Calipash and calipee

de Kock, Willemien

DOI:
10.33612/diss.691517453

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
https://doi.org/10.33612/diss.691517453
Calipash and calipee: population dynamics and foraging ecology of archaeological sea turtles

PhD thesis

to obtain the degree of PhD at the
University of Groningen
on the authority of the
Rector Magnificus Prof. C. Wijmenga
and in accordance with
the decision by the College of Deans.

This thesis will be defended in public on

Thursday 13 July 2023 at 14.30 hours

by

Willemien de Kock

born on 1 October 1991
in Den Helder
Supervisors
Prof. D.C.M. Raemaekers
Prof. P.J. Palsboll

Co-supervisor
Dr. C. Çakırlar

Assessment Committee
Prof. S. Ikram
Prof. H. Huisman
Prof. B. Wertheim
Abstract

Sea turtles (superfamily Chelonioida) inhabit tropical and subtropical waters throughout the world. Due to their often shallow foraging locations, and females leaving the water to lay their eggs on beaches, they have been often-exploited marine vertebrates in many geographic areas throughout history. In this thesis we explore the bioarchaeology of some of the oldest known exploited sea turtles from the Levant, dating to the Middle Holocene. However, we also explore more recent exploitation by analysing sea turtle bones found during archaeological excavations in the Netherlands. Throughout the thesis a historical ecology approach is used to better understand factors such as foraging ecology, genetic connectivity, archaeological bone preservation, provenance and trade. By investigating archaeological sea turtle bones, light is shed on pre-industrial sea turtle ecology and the nature of exploitation by humans.

Of the seven sea turtle species currently swimming in the world’s oceans, only two breed and nest in the Mediterranean Sea. These are the green turtle (*Chelonia mydas*) and the loggerhead turtle (*Caretta caretta*), both endangered. This research focuses on green turtles, which nest solely on the Levantine coast. A current knowledge gap is baseline data indicating past connectivity and foraging ecology of these aquatic reptiles. This PhD dissertation sets out to understand more about ancient populations of Mediterranean sea turtles, and the modes and effects of early exploitation by humans. Nowadays, the Mediterranean green turtle population is very small. Nesting predominantly occurs in Turkey and Cyprus (with minor nesting activity throughout the Levant), and 80 percent occurs on just 5 beaches. Historical hunting of sea turtles had catastrophic effects on populations around the world, the famous example is the post-Columbian era plunder of the Caribbean to supply the British aristocracy with green turtle soup during Victorian times. But the United Kingdom was not the sole large-scale exploiter of sea turtle stocks, as the Netherlands too were extracting from Caribbean seas in colonial times. While the intensive hunting of sea turtles in the eastern Mediterranean pre-dates this massive nineteenth century exploitation by at least 4000 years, we don’t know the biological characteristics of sea turtle populations available to ancient Levantines.

We have access to three separate coastal levantine sites that have yielded significant numbers of sea turtle bones. Tell Fadous-Kfarabida in Lebanon has turtle remains from the Early Bronze Age (2700-2000 BC) and Tell el-Burak has turtles from 2000 BC onward, the Middle Bronze Age, Iron Age, and Medieval occupations. The Turkish archaeological site Kinet Höyük in the Gulf of İskenderun has relatively the most sea turtle bones found in the eastern-Mediterranean, these date from the Middle and Late Iron Age (800 BC - 550 BC). This last site is adjacent to an important modern green turtle nesting site. It is difficult to quantify the intensity of sea turtle exploitation in ancient times, but by combining marine biology and zooarchaeology with some modern tools we hope to gain a better understanding. Also analysed are two more recent archaeological sea turtle bones, found in the Netherlands. Sea turtles are very rare finds in Dutch archaeology, however their presence does tell a story about historic sea turtle exploitation and export. By analysing two ‘stray’ finds of sea turtle carapace from rescue excavations using ZooMS and stable isotope analyses, we were able to explore this topic further.
Using modern DNA, the further back in time we look, the more difficult it becomes to identify bottleneck events such as periods of intense hunting. Ancient DNA (aDNA) is the genetic material that remains in ancient specimens, such as bones. In this PhD the aim is to extract this (often highly degraded) DNA in order to gain a more precise understanding of eastern-Mediterranean sea turtle populations from as early as 2500 BC. The success particularly depends on DNA preservation in the ancient sea turtle bones, something that is generally low in material from the eastern Mediterranean. If species identification is the goal, palaeoproteomics (specifically the ZooMS technique) can provide a low cost and effective solution (due to the longer preservation time of bone collagen). In order to better understand past environments, it is also useful to compare past and present diets. To reconstruct the trophic level of an individual animal, researchers can utilise stable isotope analysis. We are what we eat, and animals assimilate atoms, including stable isotopes into our tissues from our diet, so also into our bones. The position in the food web can be estimated from nitrogen stable isotope ratios (\( \delta^{15}N \)). For herbivorous species such as the green sea turtle, carbon stable isotope ratios (\( \delta^{13}C \)) can be used to reconstruct which marine vegetation they fed on, and sometimes the area where they were feeding.

By establishing the species (ZooMS) and stable isotope ratios (\( \delta^{13}C \), \( \delta^{15}N \) and \( \delta^{34}S \)) of archaeological sea turtle remains from the Levant, in Chapter 2 we were able to statistically assign ancient green turtles to modern foraging grounds on the North African coast. In Chapter 3, the same biomolecular archaeological tools were used to explore trade of sea turtles by the Netherlands. The possibilities of aDNA analysis for sea turtle archaeology are explored in Chapter 4, with particular emphasis on DNA preservation in archaeological samples. This research hopes to build stronger bridges between marine biology and zooarchaeology to gain a better understanding of ancient and historical sea turtle exploitation, and the ecology of sea turtles in the past. The results generated as part of this thesis have relevance to current debates on nature preservation, and demonstrate opportunities for the past to inform the present.
# Table of Contents

List of Figures ................................................................................................................................. 5
List of Tables .................................................................................................................................. 12
Dedication ....................................................................................................................................... 15
Acknowledgements ......................................................................................................................... 16
Author’s Declaration ....................................................................................................................... 18
Introduction .................................................................................................................................... 19

Chapter 1: Potential applications of biomolecular archaeology to the echistory of sea turtles and groupers in Levant coastal antiquity .......................................................... 21

Chapter 2: Threatened North African seagrass meadows have supported green turtle populations for millennia ............................................................................................................ 28

Chapter 3: Sea turtle shells in the Netherlands: Zooarchaeology by mass spectrometry and stable isotope analysis identify species and provenance ........................................... 98

Chapter 4: Ancient and modern genomics of Mediterranean and Atlantic green turtles (Chelonia mydas), and the challenges of aDNA analysis ....................................................... 121

Chapter 5: Conclusions .................................................................................................................... 144
  5.1. Applications of molecular bioarchaeology ............................................................................. 144
  5.2. Species identification and ancient foraging ground use ....................................................... 145
  5.3. Sea turtle export: Archaeological sea turtles from the Netherlands .................................... 146
  5.4. Ancient DNA and sea turtles ............................................................................................... 147
  5.5. Closing Remarks and Prospects ......................................................................................... 148

References ...................................................................................................................................... 150
List of Figures

Chapter 1

Figure 1. Map depicting the northern coastal levant and three archaeological sites (black squares) with grouper and sea turtle assemblages; Kinet Höyük, Tell el-Burak, and Tell Fadous. Yellow triangles indicate current green turtle (*C. mydas*) breeding sites, green diamonds indicate current known nesting locations with at least 100 clutches per year, and pink diamonds indicate two sites in Lebanon with minor (4–14 clutches per year) nesting activity (data adapted from (Casale et al., 2018)). Blue circles indicate current fishing ports on the Turkish Levant that land a considerable 'Best Day's Catch' for *E. aeneus* (White Grouper) [at least 40 kg, maximum length at least 80 cm] and/or *E. marginatus* (Dusky Grouper) [at least 10 kg, maximum length at least 60 cm], data adapted from (Mavruk et al., 2018). .......................................................... 23

Figure 2. Infographic depicting the steps associated with analysing zooarchaeological remains, analysis tools and possible insights they can provide.......................................................... 24

Chapter 2

Figure 1. Map of the Eastern Mediterranean. Three archaeological sites with sea turtle remains are marked, with an indication of the assemblage nature (highly fragmented at the southern Levantine sites) and bone elements analysed at each site. Modern Mediterranean green turtle post-nesting migration routes back to foraging grounds (white and red stripes) are taken from Stokes et al. (2015) as well as the nesting distributions (red circles), the largest circles indicate 100 nests or more per year, smallest circles indicate less than 50 nests. Gulf of Bomba (*n*=7, blue triangles), Egyptian (*n*=8, purple triangles), Turkey/Cyprus (*n*=3, pink triangles) and West Libyan (*n*=7, green triangles) green turtle foraging grounds are presented as the final location of satellite tracked modern turtles (*J*). Images of the two sea turtle species which nest and breed in the Mediterranean (*C. mydas* and *Caretta caretta*) are shown. Figure created with assistance from S.E. Boersma.......................................................... 29

Figure 2. (A) Published (Harvey et al. 2019) and novel ZooMS biomarkers distinguishing *C. mydas* and *C. caretta*. The mass to charge ratio (m/z)
displayed for each biomarker is the sum of the mass of the peptide amino acid sequence and any mass shifts due to potential posttranslational modifications, namely hydroxyproline (+16) and deamidation (+1). MALDI spectra for each novel ZooMS biomarker (B-H) denoting the ion coverage from nanoLC-MS/MS next to the spectra for each species (C. mydas, in green; C. caretta in orange)…………………………………………………………… 31

Figure 3. (A) Comparisons between species identification methods for sea turtles from turtle humeri (n=27) and our total bone assemblage (n=124). The dashed black line indicates the number of remains identified by the published COL1α1 586–618 (Harvey et al. 2019) peptide, the red line indicates the total experiment number for each facet. Comparative osteometry/osteology was not possible on the total assemblage due to lack of existing morphological identification criteria and the shattered nature of the remains. Donut charts of sea turtle species composition at three Levantine archaeological sites are shown in (B) for confident ZooMS identification with our 7 novel biomarkers, and in (C) for less confident ID (ie. visible peaks that did not make the mMass peak picking threshold)........................................................................................................................................... 32

Figure 4. Mediterranean sea turtle stable isotope values. Ancient SIA bagplots (A-C) are of archaeological Levantine sea turtle bones (n=45). Species identifications are from palaeoproteomics analysis. We also present scatterplots of modern data used in Discriminant Analysis (D-F; ancient C. mydas remains (grey points, indicated by ellipses) overlaid with stable isotope values in samples from of (Suess-corrected(13)) satellite tracked modern Levantine C. mydas (diamonds). The foraging areas of the modern turtles are; Bomba (n=7), Egypt (n=8), Turkey/Cyprus (n=3), and West Libya (n=5). All δ34S data are depicted in plots A-C, and a subset of δ34S data, subjected to very stringent (Supplementary Information) filtering in plots E-F (n=32)......................................................................................................................... 33

Figure S1. LC-MS/MS spectra of peptide COL1α1 324-341 for (A) C. mydas and (B) C. caretta. Grey shading indicates the amino acid substitution differentiating the two species……………………………………………………………………………………………… 66

Figure S2. LC-MS/MS spectra of peptide COL1α1 713-740 for (A) C. mydas and (B) C. caretta. Grey shading indicates the amino acid substitution differentiating the two species……………………………………………………………………………………………… 67

Figure S3. LC-MS/MS spectra of peptide COL1α1 825-844 for (A) C. mydas and (B) C. caretta. Grey shading indicates the amino acid substitution differentiating the two species……………………………………………………………………………………………… 68
Figure S4. LC-MS/MS spectra of peptide COL1α1 893-914 for (A) *C. mydas* and (B) *C. caretta*. Grey shading indicates the amino acid substitution differentiating the two species………………………………………… 69

Figure S5. LC-MS/MS spectra of peptide COL1α2 393-402 for (A) *C. mydas* and (B) *C. caretta*. Grey shading indicates the amino acid substitution differentiating the two species………………………………………… 70

Figure S6. LC-MS/MS spectra of peptide COL1α2 393-414 for (A) *C. mydas* and (B) *C. caretta*. Grey shading indicates the amino acid substitution differentiating the two species………………………………………… 71

Figure S7. LC-MS/MS spectra of peptide COL1α2 574-587 for (A) *C. mydas* and (B) *C. caretta*. Grey shading indicates the amino acid substitution differentiating the two species………………………………………… 72

Figure S8. LC-MS/MS spectra of peptide COL1α1 10-42 for (A) *C. mydas* and (B) *C. caretta*. There are no amino acid differences between the two species…………………………………………………………………. 73

Figure S9. Boxplots showing δ¹³C values from (A) all archaeological *C. mydas* (n=45), (B) archaeological *C. mydas* samples excavated at Kinet Höyük (n=37) and Tell Fadous-Kfarabida (n=8), and (C ) species differences between *Caretta caretta* (n=25) and *Chelonia mydas* (n=45)................. 75

Figure S10. Boxplots showing δ¹⁵N values from (A) all archaeological *C. mydas* (n=45), (B) archaeological *C. mydas* samples excavated at Kinet Höyük (n=37) and Tell Fadous-Kfarabida (n=8), and (C ) species differences between *Caretta caretta* (n=25) and *Chelonia mydas* (n=45)................. 79

Figure S11. Boxplots showing δ³⁴S values from (A) archaeological *C. mydas* from Kinet Höyük (n=32), (B) archaeological *C. mydas* samples excavated at Kinet Höyük (n=30) and Tell Fadous-Kfarabida (n=2), and (C ) species differences between *Caretta caretta* (n=7) and *Chelonia mydas* (n=32)…………………………………………………………………. 82

Figure S12. Scatterplots of the collagen concentration (ng/uL) from BCA assays which determine the total concentration of protein for a subset of ancient samples (n=53). Plots a-b show stable isotope signals, whereas plots d-e show δ³⁴S quality control criteria, with the acceptable range according to Nehlich and
Richards (2009) for each variable shown in pink shading…………………………………………………………………. 87

Figure S13. Linear regressions between δ\textsuperscript{13}C and δ\textsuperscript{15}N data measured at University of York BioArCh Laboratory, University of Groningen CIO laboratory, and University of Glasgow SUERC laboratory. Samples measured at BioArCh and SUERC are from the same collagen extraction, samples measured at the CIO are from a separate collagen extraction of the same bone……………………………………………………………………. 91

Figure S14. (A) δ\textsuperscript{13}C\textsubscript{DIC} reconstructions (grey circles and green triangles) from Sisma-Ventura et al. (2014), with dark grey line showing ‘loess’ smoothing of the 20 year averaged data. The Industrial Revolution (AD 1760 – 1830) is highlighted in orange following the example by (34). The purple highlight indicates the period of construction for the Suez Canal (1859-1870), and red indicates the construction of the Aswan High Dam on the Nile (1960-1970); two large-scale anthropogenic activities which affected the average contribution of δ\textsuperscript{13}C-depleted freshwater into the eastern-Mediterranean. Green triangles are the 10 most recent points from the Sisma-Ventura et al. (2014) δ\textsuperscript{13}C\textsubscript{DIC} data, which we have used to build a linear model (B) to be used in applying Suess corrections to modern turtle δ\textsuperscript{13}C data from different years in order to compare to archaeological specimens……………………………………………………………… 92

Figure S15. Stable Isotope values of ancient loggerhead turtle remains (grey points) overlaid with modern stable isotope values (diamonds) for (Suess corrected) satellite tracked Levantine loggerhead turtles. (A) δ\textsuperscript{15}N vs δ\textsuperscript{13}C (n=30 for ancient data, n=19 for modern data), (B) δ\textsuperscript{15}N vs δ\textsuperscript{34}S (n=7 for ancient data, n=12 for modern data)...................................... 93

Figure S16. Comparisons between Linear Discriminant Analysis (LDA, outer circles) assignments and Flexible Discriminant Analysis (FDA, inner circles) assignments of (A-B) 165 modern green turtles from Cyprus (21) and (C-D) 32 ancient green turtles analysed in this study using δ\textsuperscript{13}C, δ\textsuperscript{15}N and δ\textsuperscript{34}S. Foraging ground assignments with 83% posterior probability are displayed (an order of magnitude increase over random odds). Samples not assigned at the posterior probability threshold are classified as unidentified (grey)................................................................. 94
Chapter 3

Figure 1. (A) Map of the Netherlands showing the archaeological origin of two sea turtle bones. (B) Archaeological turtle rib from Leeuwarden, and (C) archaeological turtle rib from Schagen.......................................................... 99

Figure 2. (A) MALDI spectra of the COL1α1 586-618 biomarker (Harvey et al. 2019) in extracted collagen from archaeological turtle specimens from Schagen with C. caretta ZooMS marker (above) and Leeuwarden specimen with a C. mydas ZooMS marker (below). (B) Values from stable isotope analysis (δ¹³C and δ¹⁵N) of both archaeological turtle bones................................................................. 103

Figure 3. (A) Bagplots of modern global stable isotope δ¹³C and δ¹⁵N values of C. mydas from East Atlantic (n=72, (Ferreira et al. 2021; Hancock et al. 2018)), Eastern Mediterranean (n=196, (Bradshaw et al. 2017)), Indian Ocean (n=86, (Burkholder et al. 2011)), Pacific (n=74, (Shimada et al. 2014)), South-West Atlantic (n=171, (Gama et al. 2021)), and West Atlantic (n=434, (H. B. Vander Zanden et al. 2013; Hannah B. Vander Zanden et al. 2015)). The stable isotopic signal of the archaeological Leeuwarden C. mydas sample is indicated by an orange triangle. Modern values have been corrected (Dombrosky 2020), with a Suess correction of 1.8. Linear Discriminant Analysis results are displayed (B) in table form, with a posterior probability of group membership displayed to two decimal places…………………………………………………………….. 104

Figure S1. Archaeological turtle bone found in Schagen, with accompanying note............................................................................................................................................ 112

Figure S2. Archaeological turtle bone found in Leeuwarden, previously misidentified as a whale rib, now stored at the archaeological depot in Nuis, Groningen................................................................. 112

Figure S3. Menu, unknown date and location. Collection number PR03036.049.............................................................................................................................................. 113

Figure S4. Menu for a dinner hosted by the province of Frisia for King Willem III when he visited the region, 14th of May 1873. The entree was sea turtle soup. Collection number PR03036.058........................................................................................................ 114

Figure S5. Menu for a dinner to celebrate the new railway line between Makkum and
Harkezijl on 18th of May 1898. The entree was sea turtle soup. Unknown location in Friesland. Collection number PR03036.012

Figure S6.

Menu for a dinner on 26th September 1877 for an unknown reason, the entree was sea turtle soup. Collection number PR03036.045

Figure S7.

Menu including an entree of sea turtle soup. Unknown date, location. Collection number PR03036.017

Figure S8.

Collection number PR03036.021. Menu of a meal in 1856 to celebrate and remember the Groninger en Franeker Studenten-Compagnie

Figure S9.

Menu for a meal in Leeuwarden in 1860. Organised by the Maatschappij tot bevordering van Nijverheid. Collection number PR03036.059

Figure S10.

A dinner in Friesland serving mock turtle soup in 1957. Fries museum collection number PR03036.038

Figure S11.

An example of mock-turtle soup advertised in a newspaper Algemeen Handelsblad 14-12-1832

Chapter 4

Figure 1. Images from 5300 Fragment Analyzer in NGS mode of two samples (W21 from Kinet Höyük and W73 from Tell Fadous-Kfarabida). Curves from Double-Stranded and Single-Stranded library protocols are shown for the two samples. Adapter dimer is shown in the Single-Stranded libraries, and significantly bigger in the W73 sample.

Figure 2. (A) Percentage endogenous C. mydas DNA from 50 ancient Mediterranean double stranded shotgun sequencing screening reads, and (B) boxplots of endogenous percentage comparing the two sites Kinet Höyük (n=42) and Tell Fadous-Kfarabida (n=8).

Figure 3. (A) Percentage endogenous C. mydas DNA in sequencing reads from 10 ancient Mediterranean sea turtle samples and (B) mean fragment length from the same samples. Represented are shotgun screening of double stranded libraries (pink triangles), mtDNA hybridisation capture of single
stranded libraries (green circles) and SNP hybridisation capture of single stranded libraries (orange squares). The fragment lengths of the three library types are further compared using box plots (C) and the fragment lengths in samples from the two archaeological sites Kinet Höyük and Tell Fadous-Kfarabida (irregardless of the library type) are also compared using boxplots (D)................................. 129

Figure 4. Phyllogenetic tree produced from IQ-Tree, with 1000 bootstraps. Modern Caribbean samples have been included, but their clades collapsed (blue colour). Mediterranean samples without variation have also been collapsed (orange colour). Bootstrap percentages are included at the nodes................................................................. 130

Figure S1. Per-sample plots of 10 ancient Mediterranean C. mydas mitogenome sequences showing the frequency of cytosine to uracil (thymine) transitions at the initial base of the 5’ strand end, Produced by the MapDamage software................................................................. 143

Figure S2. Per-sample plots of 32 historical museum (Naturalis) C. mydas mitogenome sequences showing the frequency of cytosine to uracil (thymine) transitions at the initial base of the 5’ strand end, Produced by the MapDamage software................................................................. 143

Chapter 5

Figure 1. Infographic depicting the steps associated with analysing zooarchaeological remains, analysis tools and possible insights they can provide. Reproduced from Winter, de Kock et al. (2021)........................................................................................................ 145

Figure 2. (A) Percentage endogenous C. mydas DNA in sequencing reads from 10 ancient Mediterranean sea turtle samples in three library types (shotgun screening of double stranded libraries [pink triangles], mtDNA hybridisation capture of single stranded libraries [green circles] and SNP hybridisation capture of single stranded libraries [orange squares]). (B) Boxplots showing fragment lengths in samples from the two archaeological sites Kinet Höyük and Tell Fadous-Kfarabida (irregardless of the library type).................................................................................. 148
Chapter 2

Table S1. Species Identifications using peptide A1T55/56 (F) published by Harvey et al. (2019), and 7 novel ZooMS biomarkers. Archaeological samples suspected to be Softshell turtles (*Trionyx triunguis*) from the m/z of the A1T55/56 peptide were excluded from further analysis, which focussed on differences between the two sea turtles. Samples are identified as either *Chelonia mydas* or *Caretta caretta* depending on whether the MALDI spectrum showed the peak for that respective species which achieved the mMass peak-picking thresholds in at least 1 replicate. A peptide is marked as 'Unidentified' if neither of the two species' peaks were found. Strikethrough indicates manually observing a biomarker peak in at least 2 replicates, where the MALDI peaks do not achieve the mMass peak-picking thresholds in any replicate. An asterix (*) indicates that the peak was confidently found by mMass, however the other species’ peak was also observed manually, although not high enough to achieve the mMass peak-picking thresholds. ‘Both peaks present’ describes the uncommon event that both species' biomarkers were present in the spectra. 49

Table S2. Species identification of samples unable to be identified to species using the A1T55/56 (F) ZooMS biomarker published by Harvey et al. (2019). The m/z positions for all seven novel biomarkers were observed in mMass. Confident identification describes a MALDI peak detected by mMass in at least 1 replicate. Less confident identification describes manually observing a biomarker in at least 2 replicates, where the MALDI peak does not achieve the mMass peak-picking thresholds. 51

Table S3. Sample information of LC-MS/MS reference samples. 51

Table S4. List of all LC-MS/MS peptides found in *Caretta caretta* reference spectra. X in the first column indicates that an amino acid (as analysed in MaxQuant MS/MS spectra ) was not well covered by b or y ions. Sequences in green indicate peptides mapped to the *C. mydas* reference COL1 sequence. 52

Table S5. Archaeological stable isotope data of $\delta^{13}$C and $\delta^{15}$N (CIO, University of Groningen and BioArch, University of York), and $\delta^{34}$S (SUERC, University of Glasgow). Only mass-spectrometry measurements which pass C:N ratio quality control (C:N between 2.9-3.6) are presented. All
sulphur isotope values are shown, whether passing or failing the quality criteria. If a diagenic indicator for δ³⁴S quality (%S, C:S ratio or N:S ratio) falls outside the acceptable range described by Nehlich and Richards (2009), the value is bolded and the cell highlighted in grey. If more than one diagenic indicator falls outside the acceptable range, the sample fails sulphur QC following the method used by Rand, Freiwald and Grimes (2021), this is indicated by strikethrough. Absence of data is indicated by - ………………………………………………………………………………………………………… 57

Table S6. Posterior probabilities of *C. mydas* foraging grounds, from Linear Discriminant Analysis using δ¹⁵N and δ¹³C stable isotope values. Samples that exceed 80% posterior probability are highlighted in green. Leave-one-out cross validation plots of the model training data are provided above the table……………………………………………………………………………………………………… 58

Table S7. Posterior probabilities of *C. mydas* foraging grounds, from Linear Discriminant Analysis (LDA, left) and Flexible Discriminant Analysis (FDA, right) using δ¹⁵N, δ¹³C and δ³⁴S stable isotope values. Samples that exceed 80% posterior probability are highlighted in green. Leave-one-out cross validation plots of each model training data are provided above each table……………………………………………………………………………………………………… 59

Table S8. Posterior probabilities of *C. mydas* foraging grounds, Flexible Discriminant Analysis (FDA, left) using δ¹⁵N, δ¹³C and δ³⁴S stable isotope values. Samples that exceed 83% posterior probability (an order of magnitude increase over random odds) are highlighted in green. FDA of δ³⁴S (right) was used to discriminate between ancient samples which mapped to the TCWL group (Turkey-Cyprus and West-Libya combined) in FDA 1. Samples with a posterior probabilities at least 1 order of magnitude greater than random odds (≥ 0.94) are highlighted in green (an order of magnitude increase over random odds). Leave-one-out cross validation plots of each model training data are provided above each table……………………………………………………………………………………………………… 60

Table S9. Posterior probabilities of *C. caretta* foraging grounds, from Linear Discriminant Analysis using δ¹⁵N and δ¹³C stable isotope values. Samples that exceed 75% posterior probability are highlighted in green. Leave-one-out cross validation plots of the model training data are provided above the table……………………………………………………………………………………………………… 61

Table S10. Posterior probabilities of *C. caretta* foraging grounds, from Linear Discriminant Analysis using δ¹⁵N, δ¹³C and δ³⁴S stable isotope values. Samples that exceed 75% posterior probability are highlighted in green.
Leave-one-out cross validation plots of the model training data are provided above the table…………………………………………………………. 61

Table S11. Stable Isotope Data, and additional information on modern green turtles tracked using satellite telemetry from Cyprus (Bradshaw et al. 2017)…..62

Table S12. Stable Isotope Data, and additional information on modern loggerhead turtles tracked using satellite telemetry from Cyprus (Haywood et al. 2020)………………………………………………………………………………… 62

Table S13. Pearson’s product-moment correlations of δ34S values of sea turtle collagen (n=71), with CS Molar, NS Molar and %S. No significant correlations are found……………………………………………………….. 86

Chapter 3

Table S1. Sample information of two analysed archaeological sea turtle bones excavated in the Netherlands……………………………………….. 112

Table S2. Leave-one-out cross validation of Linear Discriminant Analysis model of global Chelonia mydas δ13C and δ15N per region. Rows are actual regions, and columns are predicted regions. Numbers displayed as percentage of points assigned to each region, shaded squares indicate the percentage correctly assigned for each region…………………………………..113

Chapter 4

Table S1. Master sample list of all ancient and historic C. mydas samples extracted and sequenced, with the library type and DNA concentrations at different stages of the wet-lab protocol……………………………………………………. 141
Rob, this one is for you.
Acknowledgements

As I write this, far too late in the process, I am very worried I will not do everybody justice in these acknowledgements. The truth is that I am very grateful to have had this glorious opportunity. Although a PhD is traditionally the work of one person, in my case I have been very fortunate to work with such a large number of people and therefore this work is collaborative in essence. I fear that I have inevitably forgotten to thank somebody; to all those who helped me, please know that your contribution was invaluable.

Canan, this project was your baby and you worked so hard to set everything up for it, for me to then take the research in my own direction. You have supported me throughout, especially during the corona hell when secondments were being cancelled left, right and centre and I was in despair thinking I would never generate the data I needed to EVEN BEGIN my analysis. In hindsight I should have been calmer, but I’m thankful you were there. I’m looking forward to continuing working together. I’m lucky to have you as a mentor.

Per, your honest, calm and patient attitude has been a comfort. You have been understanding and kind, especially in the face of delays and extreme time constraints. I’m very impressed by the passion you have for your work, and especially by your high standards for science. I’ve learned so much, and I’m grateful.

Daan, thank you for helping me navigate through this process. I’m particularly grateful for your swift action when I had to rush back to New Zealand, and subsequent bereavement leave. I felt very supported. Morten, your can-do attitude is inspiring. Thanks for letting me visit, and giving me access to the lab and training. We didn’t see each other much because of the pandemic and your move to Perth, but I appreciate that you made it possible for me to come to Copenhagen at such a strange time. Michelle, I spent two months in York while the UK was in lockdown. Looking back, it seems amazing that you managed to organise it. At the time I was just desperate to generate some data, thanks for being patient with me but also humouring my ideas. I’ve really enjoyed working with you. Alberto, you always made our collaborations a priority and you supported me so much. I have been inspired by your enthusiasm to always push a bit further. From the start, you have been incredibly generous with your time. I have been inspired by your passion to always push a bit further. From the start, you have been incredibly generous with your time. I’m very thankful.

Jurjan, thanks for blazing a path with the sea turtles – I’m really looking forward to our collaborations going forward.

Martine, because of you I am much more confident in any lab. You didn’t just show me how to do the things, but allowed me to understand exactly what was happening at each step. This made everything less mysterious. Your training allowed me to be calm and do things correctly, and I’m grateful. For me, one of the most important parts of my PhD without a doubt. Jesper, thank you so much for your time. I arrived in Copenhagen with a bunch of old sea turtle bones and you took it in your stride. Thanks for teaching me all you did. Matt, my time at BioArCh was great despite the pandemic. You kept the show on the road, and I appreciate that you trusted us to do things properly even when you couldn’t physically be there because somebody tested positive AGAIN. Thanks for the training. Mike, you have also been very generous with your time and letting me train at the CIO. It was invaluable.

I would like to thank my colleagues at GIA, and GELIFES. You were always friendly and generous despite the fact I was often absent due to being at the other institute, or on secondment or working from home. Special thanks go to Francesca, Pir, Safoora, Martina, Manuela, Yakamoz, Robin, Marcos, Yacine, Hannah, Jolijn, Merita, Nathalie, Jildou, Shyama, Sean, Pinar, and Dimitris. I’m also grateful to Marijke, Inge, Nadia and Hinke for their help.

I would also like to acknowledge people I met on my secondments. In Copenhagen, I’m very grateful to Maria, Agnete, Tejs and Klaus for welcoming me into their lab group and helping me. Other people I met at the Globe; Meaghan (my MaxQuant guru), Max, Abby, Fabiana and Liam. Thanks for all the
help and I really enjoyed spending time with you guys. Matthew C, a lot of things wouldn’t have happened if it wasn’t for you, thanks for your enthusiasm and letting me visit your lab. Also the whole crew at BioArCh, thank you and especially to Michelle D, Efrossini, Maddie and Maria for help along the way.

I’m grateful to everybody in the SeaChanges network, especially David, Nell and Catherine. I want to thank the ESRs; Lulú, Rachel B, Lane, Katrien, Liz, Lucía, Marie, Adam, and Magie. Extra thanks to Giulia and Laura who have collaborated with me, thanks for giving me your time and company.

Next thanks are for the core crew. Emily I’m very grateful I met you on this journey, your passion has been inspiring for me both personally and professionally. Nobody loves biology the way you do. Thanks for your support, teachings and time. Fabricio you’ve been a rock in the storm. I’ve relied on you a lot. Thank you, thank you, thank you! You are one of the most capable, nice and funny people I know. Rachel my dear, I cannot deny that this PhD journey has been rather defined by having you next to me, both figuratively and literally. With that signature determination, you started planning things together, first professional but also in our personal lives. The last three years have been a lot richer for it. Thank you.

The journey to the PhD started a long time ago, and countless people have contributed to it. I’m thankful for my family and friends. Jelmer, thanks for our discussions before I even applied for the position. Various people from Katikati College, Toi Ohomai Marine Studies, Waikato University, NIWA, ACES, and HCMR have helped me a lot to get here. Special thanks go to Fleur, Chrissen and Eugenia for all that they did.

Antonio (who calls you that?), you have gone through two PhDs now - yours and mine. No more, I promise, it’s done. Writing a thank you in words feels small, because you have been there every day. You saw it all, you supported me, and you humoured me when I was probably just having a textbook PhD moment. We spoke about my research every. single. day. I think you know it as well as I do. Thanks for loving me and not doubting that I would get it done. Te amo, let’s move on now.

Orselien, thank you for dressing me in baby clothes with fish on them. I don’t ever, ever remember thinking I couldn’t do something I wanted to do. It really, never occurred to me, and that’s due to you. You are the most interested and interesting person I know, and you are my sounding board. So here I am with a finished PhD thesis. These last years haven’t been easy, but I’m proud of both of us. We gave ‘em hell. Love you.

Rob, my final thanks are for you with all my love. You saw the start of this endeavour and it breaks my heart that you didn’t get to see the end, you would have loved it. However, isn’t the true magic really in the beginning? The imagination of the project. The purchase of the sailboat. The setting of the fyke. The journey, not the destination. Especially in this way, I am you, and we are the generations.
Author’s Declaration

I declare that this dissertation is a presentation of original work and I am the sole author. This work has not been previously presented for an award at this, or any other university. All sources are acknowledged as references. The work carried out as part of this dissertation has resulted in the following publications:


Introduction

Although archaeological sea turtles are found at many coastal sites, osteological or biomolecular archaeological studies have been few and recent (Koolstra et al. 2019; Harvey et al. 2019; Conrad et al. 2018; Rand et al. 2021). This thesis focuses on exploring the possibilities of biomolecular archaeology to answer historical ecological questions about sea turtles in the pre-industrial past.

Archaeological sea turtle remains have been found along the Levantine coast spanning periods from the Neolithic to the Late Iron Age (Çakırlar, Koolstra, and Ikram 2021). While anthropogenic, presumably these are mostly representative of nesting or breeding populations, from the proximity to modern nesting beaches. Traditional osteological and osteometrical methods can answer only few of our questions regarding these ancestral populations and how humans interacted with them (Koolstra, Küchelmann, and Çakırlar 2019), while biomolecular tools are particularly advantageous to study highly fragmented assemblages, by shedding more definitive light on species identifications, and population characteristics such as population connectivity, size, foraging ecology, and nesting grounds.

This dissertation contains 4 research chapters; the first is a review of the current state of the art in biomolecular archaeology of marine vertebrates on the Levant, the second explores the foraging ecology and habitat use of ancient sea turtles in the eastern Mediterranean, the third is a biomolecular case study of two archaeological sea turtle bones found in the Netherlands, and the fourth focuses on the opportunities and challenges of ancient DNA to resolve questions about connectivity of ancient Mediterranean green turtles.

We seized an opportunity: The advent of palaeoproteomics, and subsequent development of the Zooarchaeology by Mass-Spectrometry (ZooMS) method of species identification (Buckley 2017), offers an efficient and cost effective technique to identify large numbers of samples with minimal destruction. ZooMS collagen biomarkers distinguishing between sea turtle species have been developed (Harvey et al. 2019), with three peptides published which discriminate between the green turtle (*Chelonia mydas*) and the loggerhead turtle (*Caretta caretta*), these are the two sea turtle species which nest and breed in the Mediterranean. Harvey et al. (2019) also published initial collagen sequences for extant sea turtles, though LC-MS/MS sequencing of additional *C. caretta* is warranted if additional biomarkers are to be found.

Increasing the number of ZooMS biomarkers discriminating between species also increases the probability of making a species identification from ancient and highly degraded collagen. Chapters 3 and 4 utilise this method to determine the species of archaeological sea turtle bones, both from the ancient Levant and the Netherlands.

Chapter 4 also takes advantage of sea turtles’ strong foraging ground fidelity, which delivers an exciting research opportunity as animal tissues (eg. skin, bone) reflect the isotope geochemistry of both their diet and the foraging location. Additionally, sea turtles have highly specific niches to avoid interspecies competition, this means that different sea turtles species usually forage in different habitats. The herbivorous green turtle preferentially utilises seagrass meadows as foraging ground (Cardona et al. 2010), these habitats are considered to be one of
the most threatened marine ecosystems (Waycott et al. 2009). Loggerheads demonstrate benthic or pelagic foraging (Blasi et al. 2018), but also have high fidelity to their over-wintering grounds (Godley et al. 2003). There can be strong distinctions between foraging areas, especially for benthic feeders such as green turtles. This has been demonstrated in modern Mediterranean green turtles, which exhibited differences between several distinct foraging locations from Egypt to West Libya, where SIA (δ¹³C, δ¹⁵N and δ³⁴S) of turtle epidermis showed a unique signal (Bradshaw et al. 2017). In terms of bioarchaeology, ancient sea turtle bones are information vaults which can inform us about the habitat these populations were using in the past, and whether the fidelity to the same areas has endured over time. Exploring the population genetics of ancient Mediterranean sea turtle remains could resolve how similar past populations were to the present day.

Using aDNA analysis, recently two gold rush-era green turtles were identified to species (Conrad et al. 2018). However there have been no studies to date using aDNA from sea turtles for population genomics. Modern green turtle genomics has revealed that the population from the East Atlantic diverged into other geographic areas after the last glacial period (van der Zee et al. 2021), highlighting the potential of using a genomic approach to research the origins of Mediterranean turtle populations. However, warm climatic conditions often lead to poor DNA preservation in archaeological remains (Reed et al. 2003). This means that the feasibility of using aDNA for sea turtle population genomics should be explored. Chapter 5 focuses on the preservation challenges, possibilities, and future directions of aDNA research of archaeological green turtles.

Although much of the thesis is focussed on ancient archaeological sea turtles from the Levant, in Chapter 4 we also explore biomolecular archaeology of more recent archaeological sea turtles found in the Netherlands. Sea turtles are not common in the Netherlands, therefore we set out to investigate the species and stable isotope ratios of two archaeological bones found in the Netherlands. The bones are from different archaeological periods, and therefore we wanted to investigate their differences using bioarchaeology. In doing so aspects of history, culinary culture and turtle exploitation and export are investigated.

Here we focus on the exploitation of marine turtles, but our goal is to highlight the importance of marine zooarchaeology in general. Chapter 2 serves as a more detailed introduction to the thesis topic, and introduces the main biomolecular archaeological techniques used within this PhD research.