Marine historical ecology of groupers (Epinephelidae) in the eastern Mediterranean utilising zooarchaeology, dietary isotopes, collagen sequencing, and peptide mass fingerprinting

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 11.00 hours

by

Rachel Marie Winter

born on 10 February 1994
in Spokane, Washington
United States of America
Supervisor
Prof. D.C.M. Raemaekers

Co-supervisor
Dr. C. Çakırlar

Assessment Committee
Prof. P.L. Horvatovich
Prof. M. Bariche
Prof. S. Ikram
Abstract

Marine resources have been exploited in the eastern Mediterranean for millennia, forming an important dietary resource for many coastal settlements. Being highly organoleptic fishes, groupers represent one of the most ubiquitous ichthyofaunal finds since the Neolithic in eastern Mediterranean archeological contexts. Groupers (Epinephelidae) are large, solitary, apex predatory fishes found globally in coastal rocky reef ecosystems in temperate, subtropical, and tropical marine waters. Due to various life history traits such as slow time to maturity, sequential hermaphroditism, and the formation of spawning aggregations, groupers are particularly vulnerable to the effects of overfishing. They have been heavily fished in the eastern Mediterranean leading to several species being classified as ‘vulnerable’ and ‘endangered’ by the International Union for Conservation of Nature (IUCN). Abundant, large predators, such as groupers, are essential for maintaining healthy ecosystems. Their high frequency in Mediterranean archaeological contexts and importance to marine ecosystems makes them key taxa for gaining long term perspectives on local fishing in the eastern Mediterranean and for establishing historical baselines.

This work employs three methods to gain long term perspectives on grouper fisheries as well as to establish historical ecological baselines for Mediterranean groupers in the Levant (Chapter 2). The material analysed for this thesis comprises fish bones from three eastern Mediterranean coastal archaeological sites. The bulk of material studied comes from Kinet Höyük (ca. 3000 - 50 BC and 8/9th c. - 14th c. AD), located in the most northeastern corner of the Mediterranean Sea in Iskenderun Bay, Turkey with additional material coming from two coastal sites located in present day Lebanon, Tell el-Burak (975 - 334 BC) and Tell Fadous-Kfarabida (ca. 3000 - 1550 BC). Osteometrics were used to analyse grouper and comber (fishes with similar ecology to groupers which are osteomorphologically indistinguishable from groupers) bones from all three sites to reconstruct catch sizes in the past and assess for fluctuations in the size structure of past grouper populations. Stable isotopes analysis, $\delta^{13}$C and $\delta^{15}$N, of archaeological fish bones was undertaken to reconstruct the foraging ecology of ancient marine ichthyofauna in the eastern Mediterranean with a special emphasis on groupers. Lastly,
proteomic analysis of four grouper species in the Mediterranean was carried out for de novo reconstruction of collagen type I amino acid chains (using LC-MS/MS data) to advance efforts of using paleoproteomics to distinguish ichthyoarchaeological material using Zooarchaeology by Mass Spectrometry (ZooMS) (MALDI-TOF data) and propose a phylogenetic tree for the species studied.

Catch size reconstruction of groupers and combers in the Bronze and Iron Age Levant, provides evidence for impressively large groupers in the past (~150 cm Total Length [TL]) sizes which are rarely seen today, even within well-enforced and long established Marine Protected Areas (MPAs) (Chapter 3). At the longest occupied site, Kinet Höyük, local grouper fishing efforts were intense enough to have brought about either a change in the size structure of local grouper populations or elicited a behavioural response of groupers moving to deeper waters by the Hellenistic period. Dietary stable isotopic data ($\delta^{13}$C and $\delta^{15}$N) from 137 archaeological fish bones was used to assess Levantine marine ichthyofaunal foraging strategies in the Middle to Late Holocene (Chapter 4). This data suggests that in the past, marine fish consumed a wider range of trophic resources than they do today. By comparing the isotopic niches of past trophic groups, we also see that the highest overlap in shared trophic resources is among fish from lower and middle trophic levels, indicating that these trophic groups may be more vulnerable to competition for trophic resources such as has been presented by non-indigenous species. Collagen type I sequences were reconstruction of four species of Mediterranean groupers: Epinephelus marginatus, Epinephelus aeneus, Epinephelus caninus, and Epinephelus costae (Chapter 5). Adequate variability in collagen sequences between grouper species generated what is currently the best supported phylogenetic tree for Mediterranean groupers within the Epinephelus genus. Twenty three novel ZooMS peptide biomarkers were found and validated, enabling species-level identification of archaeological grouper bones in the Mediterranean. A case study applying ZooMS to archaeological grouper and comber bones from Kinet Höyük demonstrates 4000 years of abundance and dominance of the white grouper, E. aeneus, in Iskenderun Bay, Turkey. As a result of combining ZooMS identifications with catch size reconstructions from the same archaeological fish bones, we have shown that E. aeneus is capable of growing ca. 30 cm TL larger than has been believed possible.
# Table of Contents

List of Figures .................................................................................................................. V

List of Tables .................................................................................................................... XI

Acknowledgements .......................................................................................................... XIV

Author’s Declaration ....................................................................................................... XIX

Chapter 1: Introduction to marine historical ecology, Mediterranean marine ecosystems, and research framework .............................................................. 1

1.1 The cumulative, global depletion of marine resources ............................................. 1

1.2 Marine historical ecology background and key concepts .................................... 2

1.3 Mediterranean marine ecosystems and their historical exploitation .................... 3

1.4 Selection of study species and scope ..................................................................... 4

1.5 Research questions ................................................................................................. 6

Chapter 2: Potential Applications of Biomolecular Archaeology to the Ecohistory of Sea Turtles and Groupers in Levant Coastal Antiquity .............................................. 15

Chapter 3: Catch of the Day: Abundance and Size Data of Groupers (Epinephelidae) and Combers (Serranidae) from Middle to Late Holocene Levantine Archaeological Contexts ......................................................................................... 37

Supplementary Material ................................................................................................. 72

Chapter 4: Pre-Lessepsian isotopic niche spaces: using paleoecological proxies to assess the impact of ongoing bioinvasions in the eastern Mediterranean Sea ................................................................. 136

Supplementary Material ................................................................................................. 157

Chapter 5: Grouping groupers: collagen sequencing and peptide mass fingerprinting demonstrates 4000 years of *Epinephelus aeneus* dominance in the northeastern Mediterranean ......................................................................................... 178

Supplementary Material ................................................................................................. 208

Chapter 6: Conclusions from a multi-methodological approach to the long term ecology and exploitation history in the eastern Mediterranean of Epinephelidae ................................................... 243

6.1 Summaries of thesis chapter ................................................................................... 243
6.1.1 Insights from Ancient Grouper and Comber Catch Size Data
(research question 1) ................................................................. 243

6.1.2 Isotopic Niche Spaces, Foraging Ecology, and ancient eastern
Mediterranean Ichthyofauna (research question 2) .................... 244

6.1.3 Grouping groupers using collagen sequencing and peptide mass
fingerprinting (research question 3) .......................................... 245

6.2 Grouper ecohistory in the context of current marine historical ecology
research .................................................................................. 246

6.3 Future prospects and directions in Mediterranean marine historical
ecology .................................................................................... 247

6.3.1 Connecting marine historical ecology with policy ............... 247

6.3.2 Prospects ............................................................................ 248

Appendices with additional data generated during the thesis .......... 253

Appendix 1. δ¹³C and δ¹⁵C stable isotope results from archaeological Levantine
freshwater fishes ..................................................................... 253

Appendix 2. δ³⁴S stable isotope results from archaeological Levantine
fishes ....................................................................................... 255
List of Figures

Chapter 2

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). ..............................................................18

Figure 2  Infographic depicting the steps associated with analysing zooarchaeological remains, analysis tools and possible insights they can provide. .................................................................19

Chapter 3

Figure 1  Archaeological site locations along the Levantine coast in the eastern Mediterranean: Kinet Höyük in present day Turkey and Tell Fadous-Kfarabida and Tell el-Burak in present day Lebanon.........................41

Figure 2  (A) Size distribution of groupers and combers recovered from Tell el-Burak (hand collection) and (B) Tell Fadous-Kfarabida (hand collection and sieving). .................................................................53
Figure 3  (A) Size distribution of groupers and combers from Kinet Höyük from hand collected and (B) sieved recovery methods……………………55

Figure 4  (A) Estimated size of groupers and combers from hand collected and (B) sieved material by time period at Kinet Höyük as estimated by osteometrics…………………………………………………………….56

Figure 5  (A) Size distribution of all combers and groupers from Kinet Höyük as estimated by osteometrics and comparison with a reference collection. (B) Hypothetical size distribution of groupers and combers at Kinet Höyük if all soil excavated at the site was also sieved…………………58

Figure S1  Total Length (TL) (mm) and Morales-Muñiz and Rosenlund (1979) Measurement 1 of the articular from the metrics provided in Table 3…75

Figure S2  Total Length (TL) (mm) and Morales-Muñiz and Rosenlund (1979) Measurement 3 of the articular from the metrics provided in Table 3…75

Figure S3  Total Length (TL) (mm) and maximal height of the margo posterior (MHMP) of the quadrate as described in Desse and Desse-Berset (1996) from the metrics provided in Table S3…………………………………76

Figure S4  Total Length (TL) (mm) and transverse diameter of the articular surface (TDA) of the quadrate as described in Desse and Desse-Berset (1996) from the metrics provided in Table S3…………………………………76

Chapter 4

Figure 1  Map of the eastern Mediterranean showing the archaeological sites that the ancient fish bones are from and the landing sites where commercially
caught fish were purchased by 12. Archaeological sites (indicated by black circles) include Kinet Höyük in Turkey, Tell Tweini in Syria, and the remaining three sites which are all in Lebanon: Tell Fadous, Sidon, and Tell el-Burak. Landing sites (indicated by white triangles), Tripoli, Batroun, and Beirut, are all on the Lebanese coast. Ancient fish bones shown include: a. articular of a grouper (Epinephelidae), b. caudal vertebra of a jack (Carangidae), c. precaudal vertebra of a grey triggerfish (Balistes capriscus), d. caudal vertebrae from a Scombridae, e. premaxilla of a grouper (Epinephelidae) or comber (Serranidae), f. premaxilla of a dentex (Dentex sp.), g. caudal vertebrae of a mullet (Mugilidae), h. premaxilla of a sea bream or porgy (Sparidae), and i. dentary of a gilthead seabream (Sparus aurata). ……………………..136

Figure 2 Biplot of bulk $\delta^{13}C$ and $\delta^{15}N$ values from ancient marine fish bone collagen. Data combined from this study (n=139) (Fuller et al. 2020; Schutkowski and Ogden 2011)…………………………………………………………...142

Figure 3 KUD of groupers (Epinephelidae) and combers (Serranidae) divided by size groups at contour levels of 50, 75, and 95. The table provides the INS sizes at various contour levels, the IPI of each size category, and sample sizes……………………………………………………………144

Figure 4 KUD at contour levels of 40, 75, and 95 of Middle and Late Holocene eastern Mediterranean ichthyofaunal trophic groups: Benthic Carnivores (BC), Generalised Carnivores (GC), Omnivores (O), and Piscivores (PI)…………………………………………………………………….145

Figure 5 KUD model of INS at contour levels of 40, 75, and 95 comparing the full ranges of ichthyofauna for the Middle to Late Holocene, Modern Lessepsian, and Modern indigenous Mediterranean groups. Table A provides INS sizes, IPI, and sample sizes for each group and Table B
shows the degree of INS overlap between the modern, indigenous Mediterranean fish (MED) and modern, Lessepsian fish (LES).
Figure 3  ZooMS identifications of Epinephelidae and Serranidae bones from Kinet Höyük (see Table S2)…………………………………………...191

Figure 4  Peptide mass fingerprints of two archaeological samples, KT 23570 and KT 23564, showing the peaks corresponding to the species specific forms of COL1α1 586. ……………………………………………………………192

Figure S1  The evolutionary history was inferred by using the Maximum Likelihood method and General Reversible Mitochondrial + Freq. model (Adachi and Hasegawa 1996). The tree with the highest log likelihood (-15662,28) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the JTT model, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0,1689)). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site (next to the branches). This analysis involved 8 amino acid sequences. All positions with less than 25% site coverage were eliminated, i.e., fewer than 75% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option). There were a total of 4255 positions in the final dataset. Evolutionary analyses were conducted in MEGA11(Tamura et al. 2021)…………………………………………………………………..205

Figure S2  Amino acid sequence and corresponding MALDI-TOF peak of COL1α2 568……………………………………………………………………235

Figure S3  Amino acid sequence and corresponding MALDI-TOF peak of COL1α3 934 shorter peptide……………………………………………….235
Figure S4  Amino acid sequence and corresponding MALDI-TOF peak of COL1α3
934 longer peptide...........................................................................236

Figure S5  Amino acid sequence and corresponding MALDI-TOF peak of COL1α1
586....................................................................................................236

Figure S6  Amino acid sequence and corresponding MALDI-TOF peak of COL1α3
271....................................................................................................236

Figure S7  Amino acid sequence and corresponding MALDI-TOF peak of COL1α2
662....................................................................................................237

Figure S8  Amino acid sequence and corresponding MALDI-TOF peak of COL1α1
793....................................................................................................237

Figure S9  Amino acid sequence and corresponding MALDI-TOF peak of COL1α2
361....................................................................................................238

Figure S10  Amino acid sequence and corresponding MALDI-TOF peak of COL1α1
705....................................................................................................238

Figure S11  Amino acid sequence and corresponding MALDI-TOF peak of COL1α3
238....................................................................................................239
List of Tables

Chapter 1

Table 1  Chronology of time periods associated with archaeological sites included in this thesis

Chapter 3

Table 1  Dating of the contexts from which the fish assemblages derive. Details of the phasing by site and dates are taken from the following sources as well as personal communication with excavation directors (Beach and Luzzadder-Beach 2008; Gates 2015, 2013, 2004; Genz et al. 2016; Kamlah and Sader 2019; de Miroschedji 2013).

Table 2  Size ranges for grouper and combers as estimated via osteometrics from hand collected and sieved material

Table S1  Size estimations via osteometrics of groupers and combers by time period from Kinet Höyük hand collected material

Table S2  Size estimations via osteometrics of groupers and combers by time period from Kinet Höyük sieved material

Table S3  Measurements of Epinephelidae and Serranidae bones used for size reconstructions in the present study

Table S4  Details of Epinephelidae and Serranidae from which size estimations were done via comparison to specimens in the reference collection at the Royal Belgian Institute of Natural Sciences in Brussels
Table S5  Measurements taken from and Specimen Details of groupers in the Jean Desse and Nathalie Desse-Berset Modern Reference Collection……..127

Table S6  Abbreviations for Time Periods used in Tables S3 and S4………………131

Table S7  Abbreviations for measurements used in size estimations………………131

Chapter 4

Table S1  Overview of the archaeological fish bones sampled in this study, (Fuller et al. 2020), and (Schutkowski and Ogden 2011) as well as the trophic groups assigned to each taxa………………………………………………154

Table S2  INS at various contour levels, IPI, and sample size of trophic groups of marine fishes from eastern Mediterranean archeological sites calculated using the KUD model in rKIN………………………………………………155

Table S3  Overlap of INS of archaeologically derived trophic groups of marine fishes utilising the KUD model in rKIN………………………………………………155

Table S4  Full quality control data pertaining to fish bones samples and analysed. Highlighted and italicised samples indicate unacceptable C%, N%, C:N ratio, or too much error between duplicates (indicated with * next to the problematic quality control criteria). Highlighted and bold results indicate collagen yields which were too low or too low of quality for analysis……………………………………………………………………156

Chapter 5

Table 1  Percentage of collagen type I sequences of Mediterranean Epinephelus spp. which were able to be reconstructed from modern LC-MS/MS data. Amino acids were only considered well covered if they were recovered in at least two unique, razor peptides. Amino acids which were only recovered in one peptide are thus not included in calculating the values above. The start of the mature peptide chain was determined following (Brown et al. 2021)…………………………………………………………185
Table 2  Species-specific peptides identified using LC-MS/MS and MALDI-TOF data for Mediterranean *Epinphelus* spp. Peptide names follow nomenclature proposed by Brown (et al. 2021). .................................189

Table S1  Archaeological samples for ZooMS.................................208

Table S2  Modern samples for LC-MS/MS and ZooMS analyses.........210
Acknowledgements

I really do not know where to begin in thanking everyone that was a part of my journey in completing this PhD. Truly, the list of people that contributed, professionally, personally, and oftentimes in both manners, to my life over the last three and half years is innumerable.

First and foremost, I would like to extend my deepest gratitude and thanks to my supervisor, Dr. Canan Çakırlar, and to my promotor, Prof. Dr. Daan Raemaekers. Thank you both for selecting me for this position, supporting me through the thesis, and the opportunity to grow as an individual and academic in Groningen. To Canan, thank you for keeping me focused on my work (instead of running after ideas in far too many directions), keeping me motivated, steadfast and tireless support, and your diligence when reviewing my work - which has surely made me a stronger, more critical researcher.

In addition to my supervisors in Groningen, I am so thankful to the research partners I’ve had outside of the Netherlands. Prof. Dr. Michelle Alexander, thank you for the guidance, idea exchange, and humour I’ve been privileged to know through both my Master’s degree and now also PhD thesis. Thank you to Dr. Alberto Taurozzi for supervising my lab work (accompanied by plenty of thought provoking and entertaining conversations), your friendship, wisdom on academia and life, and patience while I faced the steep learning curve of paleoproteomics. My project (and life) have benefited immensely from having the brilliant marine ecologists, Dr. Paolo Guidetti and Dr. Elena
Desiderà, as collaborators. Thank you both for teaching me so much about Mediterranean marine ecology, refining my ideas and word choices, and enthusiasm for our research ventures.

I hope it goes without saying, but I wouldn’t have made it this far in my studies if not for the support of excellent mentors earlier in my career, for that I extend many thanks to Susan Thurston-Myster, Skip and Phyllis Messenger, and Andre Carlo Colenese. I would also like to thank Matthew Collins for all of the support and encouragement he has shown me over the years, from encouraging me to come to York for my Masters to welcoming me into his lab group in Copenhagen during my PhD and listening to my research ideas with such earnest enthusiasm.

I don’t think I can (or ever will) find the words to do justice to how much I love and thank my parents for their unwavering love and unconditional support in pursuing my dreams. I know that moving an ocean away to pursue academia has put thousands of miles between us, but I am incredibly lucky to have parents that raised me to take such risks and follow my heart. No matter how far I venture, Minnesota will always be home and I am forever grateful to always know that my biggest cheerleaders are there. On the family note, thank you to my little brother, Sean, for always taking an interest in what I am up to and also supporting me from afar.

Moving to Groningen in 2019, I was insanely lucky to have several fellow SeaChanges ESRs here with me: Willemien de Kock, Fabricio Furni, and Emily Ruiz Puerta. I have
so many fond memories with the three of you and am so thankful to have had your support and friendship during the PhD; from travels together, much reggaeton and dancing, the collective support through the thesis, and so much more. During my time in Groningen, I have met and found friendship in so many wonderful individuals and I really thank each of them for keeping me sane, helping me unwind, and encouraging me through this thesis! I am thankful to have enjoyed so many borrels (appertivos), dinners, and adventures with Safoora Kamjan, Dimitris Filiooglou, Martina Parini, Merita Dreshaj, Manuela Ritondale, Pinar Erdil; detailed story telling, baking, and movie nights with Matilda Siebert; game nights and dinners with Erik Stoter and Youri van den Hurk; and bouldering with Nathalie Brusgaard, Jordy Aal, Jildou Kooistra, and John Turco. While in the GIA and more generally around Groningen, many thanks for coffees, friendship, and late nights to Stefanny Ramirez, Marcos Suárez Menéndez, Mario Gabualdi, Antonio Guiliani, Tom van Luinen, Pir Hoebe, Jolijn Erven, Anna Moles, Ben Lapierre, Francesca Bulian, Francesca Slim, Audrey Crabbé, Pinar Özküurt, Tommaso Della Seta, Ana Smuk, and Jos and the late Inge van der Schot.

My time so far in Groningen would absolutely not be what it has been without my wonderful and adoring partner, Remco Bronkhorst. Thank you for the many adventures, naturally encompassing hunebedden and the Waddenzee, laughter, keeping me sane (or something like it), and helping me relax and enjoy life outside of work, especially in the final stages of my thesis.

I have a number of friends in the UK and back in the US who have provided me with welcomed reprieve from the PhD in the form of video and phone calls and, when covid
and distance made possible, memories in person. A lot of love and thanks to the Shaw family: Ani, Gus, and Hal (and Rosie) for all of the calls, support, and friendship. I am perpetually grateful to Maja Proescholdt and Esther Fagelson for countless hours of phone calls, ridiculously long text messages, both coming to visit me in the Netherlands and both hosting me in the UK. I am really thankful to you two for the unconditional support, ample encouragement, and friendship you’ve both shown me over the years. I also would like to thank Belle (and Rhonda, of course), Becca Robson, Andrew Graham, my aunt, Kay Winter, Katherine Tomscha, Chelsea Palmateer, Desiree Haggberg, Ellen Hoffman, Courtney Rossbach (and Mani), Kyle Knapp, and Meghan Fransen.

The existence of my PhD project is thanks to the creation of the SeaChanges ITN, so my sincerest gratitude to everyone involved with the network but especially our PI, David Orton. David, thank you for your kindness, advice, and support during this project. From the SeaChanges, I would like to also extend my personal thanks to Marie Petitguyot, Rachel Blevis, Laura Corto, Lane Atmore, Katrien Dierickx, Nell Booker, and Catherine Taylor.

During this PhD, I had the pleasure of travelling and working in several countries around Europe, making many new colleagues and friends along the way. Starting in Groningen, I am forever grateful to Dr. Mike Dee for opening his lab (and the occasional weekend) to me and Willemien during the first covid summer to do our isotope work. Also in Groningen, I owe many thanks to Dr. Sean Desjardins, Flip Kamer, and Prof. Dr. Sofia Voutsaki for their time, support, and wisdom as I navigated
the PhD. In York, the BioArCh lab group has always provided me with a warm reception and made me feel more than welcomed. I am especially thankful to Maddy Bleasdale, Mik Lisowski, Matthew von Tersch, Mackenzie Masters, and Virginia Harvey. From my time in Copenhagen, many thanks to Meaghan Mackie, Abby Ramsøe, and Max Ramsøe for the good company, pub quizzes, and technical (and moral) support. While away from Groningen and attending conferences, thank you to Christina Cheung for ensuring I never felt alone in new places and your enthusiasm for fish.
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:


*Upon prior agreement, the first article (Chapter 2), was written as a literature review with Willemien de Kock as a joint, first author. It does not form as a core component of the thesis but rather as a shared introduction to our respective theses and was carried out during the COVID-19 pandemic due to uncertainties of accessing laboratories and carrying out the bulk of the work for this thesis.