; Seminoff et al. 2012a). Hunting of sea turtles from the mid-19<sup>th</sup> through the mid-20<sup>th</sup> century seems to have been one driver of this decline (Caldwell 1963; Conrad and Pastron 2014; Early-Capistrán et al. 2017; Nichols 2003; O'Donnell 1974), but previous studies have not explored the potential role of habitat change in the historic turtle population crash (Early-Capistrán et al. 2017; Plotkin et al. 2012; Delgado-Trejo and Alvarado-Diaz 2012).

As habitat change contributes to declining sea turtle abundance today (Hawkes et al. 2009; Saba 2012) and known sea surface temperature changes have occurred since the mid-19<sup>th</sup> century in the eastern Pacific Ocean (Douglas 1980), it is possible that changes in turtle diet, reflecting the habitat and sea surface temperature changes, contributed to sea turtle population declines in the 20<sup>th</sup> century.

Research on this topic has likely not occurred previously for two logistical reasons: a lack of credibly-dated historic zoological and zooarchaeological specimens; and difficulties in determining the correct taxon of those specimens, particularly those from archaeological sites. Whalers, mariners and maritime passengers, the primary groups hunting sea turtles during the 1800s, typically discarded turtle carcasses overboard in open water after consuming the animals (Conrad and Pastron 2014; O'Donnell 1974), with very few specimens, if any, arriving in museums for curation. Compounding the paucity of specimens is the difficulty in identifying sea turtle remains using bone morphology (e.g., Armitage 2013; Frazier 2005; Smith et al. 2007). Different sea turtle species share gross morphological skeletal characteristics (Wyneken 2001). Elements that are distinct morphologically (e.g., entoplastron) are

\* University of New Mexico, US

† National Oceanic and Atmospheric Administration, US

‡ University of Oklahoma, US

§ Sonoma State University, US

|| University of Kansas, US

¶ Archeo-Tec: Consulting Archaeologists, US

Corresponding author: Cyler Conrad ([cylerc@unm.edu](mailto:cylerc@unm.edu))

easily fragmented and often absent from skeletal zooarchaeological collections.

Here, we use ancient DNA analysis to identify two archaeological sea turtle specimens that date to the middle 19<sup>th</sup> century to species level. We then compare the carbon ( $\delta^{13}\text{C}_{\text{col/ap}}$ ), nitrogen ( $\delta^{15}\text{N}$ ), oxygen ( $\delta^{18}\text{O}_{\text{ap}}$ ) and hydrogen ( $\delta\text{D}$ ) isotopic data from archaeological and modern specimens to assess dietary change between 19<sup>th</sup> century and present-day eastern Pacific Ocean sea turtles and what it may indicate about the influence of habitat modification on 20<sup>th</sup> century sea turtle populations.

### 1.1. Gold Rush Sea Turtle Exploitation

The human population increase associated with the California Gold Rush led to a shortage of food in the booming towns and cities throughout the San Francisco Bay and the Sierra Nevada region (Bancroft 1888; Soulé et al. 1854). Eastern Pacific sea turtles were one resource exploited to satisfy this need (Conrad and Pastron 2014). The maritime migration to the gold fields created a large demand for food resources, and this coincided with the proliferation of sea turtle hunting in northwestern Mexico and eventually a direct sea turtle trade bringing turtles from the Baja California area to northern California during the 1850s and after (Conrad and Pastron 2014).

The transport of sea turtles to be used as food is seen in the archaeological record. At Thompson's Cove (CA-SFR-186H), located near the Financial District of modern-day San Francisco, excavations recovered a single sea turtle (Cheloniidae) flipper phalanx in food refuse deposits dating to the early 1850s (Figure 1; Conrad et al. 2015; see Pastron and Bruner 2014 for additional chronological information). Morphological similarity in sea turtle phalanges (with the exception of leatherback sea turtles [*Dermochelys coriacea*]; Wyneken 2001) did not allow a species identification, but this specimen matched the size and morphology of a comparative green sea turtle skeleton from the Museum of Vertebrate Zoology, Berkeley.

At CA-SFR-195H, an archaeological site on the San Francisco waterfront, excavations recovered the remains of a single sea turtle represented by 23 carapace, plastron and appendicular skeletal elements dating to the early 1850s (Figure 1; see Praetzellis 2017 and chronological

information therein). Cutmarks on the carapace suggest this individual was butchered. A comparison with sea turtle specimens of known species at the California Academy of Sciences, San Francisco, suggested this was either a green or olive ridley (*Lepidochelys olivacea*) sea turtle.

### 1.2. Eastern Pacific Ocean Environmental Change

Global climate change impacts sea turtle populations via a complex web of physical and biological interactions (Fuentes et al. 2010a, 2010b; Hawkes et al. 2009; Saba 2012). Sea turtles are at risk of losing nesting habitat and beaches due to both rising sea levels and anthropogenic modifications to limit sea level rise (e.g., sea walls). Reproduction loss or sex-ratio skewing (i.e., feminization) is also likely, due to changes in temperature that drive turtle sex determination during incubation (Jensen et al. 2018). Most importantly for this study, changes also occur in sea turtle diets due to shifts in sea surface temperature and, thus, habitat structure and prey abundance in nearshore areas (Koch et al. 2013). The exact impacts and responses of these processes are region- and species-specific, but changes in climate clearly impact modern sea turtles (Fish et al. 2005; Fuentes et al. 2010a, 2010b). As an example, long-term shifts in atmospheric pressure, temperature, and rainfall impact sea turtle nesting patterns in northern Australia due to decreases in herbivorous food resources for turtles (especially green sea turtles) which affect fat reserves required for breeding (Limpus and Nicholls 2000; see also Saba et al. 2007 for the eastern Pacific).

In the eastern Pacific Ocean, climate-related events may also directly affect sea turtle populations by impacting food web dynamics (Chavez et al. 2003; Hernández-Carmona et al. 2011; Kahru and Mitchell 2000; Saba 2012; Turk et al. 2001). Green sea turtles are primarily herbivorous, consuming seagrass, but dietary studies suggest that this species may also exhibit omnivorous behavior depending on foraging location and condition (Amoroch and Reina 2008; Lopez-Mendilaharsu et al. 2005; Seminoff et al. 2002, 2008). Because sea turtles are sensitive to oceanic and terrestrial temperature shifts (Davenport 1997; Fuentes et al. 2010a, 2010b) and marine primary productivity is also influenced by temperature changes (Koch et al. 2013), long-term climatic changes may have impacted



**Figure 1:** Gold Rush-era sea turtle bones analyzed in this study. **A:** CA-SFR-186H phalanx and **B:** CA-SFR-195H costal carapace.

habitat conditions and resource availability for eastern Pacific sea turtles in the past. An example of this process is the decline of sea grass (*Zostera marina*) in modern eastern Pacific coastal lagoons (Riosmena-Rodriguez et al. 2013).

Few long-term sea turtle habitat studies exist for the eastern Pacific, but sea surface temperatures have fluctuated through time in this region, particularly during the mid-19<sup>th</sup> century (D'Arrigo et al. 2005). Sea surface temperatures were cooler during the 1800s than today (Kennedy et al. 2011; Rayner et al. 2003), but in the eastern Pacific Ocean the 1840s–1860s experienced an anomalous period of warm summers (~21–23.5°C) with temperatures +2.0°C greater than the period between 1671–1800 (Douglas 1980). This suggests that sea turtle diets may have shifted during the mid-19<sup>th</sup> century due to broad changes in sea surface temperature that directly affected predator-prey relationships and dietary resources (Etnoyer et al. 2006).

These background changes in sea surface temperatures and thus sea turtle habitats leave an unanswered question: were sea turtle diets significantly different in the 19<sup>th</sup> and 20<sup>th</sup> centuries? We analyze Gold Rush-era sea turtle bone stable isotopes, as a proxy for dietary and habitat ecology, and compare these values with modern specimens to determine if there is evidence for change in turtle diet between the 1850s and today.

## 2. Methods

Because sea turtles forage on a diverse range of resources, even within the same species (Jones and Seminoff 2013), identification of changing ecology is only possible if the data under consideration derive from the same species and geographic area. We therefore use ancient DNA analysis to assign a species affiliation to the archaeological specimens, and then bone collagen and apatite stable isotope analyses to identify diet (i.e., trophic status) in both archaeological turtles and modern comparatives.

Carbon ( $\delta^{13}\text{C}_{\text{col}}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) bone collagen stable isotopes provide information regarding habitat and resource use (DeNiro and Epstein 1978, 1981).  $\delta^{13}\text{C}_{\text{col}}$  is used as a proxy to differentiate sources of primary production (i.e., plants) due to isotopically defined differences in their physiological pathways (Kharlamenko et al. 2001; Maberly et al. 1992; McConnaughey and McRoy 1979) whereas  $\delta^{15}\text{N}$  is used to estimate trophic level since it predictably fractionates when moving up within the food chain (Cabana and Rasmussen 1996; Minagawa and Wada 1984; Post 2002; Reich et al. 2007). Hydrogen isotopes ( $\delta\text{D}$ ) in bone collagen (protein) are also used to trace basal energy sources in ecosystems where primary producers present a high range of isotopic variation (Estep and Dabrowski 1980; Cole et al. 2011; Doucett et al. 2007; Pagès Barceló 2018). Finally, bone apatite carbon ( $\delta^{13}\text{C}_{\text{ap}}$ ) stable isotopes also identify sources of primary production, but in terms of aggregated protein, carbohydrate and lipid macronutrients, while oxygen ( $\delta^{18}\text{O}_{\text{ap}}$ ) stable isotopes provide information regarding sources of body water, whether from consuming food (water in diet), ingesting water (sea water) or breathing (Coulson et al. 2008; Langlois et al. 2003). These stable isotopes provide a

quantifiable technique to systematically examine dietary and habitat information for both 19<sup>th</sup> century and modern sea turtles.

This mixed method approach, using ancient DNA analysis alongside the analysis of multiple isotope systems, allows for the identification of Gold Rush-era sea turtle samples and a comparison of past and present dietary ecology.

### 2.1. Ancient DNA (aDNA) extraction, PCR analysis

All pre-polymerase chain reaction (PCR) activities were conducted in the ancient DNA laboratory at the Laboratories of Molecular Anthropology and Microbiome Research (LMAMR), University of Oklahoma, Norman, OK. This laboratory is a dedicated workspace for processing degraded, aged, and low copy number (LCN) DNA samples. Precautions aimed to minimize and monitor the introduction of contamination are practiced in the laboratory.

DNA was extracted from bone samples CA-SFR-186H and CA-SFR-195H in separate batches, each accompanied by an extraction negative control to which no bone was added. Approximately 39.2 mg was carefully subsampled from CA-SFR-186H and 30 mg from CA-SFR-195H. Each subsample was submerged in 6% (w/v) sodium hypochlorite (NaOCl) for 4 min and the bleach poured off. The samples were then twice submerged in DNA-free water, with the water poured off following submersion. Samples were transferred to 1.5 mL tubes, to which aliquots of 500  $\mu\text{L}$  of *Ethylenediaminetetraacetic acid* (EDTA) were added, and gently rocked at room temperature for >48 hours. DNA was extracted following the WSU method described by Kemp et al. (2014).

DNA extracts were tested for the presence of co-extracted PCR inhibitors following Kemp et al. (2014), using ancient turkey DNA as a positive control. Neither extract contained sufficient inhibitors to prevent amplification of turkey DNA control.

Primers were designed to amplify a 167 base pair (bp) region of the cytochrome oxidase I (COI gene), spanning nucleotide position 5590 to 5756 of the loggerhead turtle (*Caretta caretta*) full mitochondrial DNA reference sequence (NC\_016923.1). Sequences of this amplicon spanning 5614 to 5734 can be used as a barcode to discriminate between all extant sea turtles (e.g., Elmeer and Almalki 2011): loggerhead turtle, green, Kemp's ridley (*Lepidochelys kempii*), olive ridley, hawksbill (*Eretmochelys imbricata*), flatback (*Natator depressus*), and leatherback (**Table 1**).

PCRs of 15  $\mu\text{L}$  contained 1 $\times$  Omni Klentaq Reaction Buffer, 0.32 mM dNTPs, 0.24  $\mu\text{M}$  of each primer, 0.3 U of Omni Klentaq LA polymerase, and 1.5  $\mu\text{L}$  of template DNA. PCR cycling conditions consisted of: 1) a 3 min hold at 94°C, 2) 60 cycles of 15 second holds at 94°C, 56°C and 68°C, and 3) a 3 min hold at 68°C. Successful amplification was confirmed by separating 2  $\mu\text{L}$  on 2% agarose gels, which were stained with GelRed and visualized under ultraviolet light. PCRs were conducted with full concentration extracts and 1:10 dilutions of those extracts.

Following unsuccessful amplification of the CA-SFR-195H specimen 1:10 dilution, Rescue PCR was employed in an attempt to replicate results from the full concentration

**Table 1:** Single nucleotide polymorphisms (SNPs) of the cytochrome oxidase I (COI) gene used to differentiate the extant species of sea turtles.

Reference position	5614	5616	5622	5625	5626	5628	5631	5637	5649	5650	5652	5661	5664	5668	5670	5673
<i>Caretta caretta</i>	A	C	C	T	G	C	C	C	A	T	A	A	A	G	T	A
<i>Chelonia mydas</i>	-	T	T	-	-	-	-	T	C	C	-	T/C	-	-	C	-
<i>Dermochelys coriacea</i>	G	T	-	C	-	T	-	-	C	C	T	-	-	-	C	G
<i>Eretmochelys imbricata</i>	-	T	T	C	-	T	-/T	-	-	C	-	-	-	-	C	-
<i>Lepidochelys kempii</i>	-	-	T	C	-	-	T	-	-	C	-	-	G	-	-	-
<i>Lepidochelys olivacea</i>	-	T	T	A	-/A	-	T	-	-	C	-	-/G	G	-/A	-	-
<i>Nattator depressus</i>	-	-	T	C	-	-	-	-	C	C	-	C	-	-	-	-

amplicon. Rescue PCR was conducted, as described by Johnson and Kemp (2016) by increasing the concentrations of dNTPs, buffer, primers, and KlenTaq by 25%. PCR reaction conditions were as described above.

Amplicons were sequenced in both directions at MC Lab (South San Francisco, CA). Sequencher (version 5.4.6) was used to align the sequences to the full *Caretta caretta* mitochondrial genome (NC\_016923.1).

## 2.2. Collagen Analysis ( $\delta^{13}\text{C}_{\text{col}}$ , $\delta^{15}\text{N}$ , $\delta\text{D}$ )

After cutting a small portion of bulk bone, we demineralized the sea turtle samples in 0.5 N hydrochloric acid (HCl) at 5°C for 24 hours and rinsed all samples to neutrality using deionized water. Lipid extraction involved immersing the samples in a solution of 2:1 chloroform:methanol ( $\text{C}_2\text{H}_5\text{Cl}_3$ ) for 24 hours (repeated three times). We sonicated samples for 15 minutes to ensure complete chemical saturation after each immersion. At the end of 72 hours we rinsed all samples to neutrality and lyophilized the samples for 24 hours. Approximately 0.5–0.6 mg of bone collagen was then placed into tin capsules for carbon ( $\delta^{13}\text{C}_{\text{col}}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) stable isotope analysis. For hydrogen ( $\delta\text{D}$ ) isotope samples, approximately 0.1–0.2 mg of bone collagen was placed into silver capsules for analysis.

Carbon and nitrogen samples were measured on a Costech 4010 elemental analyzer (Valencia, California, USA) coupled to a Scientific Delta V isotope ratio mass spectrometer at the University of New Mexico, Center for Stable Isotopes (UNM-CSI), Albuquerque, NM. We measured hydrogen samples on a Finnigan high-temperature conversion elemental analyzer (TC/EA) coupled to a Thermo Scientific Delta V Plus mass spectrometer by a Conflo IV (see Sharp et al. 2001 for details on the high temperature conversion method) at UNM-CSI. Atmospheric  $\text{N}_2$  is the internationally accepted standard used for nitrogen and V-PDB for carbon.

We weighed hydrogen standards and samples into silver capsules and allowed the samples to sit in the laboratory for at least two weeks before analysis to ensure equilibrium between the exchangeable hydrogen in tissue and local atmosphere (Sauer et al. 2009). Hydrogen data is corrected using three laboratory keratin standards ( $\delta\text{D}_{\text{non-ex}} = -174\text{‰}$ ,  $-93\text{‰}$ , and  $-54\text{‰}$ ) of which the  $\delta\text{D}_{\text{non-ex}}$  values were previously determined through a series of atmospheric exchange experiments. Since the samples under analysis are bone collagen, a UNM-CSI cow (*Bos taurus*) bone collagen standard was also analyzed and gave a within-run standard deviation of  $<1.5\text{‰}$ . Internal-lab hydrogen standards are corrected to Vienna-Standard Mean Ocean Water (V-SMOW). The  $\text{H}^3$  factor is 5.6. Weight percent carbon and nitrogen concentrations provide a measure of collagen contamination (Ambrose 1990). Sea turtle samples ranged between 2.7 and 2.9 suggesting intact and preserved collagen without contaminants. Collagen precision (SD) for within-run analyses is  $<0.1\text{‰}$  for  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}$ , and  $<1.5\text{‰}$   $\delta\text{D}$ . We report isotope values in delta ( $\delta$ ) notation, calculated as:  $[(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R_{\text{sample}}$  and  $R_{\text{standard}}$  are the ratios (e.g.,  $^{13}\text{C}/^{12}\text{C}$ ,  $^{15}\text{N}/^{14}\text{N}$ ) of the unknown and standard material. Delta values are reported as parts per thousand, or per mil (‰).

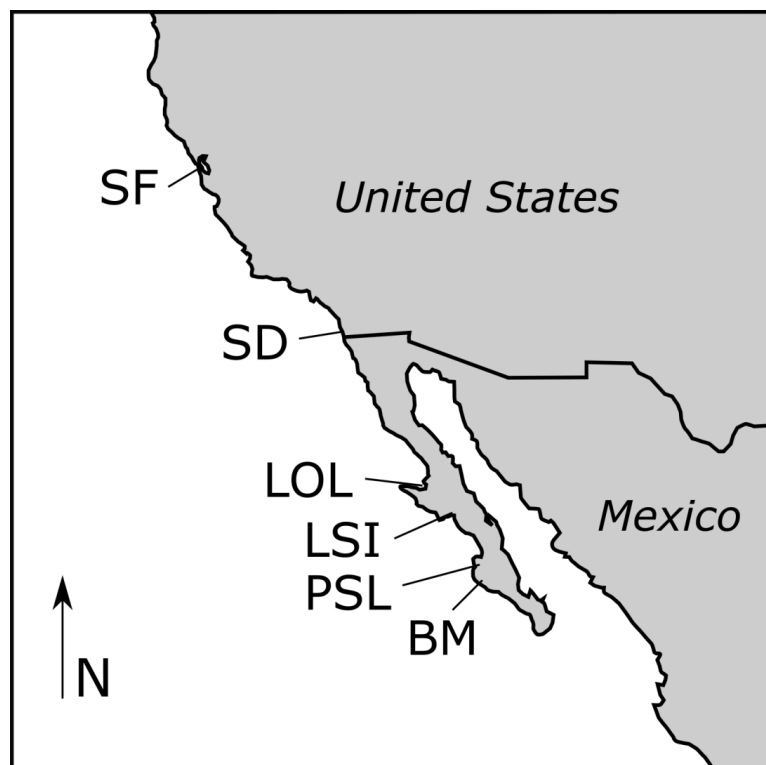
## 2.3. Apatite analysis ( $\delta^{13}\text{C}_{\text{ap}}$ , $\delta^{18}\text{O}_{\text{ap}}$ )

We powdered and homogenized bone apatite (structural carbonate) samples and cleansed both samples of organics with a treatment of 3% hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) for 24 hours. After the removal of organics the samples were rinsed to neutrality using a combination of deionized water and centrifugation. For removal of labile carbonates we treated the samples with 0.1 M buffered acetic acid ( $\text{CH}_3\text{COOH}$ ) for 30 minutes. All samples were vortexed after 15 minutes to ensure chemical saturation. Samples were rinsed to neutrality and then left to air-dry for 24 hours in a fume hood. Finally, approximately 8.0–10.0 mg of homogenized bone apatite powder was placed into exetainer vials and reacted with phosphoric acid at 50°C for 6 hours. This reaction produced  $\text{CO}_2$  for carbon ( $\delta^{13}\text{C}_{\text{ap}}$ ) and oxygen ( $\delta^{18}\text{O}_{\text{ap}}$ ) stable isotope analysis. We analyzed samples at the UNM-CSI on a Thermo Scientific GasBench (Bremen, Germany) coupled to a Delta V isotope ratio mass spectrometer. Apatite precision for within-run analysis is  $<0.5\text{‰}$  for  $\delta^{13}\text{C}_{\text{ap}}$  and  $\delta^{18}\text{O}_{\text{ap}}$  (see 2.2 for definition of delta values). Vienna Pee Dee Belemnite (V-PDB) is the internationally accepted standard used in this study for bone apatite carbon and oxygen isotopes.

## 2.4. Isotopic Comparative Data, Corrections and Analysis

Studies of living sea turtle stable isotopes are typically conducted on skin, blood and plasma tissues non-invasively to understand Cheloniidae isotopic discrimination, foraging ecology, and migration (Arthur et al. 2014; Hatase et al. 2002, 2006; Reich et al. 2007; Seminoff et al. 2006, 2009, 2012b; Shimada et al. 2014; Wallace et al. 2006). The addition of bone collagen and apatite analyses also provides insights into the latter processes, specifically foraging ecology, and for the purposes of this study, helps provide a dataset to compare modern and archaeological samples. Pioneering work on sea turtle bone stable isotopes has demonstrated their value in understanding sea turtle and ocean water physiological processes, species-specific identifications (Biasatti 2002, 2004), isotopic assimilation and paleoclimatic relationships (Coulson et al. 2008), and the effects of bone preparation protocols, ontogenetic shifts, migration, residency duration and tissue-specific isotopic discrimination (Turner Tomaszewicz et al. 2015, 2016, 2017, 2018). We include bone apatite  $\delta^{13}\text{C}_{\text{ap}}$  and  $\delta^{18}\text{O}_{\text{ap}}$  comparisons with modern leatherback, olive ridley and green sea turtles from the Caribbean (Biasatti 2002, 2004). In addition, bone collagen comparisons occur in two forms. First, a direct bone-to-bone  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}$  analysis and second, corrected bone-to-skin  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}$  analysis from eastern Pacific green sea turtles (Figure 2; Lemons et al. 2011; Lewis 2009; Rodríguez-Barón 2010; Turner Tomaszewicz et al. 2015, 2016, 2017, 2018).

As the archaeological samples were from bones that were over 150 years old, there are two corrections required to make our dataset comparable with modern eastern Pacific sea turtles. We apply a  $-0.8\text{‰}$   $\delta^{13}\text{C}_{\text{col\&ap}}$  “Suess effect” correction to the Gold Rush samples to account for changes in atmospheric carbon between the 1850s–today (Francey et al. 1999; Indermuhle et al. 1999; Leuenberger et al. 1992; Sonnerup et al. 1999), and we use the following two



**Figure 2:** Map of the location of eastern Pacific Ocean sea turtle specimens discussed in text. Archaeological: SF = 1850s Gold Rush samples from San Francisco, California. Modern: SD = San Diego Bay, LOL = Laguna Ojo Liebre, LSI = Laguna San Ignacio, PSL = Playa San Lázaro and BM = Bahía Magdalena.

equations to account for sea turtle bone-to-skin isotopic discrimination (from Turner Tomaszewicz et al. 2017):

$$\delta^{13}\text{C}_{\text{bone-to-skin}} = 0.54^* \delta^{13}\text{C}_{\text{bone}} - 8.31$$

$$\delta^{15}\text{N}_{\text{bone-to-skin}} = 0.89^* \delta^{15}\text{N}_{\text{bone}} + 2.55$$

These corrections allow our archaeological bone samples to more directly compare with bone and non-bone tissues from modern sea turtles. All analyses and visualizations were conducted in R (3.4.1) and RStudio (1.0.143) and are available open-access as source code deposited in the UNM digital electronic repository, LoboVault (Conrad et al. 2017).

### 3. Results

#### 3.1. Ancient DNA

Both CA-SFR-186H and CA-SFR-195H specimens were identified as green sea turtles (*Chelonia mydas*) based on COI sequence (Table 1). Independent amplifications for each sample (full and 1:10 concentration for CA-SFR-186H and full and rescue for CA-SFR-195H) confirm these species identifications.

#### 3.2. Gold Rush-Modern Sea Turtle Foraging Ecology

Direct bone-to-bone  $\delta^{13}\text{C}_{\text{col}}$  and  $\delta^{15}\text{N}$  comparisons indicate similarity in Gold Rush-era and modern sea turtle protein sources (Figure 3). There are no significant differences in  $\delta^{13}\text{C}_{\text{col}}$  (Wilcoxon:  $W = 25$ ,  $p = 0.16$ ) or  $\delta^{15}\text{N}$  (Wilcoxon:  $W = 17$ ,  $p = 0.82$ ) between the 1850s samples and modern green sea turtles from Playa San Lázaro, Baja California Sur, Mexico (see Turner Tomaszewicz et al. 2015;

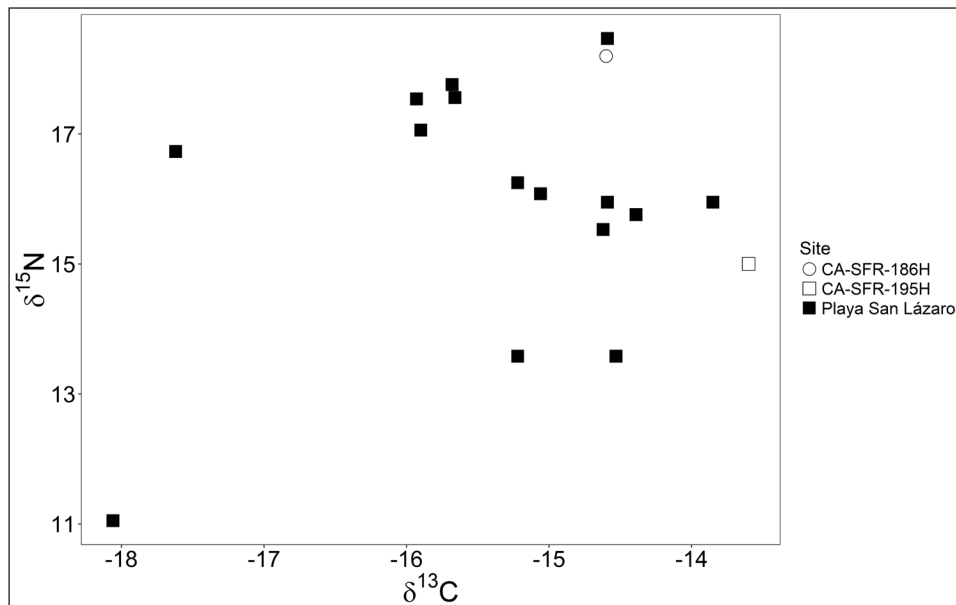
2017; 2018) when examining these isotopic systems. Only one Gold Rush sea turtle is slightly more  $\delta^{13}\text{C}_{\text{col}}$  enriched ( $-14.4\text{‰}$ ) than the mean of Playa San Lázaro turtles ( $-15.4 \pm 1.16\text{‰}$ ). However, after correcting bone collagen-to-skin (Table 2) and comparing with a larger sample of green sea turtles, the pattern of  $\delta^{13}\text{C}_{\text{col}}$  enrichment in our 1850s samples disappears (Figure 4). There are no significant differences in  $\delta^{13}\text{C}$  (Wilcoxon:  $W = 5.5$ ,  $p = 0.77$ ) or  $\delta^{15}\text{N}$  (Wilcoxon:  $W = 11$ ,  $p = 0.33$ ), and the larger sample of green sea turtle skin values encompasses the  $\delta^{13}\text{C}$  variation present between the 1850s ( $-16.6\text{‰}$  and  $-16.1\text{‰}$ ) and modern ( $-15.6 \pm 2.03\text{‰}$ ) samples.

Isotopic spacing ( $\Delta\delta^{13}\text{C}_{\text{apatite-collagen}}$ ) between Suess-corrected bone apatite and collagen is  $+11.8\text{‰}$  and  $+5.6\text{‰}$  for the specimens from CA-SFR-186H and CA-SFR-195H, respectively. The Gold Rush sea turtles have different oxygen ( $+7.5\text{‰}$  and  $-3.5\text{‰}$ ) and hydrogen ( $-3.9\text{‰}$  and  $-76.0\text{‰}$ ) stable isotope values (Table 2). The 1850s sea turtle specimen more enriched in  $\delta^{18}\text{O}_{\text{ap}}$  is also more enriched in  $\delta\text{D}$  (Table 2).

### 4. Discussion

#### 4.1. Species Identification

Both Gold Rush-era archaeological samples were identified as green sea turtles using aDNA analysis. Historical records suggest that green turtles were the species of choice for maritime passengers traveling to San Francisco during the 1850s because of their taste and relative abundance throughout the eastern Pacific (Conrad and Pastron 2014; O'Donnell 1974). However, zooarchaeological analysis was unable to distinguish these specimens as green sea turtles due to morphological similarity between



**Figure 3:** Comparison of bone collagen-to-collagen values for two Gold Rush samples from San Francisco, California and 15 dead-stranded eastern Pacific green sea turtles collected between 2004–2011 along a 45-km stretch of beach at Playa San Lázaro, Baja California Sur, México (see Turner Tomaszewicz et al. 2015).

**Table 2:** Summary of stable isotope results for the Gold Rush-era sea turtle specimens from San Francisco, California. \*Calculated following equations in Turner Tomaszewicz et al. 2017.

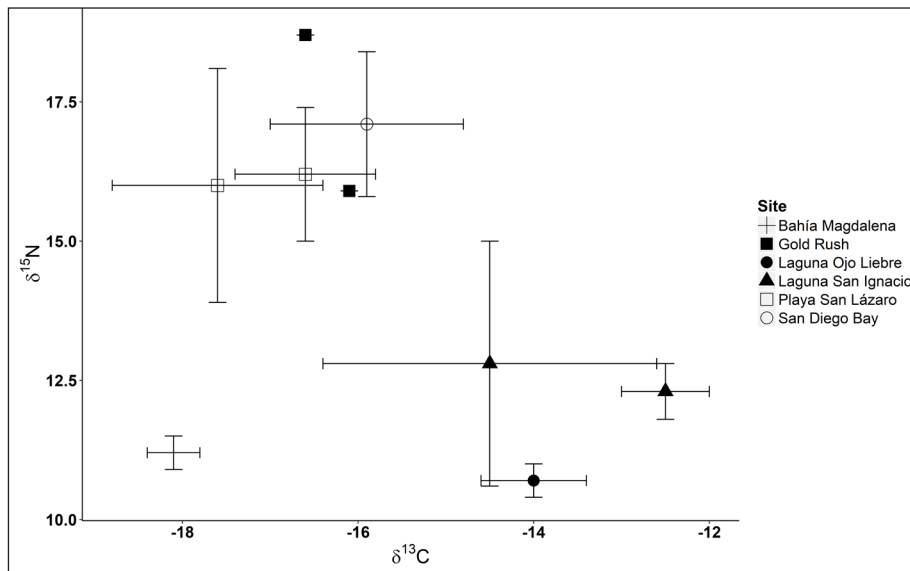
CSI ID	ST01	ST02
Taxon	<i>Chelonia mydas</i>	<i>Chelonia mydas</i>
Site Number	CA-SFR-186H	CA-SFR-195H
$\delta^{13}C_{col}$	-14.6	-13.6
$\delta^{13}C_{colSuess}$	-15.4	-14.4
$\delta^{15}N$	18.2	15.0
$\delta^{13}C_{skin}^*$	-16.6	-16.1
$\delta^{15}N_{skin}^*$	18.7	15.9
$\delta^{13}C_{cap}$	-2.8	-8.0
$\delta^{13}C_{capSuess}$	-3.6	-8.8
$\delta^{18}O_{ap}$	7.5	-3.5
$\delta D$	-3.9	-76.0
%N	15.5	15.2
%C	42.6	43.6
C:N (weight %)	2.7	2.9

the recovered elements and modern sea turtle skeletal comparatives. Only the application of aDNA techniques provided final confirmation.

Difficulty in identifying archaeological sea turtle remains is a common problem for zooarchaeologists. For example, in an analysis of archaeological sea turtle remains from the western coast of Mexico, only 4–5% of specimens were identifiable to species (Smith et al. 2007). Bone apatite studies have suggested that it is possible to identify sea turtles based on differences in carbon isotopes (Biasatti 2002, 2004), but results from our study can neither support nor refute this argument, due to our small sample size.

#### 4.2. Long-term Eastern Pacific Sea Turtle Diet

Modern eastern Pacific green sea turtles are omnivorous and consume sea grasses, algae and invertebrates, but this varies with foraging location (i.e., pelagic versus neritic) and between green sea turtle populations (Arthur et al. 2008; Bjorndal 1997; Hatase et al. 2006; Seminoff et al. 2002, 2006, 2008; Turner Tomaszewicz et al. 2018). These location-specific variables make comparisons between Gold Rush and modern sea turtle specimens difficult, especially given that both 1850s samples lack exact data on their ontogenetic age, date of capture and location of capture. However, our sea turtle stable isotope results



**Figure 4:** Comparison of corrected bone collagen-to-skin values in both Gold Rush sea turtles (see methods; following Turner Tomaszewicz et al. 2017), and bulk skin values for eastern Pacific green sea turtle populations. Mean and standard deviation bars reported, if available. Data from the following sources: Bahía Magdalena, México (Rodríguez-Barón 2010), Gold Rush, San Francisco, CA, USA (this study), Laguna Ojo Liebre, México (Rodríguez-Barón 2010), Laguna San Ignacio, México (Lewis 2009; Rodríguez-Barón 2010), Playa San Lázaro, México (Turner Tomaszewicz et al. 2017, 2018), and San Diego Bay, CA, USA (Lemons et al. 2011). The Playa San Lázaro green turtle samples represent a bulk skin dataset and a bone collagen-to-skin dataset (Turner Tomaszewicz et al. 2018).

indicate overall dietary consistency through time for  $\delta^{13}\text{C}_{\text{col}}$ ,  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}_{\text{ap}}$ . Given that bone collagen primarily reflects dietary protein sources (Ambrose and Norr 1993; Lee-Thorp et al. 1989), these values suggest consumption of a consistent protein source in the eastern Pacific (Amorcho and Reina 2007; Seminoff et al. 2002) and high intrapopulation variation in foraging (Turner Tomaszewicz et al. 2018; Pagès Barceló 2018).

The differences in  $\delta^{18}\text{O}_{\text{ap}}$  and  $\delta\text{D}$  between archaeological and modern samples have other implications. It is possible that differences in  $\delta^{18}\text{O}_{\text{ap}}$  indicate environmental differences in the eastern Pacific in the 1850s. Modern Caribbean sea turtle populations exhibit  $\delta^{18}\text{O}_{\text{ap}}$  values that are only slightly enriched ( $<+3\text{‰}$ ) from  $0\text{‰}$  (Biasatti 2002). Since, by definition, mean ocean water  $\delta^{18}\text{O} = 0\text{‰}$  (Sharp 2017) these sea turtle  $\delta^{18}\text{O}_{\text{ap}}$  values are consistent with physiological fractionation of oxygen isotopes after consuming (water from plants/animals), ingesting (sea water) or breathing water sources (Coulson et al. 2008; Langlois et al. 2003).

However, the large spread in  $\delta^{18}\text{O}_{\text{ap}}$  values in our Gold Rush samples suggests different processes: diagenetic alteration (see Koch et al. 1997; Wang and Cerling 1994) and stress induced fractionation are two possibilities. These Gold Rush-era sea turtles were removed from ocean water and were kept alive aboard ships for weeks, if not months, without food (Conrad and Pastron 2014), during their transport to San Francisco. In plants and birds, stress can cause oxygen, hydrogen (Farris and Strain 1978; Yakir et al. 1990) and nitrogen (Fuller et al. 2005; Hobson et al. 1993) isotopic fractionation. It is unknown how, or if, stress relates to oxygen isotope fractionation in sea turtles (or the potential turnover time for stress-related fractionation

in bone tissues), but we suspect that removal from their aquatic environment for an extended period may have influenced their individual  $\delta^{18}\text{O}_{\text{ap}}$  values. Analysis of sea turtle bone phosphate oxygen isotopes also indicates that bone growth occurs over a consistent and narrow body temperature range ( $\leq \pm 2.0\text{ °C}$ ; Coulson et al. 2008), providing further evidence that our Gold Rush samples fall outside of their expected known normal range of variation.

Finally, stable hydrogen isotopes from bone collagen may also provide an alternative source for understanding foraging ecology for both Gold Rush-era specimens. Hydrogen isotopes assimilate into tissues from diet and water (Hobson et al. 1999) and tend to correlate with nitrogen isotopes from protein derived food sources (Birchall et al. 2005). It is therefore likely that the  $\delta\text{D}$  values in our 1850s sea turtles reflect foraging trophic level (e.g., Pagès Barceló 2018), but their large range and relationship with  $\delta^{18}\text{O}_{\text{ap}}$  (indicating possible stress-induced fractionation) requires further investigation.

## 5. Conclusion

Eastern Pacific sea turtle populations are currently recovering from steep declines during the 20<sup>th</sup> century. The results of this study show little difference in eastern Pacific Ocean environmental and foraging dynamics since the 1850s, but sample size limits more definitive conclusions. On one hand, isotope results may support evidence suggesting that major declines in sea turtle populations derive from anthropogenic over-exploitation, egg collection, by-catch and *not* habitat change, but on the other hand, these results may simply indicate that our 19<sup>th</sup> century samples fall within the range of modern sea turtle isotopic variation due to other unknown reasons.



We emphasize the need for additional historic ecological studies combining modern and ancient samples, and future controlled feeding and dietary studies to understand how carbon, nitrogen, oxygen and hydrogen stable isotopes assimilate, discriminate, and turn over between tissues in sea turtles. With these additional data it will be possible to employ long-term testing of sea turtle remains from archaeological sites worldwide (e.g., Frazier 2003) to understand shifts in environmental and population dynamics throughout the past. These data may help identify habitat and genetic changes that can support current and future conservation and protection programs for these important, endangered species.

#### Acknowledgements

We are extremely grateful for laboratory, analysis and reference support provided by Sherry Nelson, Laura Burkemper, Viorel Atudorei, Mary Praetzelis, Adrian Praetzelis, Sandra Konzak, Jeanette Wyneken, Jens Vindum, Carol Spencer, James Delgado, Caroline Byrne, Salomeh Sheridan and Arianne Avery. Thank you to the organizers of the 2016 Stable Isotopes in Zooarchaeology Working Group Meeting, and especially to Suzie Pilaar Birch, Meghan Burchell and Catherine West. Finally, thank you to two reviewers for their detailed comments and advice.

#### Competing Interests

The authors have no competing interests to declare.

#### References

- Ambrose, SH.** 1990. Preparation and Characterization of Bone and Tooth Collagen for Isotopic Analysis. *Journal of Archaeological Science*, 17: 431–451. DOI: [https://doi.org/10.1016/0305-4403\(90\)90007-R](https://doi.org/10.1016/0305-4403(90)90007-R)
- Ambrose, SH 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: Lambert, JB and Grupe, G (eds.), *Prehistoric Human Bone: Archaeology at the Molecular Level*. Berlin: Springer-Verlag. DOI: [https://doi.org/10.1007/978-3-662-02894-0\\_1](https://doi.org/10.1007/978-3-662-02894-0_1)
- Amorocho, DF and Reina, RD.** 2007. Feeding ecology of the East Pacific green sea turtle *Chelonia mydas agassizii* at Gorgona National Park, Colombia. *Endangered Species Research*, 3: 43–51. DOI: <https://doi.org/10.3354/esr003043>
- Armitage, PL.** 2013. The Deep-Sea Tortugas Shipwreck, Florida: the Animal Bones. *Odyssey Marine Exploration*, 29: 2–19.
- Arthur, KE, Boyle, MC and Limpus, CJ.** 2008. Ontogenetic changes in diet and habitat use in green sea turtle (*Chelonia mydas*) life history. *Marine Ecology Progress Series*, 363: 303–311. DOI: <https://doi.org/10.3354/meps07440>
- Arthur, KE, Kelez, S, Larsen, T, Choy, CA and Popp, BN.** 2014. Tracing the biosynthetic source of essential amino acids in marine turtles using  $\delta^{13}\text{C}$  fingerprints. *Ecology*, 95(5): 1285–1293. DOI: <https://doi.org/10.1890/13-0263.1>
- Bancroft, HH.** 1888. *History of California Vol. VI 1848–1859*. San Francisco: The History Company, Publishers.
- Biasatti, DM.** 2002. *Stable Isotopic Profiles of Sea Turtle Humeri and Epizoic Barnacles: Implications for Ecology and Migration*. MS Thesis, Dallas: Southern Methodist University.
- Biasatti, DM.** 2004. Stable carbon isotopic profiles of sea turtle humeri: implications for ecology and physiology. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 206: 203–216. DOI: <https://doi.org/10.1016/j.palaeo.2004.01.004>
- Birchall, J, O'Connell, TC, Heaton, THE and Hedges, REM.** 2005. Hydrogen isotope ratios in animal body protein reflect trophic level. *Journal of Animal Ecology*, 74: 877–881. DOI: <https://doi.org/10.1111/j.1365-2656.2005.00979.x>
- Bjorndal, KA.** 1997. Foraging ecology and nutrition of sea turtles. In: Lutz, PL and Musick, JA (eds.), *The Biology of sea turtles, Volume 1*. Boca Raton: CRC Press.
- Cabana, G and Rasmussen, JB.** 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences*, 93: 10844–10847. DOI: <https://doi.org/10.1073/pnas.93.20.10844>
- Caldwell, DK.** 1963. The Sea Turtle Fishery of Baja California, Mexico. *California Fish and Game*, 29: 140–151.
- Chavez, FP, Ryan, J, Lluch-Cota, SE and Ñiquen, MC.** 2003. From Anchovies to Sardines and Back: Multi-decadal Change in the Pacific Ocean. *Science*, 299: 217–221.
- Cliffton, K, Cornejo, DO and Felger, RS.** 1982. Sea turtles of the Pacific coast of Mexico. In: Bjorndal, K (ed.), *Biology and Conservation of Sea Turtles*. Washington, D.C.: Smithsonian Institution Press.
- Cole, JJ, Carpenter, SR, Kitchell, J, Pace, ML, Solomon, CT and Weidel, B.** 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proceedings of the National Academy of Sciences*, 108(5): 1975–1980. DOI: <https://doi.org/10.1073/pnas.1012807108>
- Conrad, C, Gobalet, KW, Bruner, K and Pastron, AG.** 2015. Hide, Tallow and Terrapin: Gold Rush-Era Zooarchaeology at Thompson's Cove (CA-SFR-186H), San Francisco, California. *International Journal of Historical Archaeology*, 19: 502–551. DOI: <https://doi.org/10.1007/s10761-015-0297-2>
- Conrad, C, Pagès Barceló, L, Seminoff, JA, Turner Tomaszewicz, CN, Labonte, MJ, Kemp, BM, Jones, EL, Stoyka, M, Bruner, K and Pastron, AG.** 2017. Historic and modern eastern Pacific green sea turtle stable isotopes. [dataset]. University of New Mexico. [http://digitalrepository.unm.edu/anthro\\_data/5](http://digitalrepository.unm.edu/anthro_data/5)
- Conrad, C and Pastron, A.** 2014. Galapagos Tortoises and Sea Turtles in Gold Rush-Era California. *California History*, 91(2): 20–39. DOI: <https://doi.org/10.1525/ch.2014.91.2.20>

- Coulson, A, Kohn, MJ, Shirley, MH, Joyce, WG and Barrick, RE.** 2008. Phosphate-oxygen isotopes from marine turtle bone: Ecologic and paleoclimatic applications. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 264: 78–84. DOI: <https://doi.org/10.1016/j.palaeo.2008.04.008>
- D'Arrigo, R, Cook, ER, Wilson, RJ, Allan, R and Mann, ME.** 2005. On the variability of ENSO over the past six centuries. *Geophysical Research Letters*, 32: L03711. DOI: <https://doi.org/10.1029/2004GL022055>
- Davenport, J.** 1997. Temperature and the Life-History Strategies of Sea Turtles. *Journal of Thermal Biology*, 22(6): 479–488. DOI: [https://doi.org/10.1016/S0306-4565\(97\)00066-1](https://doi.org/10.1016/S0306-4565(97)00066-1)
- Delgado-Trejo, CD and Alvarado-Diaz, JA.** 2012. Current Conservation Status of the Black Sea Turtle in Miohoacán, México. In: Seminoff, JA and Wallace, BP (eds.), *Sea Turtles of the Eastern Pacific*. Tucson: University of Arizona Press.
- DeNiro, MJ and Epstein, S.** 1978. Influence of diet on the distribution of carbon isotopes in animals. *Geochimica et Cosmochimica Acta*, 42(5): 495–506. DOI: [https://doi.org/10.1016/0016-7037\(78\)90199-0](https://doi.org/10.1016/0016-7037(78)90199-0)
- DeNiro, MJ and Epstein, S.** 1981. Influence of diet on the distribution of nitrogen isotopes in animals. *Geochimica et Cosmochimica Acta*, 45(3): 341–351. DOI: [https://doi.org/10.1016/0016-7037\(81\)90244-1](https://doi.org/10.1016/0016-7037(81)90244-1)
- Doucett, RR, Marks, JC, Blinn, DW, Caron, M and Hungate, BA.** 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology*, 88: 1587–1592. DOI: <https://doi.org/10.1890/06-1184>
- Douglas, AV.** 1980. Geophysical Estimates of Sea-Surface Temperatures off Western North America Since 1671. *CalCOFI Reports*, 21: 102–114.
- Early-Capistrán, M, Sáenz-Arroyo, A, Cardoso-Mohedano, J-G, Garibay-Melo, G, Peckham, SH, and Koch, V.** 2017. Reconstructing 290 years of a data-poor fishery through ethnographic and archival research: The East Pacific green turtle (*Chelonia mydas*) in Baja California, Mexico. *Fish and Fisheries*.
- Estep, MF and Dabrowski, H.** 1980. Tracing food webs with stable hydrogen isotopes. *Science*, 209(4464): 1537–1538. DOI: <https://doi.org/10.1126/science.209.4464.1537>
- Etnoyer, P, Canny, D, Mate, BR, Morgan, LE, Ortega-Ortiz, JG and Nichols, WJ.** 2006. Sea-surface temperature gradients across blue whale and sea turtle foraging trajectories off the Baja California Peninsula, Mexico. *Deep-Sea Research II*, 53: 340–358. DOI: <https://doi.org/10.1016/j.dsr2.2006.01.010>
- Farris, F and Strain, BR.** 1978. The Effects of Water-Stress on Leaf H<sub>2</sub><sup>18</sup>O Enrichment. *Radiation and Environmental Biophysics*, 15: 167–202. DOI: <https://doi.org/10.1007/BF01323264>
- Fish, MR, Cote, IM, Gill, JA, Jones, AP, Renshoff, S and Watkinson, AR.** 2005. Predicting the Impact of Sea-Level Rise on Caribbean Sea Turtle Nesting Habitat. *Conservation Biology*, 19(2): 482–491. DOI: <https://doi.org/10.1111/j.1523-1739.2005.00146.x>
- Francey, RJ, Allison, CE, Etheridge, DM, Trudinger, CM, Enting, IG, Leuenberger, M, Langenfelds, RL, Michel, E and Steele, LP.** 1999. A 1000-year high precision record of  $\delta^{13}\text{C}$  in atmospheric CO<sub>2</sub>. *Tellus*, 51B: 170–193. DOI: <https://doi.org/10.3402/tellusb.v51i2.16269>
- Frazier, J.** 2003. Prehistoric and Ancient Historic Interactions between Humans and Marine Turtles. In: Lutz, PL, Musick, JA and Wyneken, J (eds.), *The Biology of Sea Turtles: Volume II*. Boca Raton: CRC Press.
- Frazier, J.** 2005. Marine turtles – the ultimate tool kit: A review of worked bones of marine turtles. In: Luik, H, Choyke, AM, Batey, CE and Lõugas, L (eds.), *From Hooves to Horns, from Mollusc to Mammoth: Manufacture and Use of Bone Artefacts from Prehistoric Times to the Present*. Tallinn: Tallinn Book Printers Ltd.
- Fuentes, MMPB, Limpus, CJ and Hamann, M.** 2010a. Past, current and future thermal profiles of green turtle nesting grounds: Implications from climate change. *Journal of Experimental Marine Biology and Ecology*, 383(1): 56–54. DOI: <https://doi.org/10.1016/j.jembe.2009.11.003>
- Fuentes, MMPB, Limpus, CJ, Hamann, M and Dawson, J.** 2010b. Potential impacts of projected sea-level rise on sea turtle rookeries. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 20(2): 132–139. DOI: <https://doi.org/10.1002/aqc.1088>
- Fuller, BT, Fuller, JL, Sage, NE, Harris, DA, O'Connell, TC and Hedges, REM.** 2005. Nitrogen balance and  $\delta^{15}\text{N}$ : why you're not what you eat during nutritional stress. *Rapid Communications in Mass Spectrometry*, 19(18): 2497–2506. DOI: <https://doi.org/10.1002/rcm.2090>
- Hatase, H, Sato, K, Yamaguchi, M, Takahashi, K, and Tsukamoto, K.** 2006. Individual variation in feeding habitat use by adult female green sea turtles (*Chelonia mydas*): are they obligately neritic herbivores?
- Hatase, H, Takai, N, Matsuzawa, Y, Sakamoto, W, Omuta, K, Goto, K, Arai, N and Fujiwara, T.** 2002. Size-related differences in feeding habitat use of adult female loggerhead turtles *Caretta caretta* around Japan determined by stable isotope analyses and satellite telemetry. *Marine Ecology Progress Series*, 233: 273–281. DOI: <https://doi.org/10.3354/meps233273>
- Hawkes, LA, Broerick, AC, Godfrey, MH and Godley, BJ.** 2009. Climate change and marine turtles. *Endangered Species Research*, 7: 137–154.
- Hernández-Carmona, G, Riosmena-Rodriguez, R, Serviere-Zaragoza, E and Ponce-Diaz, G.** 2011. Effect of nutrient availability on understory algae during El Niño Southern Oscillation (ENSO) conditions in Central Pacific Baja California. *Journal of Applied Phycology*, 23: 635–642. DOI: <https://doi.org/10.1007/s10811-011-9656-5>
- Hobson, KA, Alisauskas, RT and Clark, RG.** 1993. Stable-Nitrogen Isotope Enrichment in Avian Tissues due to Fasting and Nutritional Stress: Implications for Isotopic Analyses of Diet. *The Condor*, 95: 388–394. DOI: <https://doi.org/10.2307/1369361>

- Hobson, KA, Atwell, L and Wassenaar, LI.** 1999. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proceedings of the National Academy of Sciences*, 96: 8003–8006. DOI: <https://doi.org/10.1073/pnas.96.14.8003>
- Indermuhle, A, Stocker, TF, Joos, F, Flscher, H, Smith, HJ, Wahlen, M, Deck, B, Mastrolanni, D, Tschumi, J, Blunier, T, Meyer, R and Stauffer, B.** 1999. Holocene carbon-cycle dynamics based on CO<sub>2</sub> trapped in ice at Taylor Dome, Antarctica. *Nature*, 398: 121–126. DOI: <https://doi.org/10.1038/18158>
- Jensen, MP, Allen, CD, Eguchi, T, Bell, IP, LaCasella, EL, Hilton, WA, Hoff, CAM and Dutton, PH.** 2018. Environmental Warming and Feminization of one of the Largest Sea Turtle Populations in the World. *Current Biology*, 28: 154–159. DOI: <https://doi.org/10.1016/j.cub.2017.11.057>
- Johnson, BM and Kemp, BM.** 2016. Rescue PCR: Reagent-rich PCR recipe improves amplification of degraded DNA extracts. *Journal of Archaeological Science: Reports*, 11: 683–694. DOI: <https://doi.org/10.1016/j.jasrep.2017.01.006>
- Jones, TT and Seminoff, JA.** 2013. Feeding Biology: Advances from Field-Based Observations, Physiological Studies, and Molecular Techniques. In: Musick, J, Wyneken, J and Lohman, K (eds.), *Biology of the Sea Turtles, Volume 3*. Boca Raton: CRC Press. DOI: <https://doi.org/10.1201/b13895-10>
- Kahru, M and Mitchell, BG.** 2000. Influence of the 1997-98 El Niño on the surface chlorophyll in the California Current. *Geophysical Research Letters*, 27(18): 2937–2940. DOI: <https://doi.org/10.1029/2000GL011486>
- Kemp, BM, Monroe, C, Judd, KG, Reams, E and Grier, C.** 2014. Evaluation of methods that subdue the effects of polymerase chain reaction inhibitors in the study of ancient and degraded DNA. *Journal of Archaeological Science*, 42: 373–380. DOI: <https://doi.org/10.1016/j.jas.2013.11.023>
- Kennedy, JJ, Rayner, NA, Smith, RO, Parker, DE and Saunby, M.** 2011. Reassessing biases and other uncertainties in sea surface temperature observations measured in situ since 1850: 2. Biases and homogenization. *Journal of Geophysical Research*, 116: D14104. DOI: <https://doi.org/10.1029/2010JD015220>
- Kharlamenko, VI, Kiyashko, SI, Imbs, AB and Vyshkvartzev, DI.** 2001. Identification of food sources of invertebrates from the seagrass *Zostera marina* community using carbon and sulfur stable isotope ratio and fatty acid analyses. *Marine Ecology Progress Series*, 220: 103–117. DOI: <https://doi.org/10.3354/meps220103>
- Koch, M, Bowes, G, Ross, C and Zhang, X-H.** 2013. Climate change and ocean acidification effects on seagrasses and marine macroalgae. *Global Change Biology*, 19: 103–132. DOI: <https://doi.org/10.1111/j.1365-2486.2012.02791.x>
- Koch, PL, Tuross, N and Fogel, ML.** 1997. The Effects of Sample Treatment and Diagenesis on the Isotopic Integrity of Carbonate in Biogenic Hydroxylapatite. *Journal of Archaeological Science*, 24: 417–429. DOI: <https://doi.org/10.1006/jasc.1996.0126>
- Langlois, C, Simon, L and Lécuyer, CH.** 2003. Box-modeling of bone and tooth phosphate oxygen isotope compositions as a function of environmental and physiological parameters. *Isotopes in Environmental and Health Studies*, 39: 259–272. DOI: <https://doi.org/10.1080/10256010310001621146>
- Lee-Thorp, JA, Sealy, JC and van der Merwe, NJ.** 1989. Stable Carbon Isotope Ratio Differences Between Bone Collagen and Bone Apatite, and their Relationship to Diet. *Journal of Archaeological Science*, 16: 585–599. DOI: [https://doi.org/10.1016/0305-4403\(89\)90024-1](https://doi.org/10.1016/0305-4403(89)90024-1)
- Lemons, G, Lewison, R, Komoroske, L, Gaos, A, Lai, C-T, Dutton, P, Eguchi, T, LeRoux, R and Seminoff, JA.** 2011. Trophic ecology of green sea turtles in a highly urbanized bay: Insights from stable isotopes and mixing models. *Journal of Experimental Marine Biology and Ecology*, 405: 25–32. DOI: <https://doi.org/10.1016/j.jembe.2011.05.012>
- Leuenberger, M, Siegenthaler, U and Langway, CC.** 1992. Carbon isotope composition of atmospheric CO<sub>2</sub> during the last ice age from an Antarctic ice core. *Nature*, 357: 488–490. DOI: <https://doi.org/10.1038/357488a0>
- Lewis, AJ.** 2009. The feeding ecology of black sea turtles (*Chelonia mydas agassizii*) in Laguna San Ignacio Baja California Sur, Mexico. Master thesis, Bangor University, Australia.
- Limpus, C and Nicholls, N.** 2000. ENSO Regulation of Indo-Pacific Green Turtle Populations. In: Hammer, GL, Nicholls, N and Mitchell, C (eds.), *Applications of Seasonal Climate Forecasting in Agricultural and Natural Ecosystems*. Houten: Springer. DOI: [https://doi.org/10.1007/978-94-015-9351-9\\_24](https://doi.org/10.1007/978-94-015-9351-9_24)
- López-Mendilaharsu, M, Gardner, SC, Seminoff, JA 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. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 15(3): 259–269. DOI: <https://doi.org/10.1002/aqc.676>
- Maberly, SC, Raven, JA and Johnston, AM.** 1992. Discrimination between <sup>12</sup>C and <sup>13</sup>C by marine plants. *Oecologia*, 91: 481–492. DOI: <https://doi.org/10.1007/BF00650320>
- McConnaughey, T and McRoy, CP.** 1979. <sup>13</sup>C label identifies eelgrass (*Zostera marina*) carbon in an Alaskan estuarine food web. *Marine Biology*, 53: 262–269. DOI: <https://doi.org/10.1007/BF00952435>
- Minagawa, M and Wada, E.** 1984. Stepwise enrichment of <sup>15</sup>N along food chains: Further evidence and the relation between δ<sup>15</sup>N and animal age. *Geochimica et Cosmochimica Acta*, 48(5): 1135–1140. DOI: [https://doi.org/10.1016/0016-7037\(84\)90204-7](https://doi.org/10.1016/0016-7037(84)90204-7)
- Nichols, WJ.** 2003. *Biology and Conservation of Sea Turtles in Baja California, Mexico*. Ph.D. Dissertation, Tucson: The University of Arizona.

- O'Donnell, DJ.** 1974. Green Turtle Fishery in Baja California Waters: History and Prospect. M.A. Thesis, Department of Geography, California State University, Northridge.
- Pagès Barceló, L.** 2018. Using  $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ , and  $\delta^2\text{H}$  to Better Understand the Ecology of Green Sea Turtles. M.Sc. Thesis, Department of Biology, University of New Mexico.
- Pastron, AG and Bruner, K.** 2014. *Final Archaeological Resources Report for the 717 Battery Street Project, City and County of San Francisco, California*. Available from the Northwest Information Center, Sonoma, CA.
- Plotkin, PT, Briseño-Dueñas, R and Abreu-Grobois, FA.** 2012. Interpreting Signs of Olive Ridley Recovery in the Eastern Pacific. In: Seminoff, JA and Wallace, BP (eds.), *Sea Turtles of the Eastern Pacific*. Tucson: University of Arizona Press.
- Post, DM.** 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology*, 83: 703–718. DOI: [https://doi.org/10.1890/0012-9658\(2002\)083\[0703:USITET\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2)
- Praetzelis, M.** 2017. *Final Archaeological Resources Report and Data Recovery Report for 110 the Embarcadero, San Francisco, California*. Available from the Northwest Information Center, Sonoma, CA.
- Rayner, NA, Parker, DE, Horton, EB, Folland, CK, Alexander, LV, Rowell, DP, Kent, EC and Kaplan, A.** 2003. Global analyses of sea surface temperature, sea ice, and night marine air temperature since the late nineteenth century. *Journal of Geophysical Research*, 108: D14. DOI: <https://doi.org/10.1029/2002JD002670>
- Reich, KJ, Bjorndal, KA and Bolten, AB.** 2007. The 'lost years' of green turtles: using stable isotopes to study cryptic lifestages. *Biology Letters*, 3: 712–714. DOI: <https://doi.org/10.1098/rsbl.2007.0394>
- Riosmena-Rodríguez, R, Muñiz-Salazar, R, López-Calderón, J, Torre-Cosío, J, Meling, A, Talbo, SL, Sage, GK, Ward, DH and Cabello-Pasini, A.** 2013. Conservation status of *Zostera marina* populations at Mexican Pacific. In: Daniels, JA (ed.), *Advances in Environmental Research Volume 27*. Hauppauge: Nova Science Publishers.
- Rodríguez-Barón, JM.** 2010. *Afinidad Trófica a Zonas de Alimentación de la Tortuga Verde (Chelonia mydas) en la Costa Occidental de Baja California Sur, México*. M.S. Thesis, México City: Instituto Politécnico Nacional.
- Saba, VS.** 2012. Sea Turtles in the Tropical High Seas: Climate Variability, Oceanography, and Ecosystem Responses. In: Seminoff, JA and Wallace, BP (eds.), *Sea Turtles of the Eastern Pacific*. Tucson: University of Arizona Press.
- Saba, VS, Santidrián-Tomillo, P, Reina, RD, Spotila, JR, Musick, JA, Evans, DA and Paladinos, FV.** 2007. The effect of El Niño Southern Oscillation on the reproductive frequency of eastern Pacific leatherback turtles. *Journal of Applied Ecology*, 44: 395–404. DOI: <https://doi.org/10.1111/j.1365-2664.2007.01276.x>
- Sauer, PE, Schimmelmann, A, Session, AL and Topalov, K.** 2009. Simplified batch equilibration for D/H determination of non-exchangeable hydrogen in solid organic material. *Rapid Communications in Mass Spectrometry*, 23: 949–956. DOI: <https://doi.org/10.1002/rcm.3954>
- Seminoff, JA, Alfaro-Shigueto, J, Amorocho, D, Arauz, R, Gallegos, AB, Chaverri, DC, Gaos, AR, Kelez, S, Mangel, JC, Urteaga and Wallace, BP.** 2012a. Biology and Conservation of Sea Turtles in the Eastern Pacific Ocean: A General Overview. In: Seminoff, JA and Wallace, BP (eds.), *Sea Turtles of the Eastern Pacific*. Tucson: University of Arizona Press.
- Seminoff, JA, Benson, SR, Arthur, KE, Eguchi, T, Dutton, PH, Tapilatu, RF and Popp, BN.** 2012b. Stable Isotope Tracking of Endangered Sea Turtles: Validation with Satellite Telemetry and  $\delta^{15}\text{N}$  Analysis of Amino Acids. *PLoS ONE*, 7(5): e37403. DOI: <https://doi.org/10.1371/journal.pone.0037403>
- Seminoff, JA, Jones, TT, Eguchi, T, Hastings, M and Jones, DR.** 2009. Stable carbon and nitrogen isotope discrimination in soft tissues of the leatherback turtle (*Dermochelys coriacea*): Insights for trophic studies of marine turtles. *Journal of Experimental Marine Biology and Ecology*, 381: 33–41. DOI: <https://doi.org/10.1016/j.jembe.2009.08.018>
- Seminoff, JA, Jones, TT, Eguchi, T, Jones, DR and Dutton, PH.** 2006. Stable isotope discrimination ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) between soft tissues of the green sea turtle *Chelonia mydas* and its diet. *Marine Ecology Progress Series*, 308: 271–278. DOI: <https://doi.org/10.3354/meps308271>
- Seminoff, JA, Resendiz, A and Nichols, WJ.** 2002. Diet of East Pacific Green Turtles (*Chelonia mydas*) in the Central Gulf of California, México. *Journal of Herpetology*, 36(3): 447–453. DOI: [https://doi.org/10.1670/0022-1511\(2002\)036\[0447:DOEPTG\]2.0.CO;2](https://doi.org/10.1670/0022-1511(2002)036[0447:DOEPTG]2.0.CO;2)
- Seminoff, JA, Zárata, P, Coyne, M, Foley, DG, Parker, D, Lyon, BN and Dutton, PH.** 2008. Post-nesting migrations of Galápagos green turtles *Chelonia mydas* in relation to oceanographic conditions: integrating satellite telemetry with remotely sensed ocean data. *Endangered Species Research*, 4: 57–72. DOI: <https://doi.org/10.3354/esr00066>
- Sharp, Z.** 2017. *Principles of Stable Isotope Geochemistry*, 2<sup>nd</sup> Edition. DOI: <https://doi.org/10.5072/FK2GB24S9F>
- Sharp, ZD, Atudorei, V and Durakiewicz, T.** 2001. A rapid method for determination of hydrogen and oxygen isotope ratios from water and solid hydrous substances. *Chemical Geology*, 178: 197–210. DOI: [https://doi.org/10.1016/S0009-2541\(01\)00262-5](https://doi.org/10.1016/S0009-2541(01)00262-5)
- Shimada, T, Aoki, S, Kameda, K, Hazel, J, Reich, K and Kamezaki, N.** 2014. Site fidelity, ontogenetic shift and diet composition of green turtles *Chelonia mydas* in Japan inferred from stable isotope analysis. *Endangered Species Research*, 25: 151–164. DOI: <https://doi.org/10.3354/esr00616>

- Smith, CB, Kennett, DJ, Wake, TA and Voorhies, B.** 2007. Prehistoric Sea Turtle Hunting of the Pacific Coast of Mexico. *The Journal of Island and Coastal Archaeology*, 2(2): 231–235. DOI: <https://doi.org/10.1080/15564890701620338>
- Sonnerup, RE, Quay, PD, McNichol, AP, Bullister, JL, Westby, TA and Anderson, HL.** 1999. Reconstructing the oceanic  $^{13}\text{C}$  Suess effect. *Global Biogeochemical Cycles*, 13(4): 857–872. DOI: <https://doi.org/10.1029/1999GB900027>
- Soulé, F, Gihon, JH and Nisbit, J.** 1854. *The Annals of San Francisco*. New York: D. Appleton and Company.
- Turk, D, McPhaden, MJ, Busalacchi, AJ and Lewis, MR.** 2001. Remotely Sensed Biological Production in the Equatorial Pacific. *Science*, 293: 471–474. DOI: <https://doi.org/10.1126/science.1056449>
- Turner Tomaszewicz, CN, Seminoff, JA and Kurle, CM.** 2017. Stable isotope discrimination factors and between-tissue isotope comparisons for bone and skin from captive and wild green sea turtles (*Chelonia mydas*). *Rapid Communications in Mass Spectrometry*, 31: 1903–1914. DOI: <https://doi.org/10.1002/rcm.7974>
- Turner Tomaszewicz, CN, Seminoff, JA, Peckham, H, Avens, L, Goshe, L, Rodriguez-Baron, JM and Kurle, CM.** 2018. Expanding the coastal forager paradigm: Long-term pelagic habitat use by green turtles (*Chelonia mydas*) in the eastern Pacific Ocean. *Marine Ecology Progress Series*, 587: 217–234. DOI: <https://doi.org/10.3354/meps12372>
- Turner Tomaszewicz, CN, Seminoff, JA, Peckham, SH, Avens, L and Kurle, CM.** 2016. Intrapopulation variability in the timing of ontogenetic habitat shifts in sea turtles revealed using  $\delta^{15}\text{N}$  values from bone growth rings. *Journal of Animal Ecology*, 86(3): 694–704. DOI: <https://doi.org/10.1111/1365-2656.12618>
- Turner Tomaszewicz, CN, Seminoff, JA, Ramirez, MD and Kurle, CM.** 2015. Effects of demineralization on stable isotope analysis of bone samples. *Rapid Communications in Mass Spectrometry*, 29: 1879–1888. DOI: <https://doi.org/10.1002/rcm.7295>
- Wallace, BP, Seminoff, JA, Kilham, SS, Spotila, JR and Dutton, PH.** 2006. Leatherback turtles as oceanographic indicators: stable isotope analyses reveal a trophic dichotomy between ocean basins. *Marine Biology*, 149: 953–960. DOI: <https://doi.org/10.1007/s00227-006-0247-y>
- Wang, Y and Cerling, TE.** 1994. A model of fossil tooth and bone diagenesis: implications for paleodiet reconstruction from stable isotopes. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 107: 281–289. DOI: [https://doi.org/10.1016/0031-0182\(94\)90100-7](https://doi.org/10.1016/0031-0182(94)90100-7)
- Wyneken, J.** 2001. *The Anatomy of Sea Turtles*. NOAA Technical Memorandum NMFS-SEFSC-470 Miami: U.S. Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service.
- Yakir, D, DeNiro, MJ and Ephrath, JE.** 1990. Effects of water stress on oxygen, hydrogen and carbon isotope ratios in two species of cotton plants. *Plant, Cell and Environment*, 13: 949–955. DOI: <https://doi.org/10.1111/j.1365-3040.1990.tb01985.x>

**How to cite this article:** Conrad, C, Barceló, LP, Seminoff, JA, Tomaszewicz, CT, Labonte, M, Kemp, BM, Jones, EL, Stoyka, M, Bruner, K and Pastron, A. 2018. Ancient DNA Analysis and Stable Isotope Ecology of Sea Turtles (Cheloniidae) from the Gold Rush-era (1850s) Eastern Pacific Ocean. *Open Quaternary*, 4: 3, pp. 1–13. DOI: <https://doi.org/10.5334/oq.41>

**Submitted:** 19 December 2017

**Accepted:** 01 May 2018

**Published:** 30 May 2018

**Copyright:** © 2018 The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International License (CC-BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. See <http://creativecommons.org/licenses/by/4.0/>.

**]u[** *Open Quaternary* is a peer-reviewed open access journal published by Ubiquity Press.

**OPEN ACCESS** 