

Review

Leveraging palaeoproteomics to address conservation and restoration agendas

Carli Peters,^{1,*} Kristine K. Richter,² Jens-Christian Svenning,³ and Nicole Boivin^{1,4,5,6,*}

SUMMARY

Archaeological and paleontological records offer tremendous yet often untapped potential for examining long-term biodiversity trends and the impact of climate change and human activity on ecosystems. Yet, zooarchaeological and fossil remains suffer various limitations, including that they are often highly fragmented and morphologically unidentifiable, preventing them from being optimally leveraged for addressing fundamental research questions in archaeology, paleontology, and conservation paleobiology. Here, we explore the potential of palaeoproteomics—the study of ancient proteins—to serve as a critical tool for creating richer, more informative datasets about biodiversity change that can be leveraged to generate more realistic, constructive, and effective conservation and restoration strategies into the future.

WHAT IS THE SCOPE FOR CONSERVATION PALAEOPROTEOMICS?

Earth's animal species are currently disappearing at such a rapid rate that scholars have suggested our planet is witnessing what may become its sixth mass extinction event (Barnosky et al., 2011; Davis et al., 2018). Since the year 1500, over 300 vertebrate species have gone extinct (Davis et al., 2018) and one-fifth of all extant vertebrates are currently threatened with extinction (Hoffmann et al., 2010). The global loss of biodiversity—the culmination of processes of extinction, extirpation, and population decline—is the result of many millennia of intensifying human-mediated ecosystem transformation through overexploitation, habitat degradation and conversion, invasive species introductions, and other pressures (Butchart et al., 2010; Dirzo et al., 2014; Boivin et al., 2016). Human-induced global warming, together with expected growth in both global human population and per capita consumption (Dirzo et al., 2014; Barnosky et al., 2017), will likely put an even bigger strain on already vulnerable ecosystems in the future. Therefore, it is crucial to develop optimal and scientifically informed conservation, restoration, and rewilding strategies that will mediate the future loss of biodiversity and functioning of the ecosystem.

A long-term perspective on ecosystem change is critical to adapting conservation strategies to combat current climatic and environmental challenges (Willis and Birks, 2006; Scharf, 2014; Barnosky et al., 2017). Fields such as paleontology, paleobiology, archaeobiology, zooarchaeology, paleoecology, and historical ecology provide indispensable long-term data about changes to biotic communities and the role of humans in their transformation (Dietl and Flessa, 2011; Dietl et al., 2015; Fordham et al., 2020). Early ecosystem transformations include massive species losses and range declines, notably among megafauna (Sandom et al., 2014; Smith et al., 2018) and island endemics (Kouvari and van der Geer, 2018), in addition to shifts in the distribution, composition, abundance, and diversity of plant and animal communities (Boivin et al., 2016; Roberts et al., 2017). The necessity of taking such long-term data into account when assessing baselines, establishing conservation targets, and managing and restoring ecosystems is increasingly recognized (e.g., Willis et al., 2010; Dietl et al., 2015; Barak et al., 2016; Fordham et al., 2020; Boivin and Crowther, 2021).

Although these historical fields have a crucial role to play in developing informed and effective conservation approaches, their utility is hindered by important limitations, with taphonomic processes in particular yielding biased and incomplete datasets that are suboptimal for establishing baselines or evaluating climatic or anthropogenic impacts. With respect to faunal remains, the focus of our discussion, these limitations can be severe. Although preservation can sometimes be excellent, zooarchaeological and paleontological remains are more often heavily fragmented and even morphologically unidentifiable, limiting their potential to reveal long-term changes to biodiversity, understand and model processes of species

¹Department of Archaeology, Max Planck Institute for the Science of Human History, Jena, Germany

²Department of Anthropology, Harvard University, Cambridge, MA, USA

³Center for Biodiversity Dynamics in a Changing World (BIOCHANGE), Department of Biology, Aarhus University, Aarhus, Denmark

⁴School of Social Science, The University of Queensland, Brisbane, QLD 4071, Australia

⁵Department of Anthropology, National Museum of Natural History, Smithsonian Institution, Washington, DC, USA

⁶Department of Anthropology and Archaeology, University of Calgary, Calgary, Canada

*Correspondence: peters@shh.mpg.de (C.P.), boivin@shh.mpg.de (N.B.)
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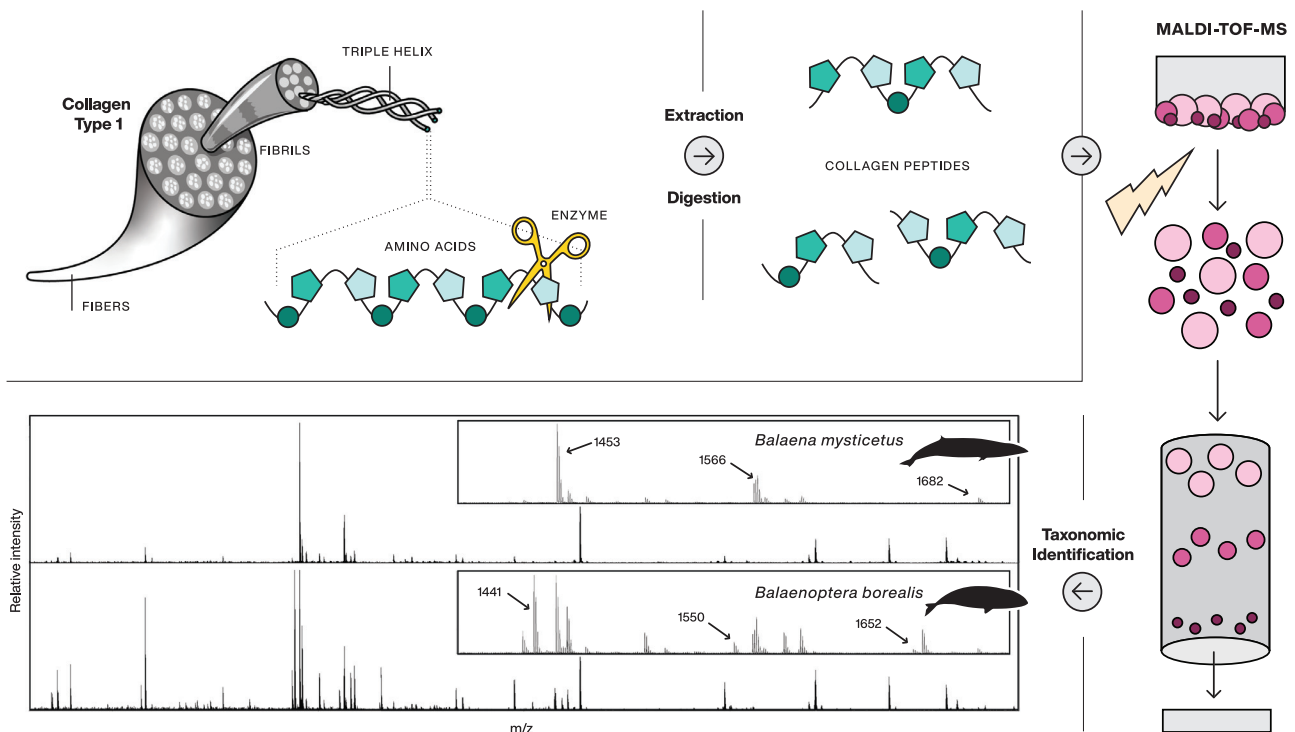


Figure 1. Schematic overview of ZooMS workflow (adapted from Brown et al., 2021a).

invasion, extinction and extirpation, and accurately and consistently measure anthropogenic alterations to ecosystems. In regions with high erosion and/or wind deflation or where cultural traditions favor mobility, zooarchaeological assemblages can even be lacking altogether.

In recent decades, biomolecular methods have significantly transformed the study of ancient faunal remains, enabling improvements in the analysis of ancient animals and assemblages. In particular, ancient DNA (aDNA) has been applied to better understand phylogenetic patterns, extinction mechanisms, and domestication processes across a wide range of faunal species (e.g., Haile et al., 2009; MacHugh et al., 2017). Ancient DNA methods have also been used to address conservation agendas (e.g., Leonard, 2008; Hofman et al., 2015; Waters and Grosser, 2016). However, challenges of aDNA preservation mean that this biomolecular approach has limited applicability, with often minimal potential in hot, humid, and tropical environments, as well as in the study of older assemblages. To address this challenge, new approaches that examine ancient proteins rather than aDNA have been adopted in the recent years. Proteins are an attractive target for conservation studies because they can be preserved over longer time periods in a wider array of contexts than aDNA and are also more resistant to degradation in warm environments (Buckley et al., 2009; Rychczynski et al., 2013; Demarchi et al., 2016; Hendy et al., 2018; Welker et al., 2019).

Two key approaches currently exist for examining ancient proteins – peptide mass fingerprinting and shotgun palaeoproteomics. Both are mass spectrometry-based approaches, which involve the detection of ionized peptides, thereby enabling the identification of peptide sequences and the characterization of proteins in a sample (Cappellini et al., 2014; Hendy, 2021). Zooarchaeology by Mass Spectrometry (ZooMS; Figure 1) is a peptide mass fingerprinting technique focused on collagen type I (COL1), a well-characterized and generally robust protein that also plays a key role in both radiometric dating and stable isotopic reconstructions of ancient diet (Pestle and Colvard, 2012). Applications of ZooMS draw on the fact that the amino acid sequence of COL1—the most abundant protein in bone, skin, antler, and dentine—varies across different taxonomic groups (Buckley et al., 2009).

ZooMS involves extraction of peptides from the targeted material, which are then analyzed using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-ToF-MS), generating a spectrum with the

mass-to-charge ratios of the individual peptides in the sample. The spectra are compared to a reference database of spectra from known taxa to taxonomically identify collagen-bearing materials. This approach is increasingly used in the field of archaeology to taxonomically identify highly fragmented and/or morphologically unidentifiable faunal remains (Buckley et al., 2017b; Sinet-Mathiot et al., 2019; Brown et al., 2021b). ZooMS is a relatively cheap and fast method (Buckley et al., 2009; Welker et al., 2015b; Richter et al., 2020), making it applicable to larger-scale assemblages than many other biomolecular methods.

In contrast to peptide mass fingerprinting in which only the predominant peptides in a sample are visualized, shotgun palaeoproteomics targets a much larger percentage of a sample's peptides by using liquid chromatography tandem mass spectrometry (LC-MS/MS) to provide a much higher resolution. The peptides in a sample are identified following two fragmentation steps. First, the mass of each peptide is detected in the first mass analyzer. The most frequently occurring peptides are further fragmented and measured again in a second mass analyzer. The amino acid sequences of the peptides in the sample can then be identified through comparison to large reference databases (Hendy, 2021). Shotgun proteomics provides the opportunity to examine phylogenetic relationships in materials that are too old or poorly preserved to yield aDNA (Rybczynski et al., 2013; Welker et al., 2015a). It can also be used to identify the sex of prehistoric individuals (Stewart et al., 2017), the presence of particular fauna, and to explore human-animal relationships from indirect sources such as dental calculus (e.g., Wilkin et al., 2021). With shotgun proteomics, it is also possible, as it is not with ZooMS, to detect posttranslational modifications of individual amino acids, allowing insight into the degradation patterns and authenticity of ancient proteins (Van Doorn et al., 2012; Cleland et al., 2015, 2021). The wealth of information that can be derived from a single sample makes shotgun proteomics more informative and versatile than peptide mass fingerprinting. However, the trade-off for this information is increased cost and time input per sample. Although studies using peptide mass fingerprinting can easily analyze hundreds or even thousands of samples (Richter et al., 2011; Brown et al., 2016, 2021b), most shotgun palaeoproteomic studies analyze only a fraction of this amount.

Protein-based methods are not new in archaeology (Abelsen, 1954; Hare and Abelsen, 1968; Newman and Julig, 1989; Johnson and Miller, 1997; Ostrom et al., 2000; Kooyman et al., 2001; Buckley et al., 2009, 2011; Cappellini et al., 2014); however, methodological improvements over the past decade (Van Doorn et al., 2011; Van der Sluis et al., 2014; McGrath et al., 2019) have seen their increasing application to a wide range of archaeological, human evolutionary, and art historical questions (for a more in-depth review about the historical perspective of ancient protein-based methods and their applications, please see Buckley (2018); Welker (2018); Villanova and Porcar (2019); Hendy (2021), and references therein). Critically, a broad array of materials are suitable for palaeoproteomic analysis, including bone (Buckley et al., 2009; Cappellini et al., 2012; Cleland et al., 2015, 2016; Welker et al., 2015b), antler (Von Holstein et al., 2014; Ashby et al., 2015), mollusc shell (Sakalauskaite et al., 2020), eggshell (Demarchi et al., 2019), ivory (Coutu et al., 2016), dentine and enamel (Cappellini et al., 2019; Welker et al., 2019), dental calculus (Warinner et al., 2014; Bleasdale et al., 2021; Wilkin et al., 2021), leather (Brandt et al., 2014), parchment (Fiddyment et al., 2015), hair (Solazzo et al., 2013), textiles (Gong et al., 2016), ceramic residues (Solazzo et al., 2008), and preserved food remains (Yang et al., 2014).

We argue that given their extraordinary advantages, ancient protein-based studies hold significant potential not only to understand the archaeological record and changes to human diets and economies through time but also to address biodiversity and conservation agendas. To date, this potential has been only minimally explored. Nonetheless, some insights have been acquired, and we review recent developments here to demonstrate the future potential of conservation palaeoproteomics. Drawing upon examples from a range of regions and time periods, we outline seven key ecological conservation issues that ancient proteins can help to address (Figure 2).

ASSESSING SPECIES RICHNESS

The global loss of biodiversity is one of the most significant threats faced by ecosystems today (Hoffmann et al., 2010; Barnosky et al., 2011; Davis et al., 2018). Estimates of species diversity and composition are crucial for making accurate inferences concerning the magnitude and rate of biodiversity loss, information that is crucial to contemporary conservation efforts (Butchart et al., 2010) as well as for informing restoration strategies (Monsarrat and Svenning, 2021). However, these estimates are often based solely on the organisms represented in modern ecosystems, not accounting for ecosystem transformations in the past.

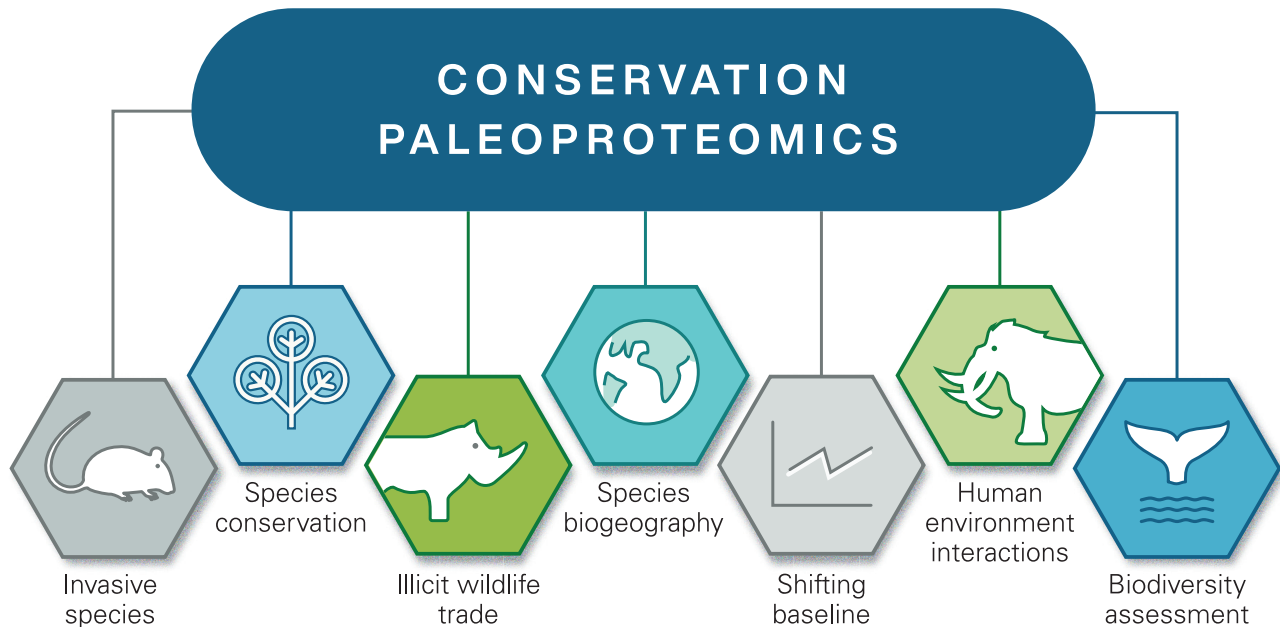


Figure 2. Seven key issues that conservation palaeoproteomics can target to help deliver more informed and effective conservation strategies.

Paleontological data have thus been recognized as a useful proxy to help better understand and contextualize biotic community structure and the dynamics of species richness over time (Dietl et al., 2015; Kidwell, 2015).

The application of palaeoproteomic techniques such as peptide mass fingerprinting has significant potential to help quantify and improve understanding of biodiversity in the past, complementing morphological analyses and increasing both the accuracy of faunal identifications as well as the number of taxonomically identified fossil remains at archaeological and paleontological sites. Among their advantages is that palaeoproteomic approaches allow the analysis of a broader diversity of materials for examining past species composition. Although the primary focus of analysis is often on a subset of morphologically identifiable specimens, palaeoproteomics offers the ability to study other faunal materials, including unidentifiable postcranial elements, bone tools, objects and their production waste (Ashby et al., 2015; McGrath et al., 2019; Jensen et al., 2020), taphonomically degraded material from owl pellets (Buckley et al., 2016; Buckley and Herman, 2019), and flowstone-encased faunal material (Harvey et al., 2019a), for example.

Perhaps its most significant advantage, however, is that ZooMS allows for the identification of fragmented faunal material. As noted, fragmented bone often makes up a significant proportion of archaeological and paleontological assemblages (Welker et al., 2015b; Brown et al., 2016; Sinet-Mathiot et al., 2019). Examples from the UK, Italy, and France show that ZooMS analyses of Late Pleistocene assemblages have significantly increased the number of identified faunal specimens, revealing a greater degree of taxonomic richness than previously understood (Welker et al., 2015b; Buckley et al., 2017b; Sinet-Mathiot et al., 2019). Proteomic analyses of fragmented remains from Final Mousterian and Uluzzian contexts at Fumane, Italy, for example, expanded biodiversity records at the site by providing evidence for two previously unidentified taxonomic groups of ecological and cultural importance, namely elephants (Elephantidae) and rhinoceroses (Rhinocerotidae) (Sinet-Mathiot et al., 2019). The analysis also increased the proportion of *Bos/Bison* specimens in the assemblage, suggesting that percussion-based carcass fragmentation of large *Bos/Bison* bone diaphyses severely fragmented the remains of this taxonomic group, making it difficult to identify the species morphologically, and biasing taxonomic assessments (Sinet-Mathiot et al., 2019). Meanwhile, peptide mass fingerprinting from the Châtelperronian contexts at Les Cottés, France, increased the number of identified faunal specimens by 30%. This was linked to a concomitant increase in taxonomic richness, with ZooMS yielding almost double the number of taxa identified through morphological analyses (Welker et al., 2015b).

Late Pleistocene contexts have so far provided the most well-developed examples of how palaeoproteomic methods can help to more accurately assess past species richness, in some cases taking researchers back to a point in time at which human impacts were more minimal. Holocene contexts also merit further investigation in this regard. Species identifications in these assemblages are often focused on large-sized animals that were exploited by humans (Crees et al., 2019), leaving taxonomic richness at the other end of the size spectrum poorly understood. Meanwhile, the study of marine diversity holds particular promise as a target of peptide mass fingerprinting because of the high variability of collagen sequences in fish species (Richter et al., 2011, 2020; Harvey et al., 2018). The ability to study morphologically uninformative marine remains with ZooMS provides great potential to significantly improve our understanding of biodiversity trends and baselines in marine ecosystems (see below).

ESTABLISHING ECOLOGICAL BASELINES

Ecological baselines often refer to ecosystem conditions perceived to predate human-mediated ecosystem transformation. These baselines are used in conservation biology to inform management strategies for species conservation and ecosystem restoration (Willis et al., 2010; Hofman et al., 2015). Ecological baselines are often based on local conditions before European colonization or widespread industrialization, drawing on the assumption that these were close to natural, prehuman baselines (Froyd and Willis, 2008). However, preindustrial as well as pre-European human impacts were frequently far more substantial than has been appreciated (Boivin et al., 2016; Ellis et al., 2016, 2021), meaning that we must look earlier in time, in some cases back to the Late Pleistocene to identify pre-anthropogenic baselines to inform conservation targets (Rodrigues et al., 2019).

Beyond this, climate change complicates the relationship between looking back to a 'natural' state and looking forward to a 'healthy' future ecosystem (Keulartz, 2016). Once again, past conditions provide critical information, for example, by providing insight into faunal functional diversity across varying climatic conditions (Svenning, 2020) and typical levels of community change (Williams et al., 2021). The documentation of ecological baselines offers insight into the climatic potential for biodiversity and mechanisms of long-term biodiversity maintenance (Svenning, 2020) as well as ecosystem responses to climatic stresses (Barnosky et al., 2017) and can thus help guide future restoration efforts under diverse climate change scenarios.

The utility of ZooMS for establishing baselines is highlighted by a number of recent studies focused on marine fauna (Biard et al., 2017; Harvey et al., 2018, 2019b; Rodrigues et al., 2018; Richter et al., 2020). Gray whale baselines, for example, have been clarified using collagen fingerprinting. A coastal whale species with extant populations in the Pacific Ocean, gray whales were also previously present in the North Atlantic, though the paucity of historical records for the species suggested they were naturally rare there (Clapham and Link, 2007). Collagen fingerprinting, together with molecular genetics and radiocarbon dating, has now clarified that the species began to decline in the Western Atlantic Basin in the Late Pleistocene (Garrison et al., 2019). Meanwhile ZooMS studies in the Western Mediterranean not only extend the known distribution of the gray whale but also reveal that the species was likely relatively common there as recently as the Roman period (Rodrigues et al., 2018). Such studies help to reveal the complex climatic and anthropogenic factors that have shaped cetacean population dynamics through time, including the role of forgotten whaling industries (Rodrigues et al., 2019). Such information can be drawn upon to more accurately evaluate population changes, supporting existing IUCN and Living Planet Index frameworks that aim to understand the impact of human activities on present-day ecosystems (Rodrigues et al., 2019).

Meanwhile, ZooMS has also provided crucial information on human exploitation of marine turtles in several regions (Harvey et al., 2019b; Peters et al., 2021; Winter et al., 2021). Species identification of sea turtles among faunal assemblages is often challenging because of the lack of robust osteomorphological reference material as well as the fragmentary nature of many turtle remains (Winter et al., 2021). ZooMS data has been combined with other molecular methods to begin to help clarify pre-commercial fisheries baselines in the Mediterranean, where the paucity of historically-informed baselines has made it challenging to gauge human impacts and the ecological potential of turtle taxa when setting conservation targets (Winter et al., 2021). The utility of ZooMS in providing historical baseline data for marine turtles has also been highlighted in the Caribbean, where the method enabled identification of less morphologically diagnostic

young turtles and highly fragmented remains from collections that had been stored for almost one hundred years in less-than-optimal microclimatic conditions (Harvey et al., 2019b).

DETECTING SHIFTS IN SPECIES ABUNDANCE AND GEOGRAPHIC RANGE

Global warming and human activities are significantly impacting the species abundance and geographic range of many faunal species. Knowing which species are likely to migrate and to where in response to climate change and other anthropogenic impacts is crucial to the development of appropriate conservation and mediation strategies. Accordingly, improving our understanding of species abundance and geographic ranges and predicting shifts in these parameters are important goals of ecological research (Willis and Birks, 2006; McGuire and Davis, 2014; Dietl et al., 2015). Meta-analyses of archaeofaunal and paleoclimatic data can provide insights into species abundance and geographic ranges during past climatic conditions, helping to improve prediction of future shifts in biogeography and guiding management strategies for the future (Lyman, 2012; McGuire and Davis, 2014; Hofman et al., 2015).

ZooMS offers useful potential for helping track species abundance and range shifts. Some of this potential is revealed in studies of cave contexts because caves are important in the life histories of many species, serving as dens for predators, hibernation locations for bats, and roosting sites for birds, for example. Caves also offer important reservoirs of paleontological specimens, trapping bones in depositional settings, which may be conducive to long-term preservation depending on geological, hydrological, and other conditions. Faunal assemblages from cave deposits are accordingly a useful resource for assessing and reconstructing past geographical species ranges (Frick et al., 2020). ZooMS analyses of Late Pleistocene microfauna remains from Pin Hole Cave, England, revealed the geographical presence of several now extirpated species, including horseshoe bat (Buckley and Herman, 2019) and moor frog (Buckley and Cheylan, 2020). Palaeoproteomic identification of eggshell remains from El Miron Cave, Spain, revealed that it once lay within the geographical range of the bearded vulture (Demarchi et al., 2019).

ZooMS has also been used to suggest shifts in species abundance. An intriguing ZooMS study of bone and antler combs from archaeological sites in Denmark shows increasing sourcing of antler from reindeer, located far to the north in the circumpolar subarctic and boreal zone. This may reflect growing pressure on local populations of red deer and a concomitant decrease in their abundance (Ashby et al., 2015). This study highlights the utility of crafted artifacts as well as production waste in reconstructing past biodiversity shifts. But caution in the interpretation of such patterns is also warranted. Another ZooMS study of Mesolithic bone points from southern Scandinavia, for example, showed that raw material selection reflected not just biodiversity changes but also cultural choices (Jensen et al., 2020).

DISENTANGLING HUMAN-ENVIRONMENT INTERACTIONS

Human activities are having a major impact on ecosystems all around the globe, but teasing human impacts apart from natural processes is not always a straightforward task. Investigation of the archaeological record can provide new insight into human-environmental relationships in the past, helping to disentangle human impacts and natural processes (Dietl and Flessa, 2011; Hofman et al., 2015; Barak et al., 2016) and supporting efforts to conserve present-day ecosystems that are threatened by human-induced climatic and environmental changes.

Studies of megafaunal extinctions are one area where ancient protein studies are poised to make an important contribution. The extinction of many megafauna species at the end of the late Quaternary led to a significant global loss of biodiversity and ecosystem function (Sandom et al., 2014; Malhi et al., 2016; Galetti et al., 2018). The exact dynamics, relative importance, and interactions of human and climatic drivers as potential drivers of these megafauna losses remain debated. Providing better data on past megafauna distributions across space and time will be important for strengthening analyses of extinction dynamics and their drivers. Yet, the underrepresentation of dated megafaunal fossils in the archaeological and paleontological records currently problematizes precise estimates of extinction chronologies and geographical ranges for many megafauna taxa (Price et al., 2018b; Swift et al., 2019). As part of combined, multidisciplinary investigations, ZooMS studies have the potential to contribute significantly to our understanding of both, especially now that peptide markers for megafauna species are becoming increasingly widely available (Buckley et al., 2011, 2017a; Van der Sluis et al., 2014; Welker et al., 2015b; Mychajliw et al., 2020). The ability of ZooMS to identify ancient hominin remains (e.g., Brown et al., 2016; Welker et al., 2016) can also contribute to

refining dispersal chronologies and clarifying the chronological overlap between human arrival and megafauna extinctions.

Parallel to efforts to understand the cause of megafaunal extinctions, there is also interest in their effects. Today there is increasing appreciation for the ecological importance of megafauna (Bakker et al., 2016; Malhi et al., 2022) and the changes to vegetation cover, ecosystem structure, biogeochemical cycling and land surface albedo that resulted from megafaunal loss (Doughty et al., 2013). Efforts to restore these lost ecosystem functions have resulted in trophic rewilding projects in which locally extirpated or novel keystone species are being reintroduced (Lorimer et al., 2015; Cortlett, 2016; Svenning et al., 2016). By contributing to a better understanding of past species ranges and the effects of megafaunal extinctions on broader ecosystems, ancient protein studies have significant potential to inform and strengthen rewilding science and practice (Dietl et al., 2015; Svenning et al., 2016). Notably, improved understanding of past megafauna distributions and ecologies is crucial for informing on megafauna recovery potential and its ecological importance (Monsarrat and Svenning, 2021), for example, in relation to large-herbivore assemblage structure (Schowanek et al., 2021) and associated effects on plant migration rates (Fricke et al., 2022). In addition, improving assessments of megafauna recovery potential is important for informing restoration efforts to enhance climate mitigation and adaptation (Malhi et al., 2022).

TRACKING THE INTRODUCTION OF NON-NATIVE SPECIES

The introduction of non-native species can have a severe impact on local biodiversity as well as ecosystem structure and function and can ultimately lead to extirpation, trophic cascades, and even extinction of native species (Gurevitch and Padilla, 2004; Hofman et al., 2015; Boivin et al., 2016; Barnosky et al., 2017). Improvements in the identification and tracking of introduced species are thus of critical concern for generating appropriate conservation strategies (Dietl and Flessa, 2011; Prendergast et al., 2017; Hofman and Rick, 2018). However, some issues are not always clear, such as knowing whether a species is introduced or endemic and the effect it had on the local ecosystems (Barak et al., 2016; Barnosky et al., 2017). Further complicating the situation are instances in which non-native species have functionally replaced exterminated native species (Lundgren et al., 2020). Data on the past is increasingly central to efforts to understand and address issues concerning introduced species and their impacts (Gurevitch and Padilla, 2004; Willis and Birks, 2006).

Palaeoproteomic methods are increasingly being employed to help track the introduction of non-native species, clarifying the status of potentially invasive species, and allowing more effective examination of their subsequent impact on ecosystems. Several studies, for example, have used peptide mass fingerprinting to track the introduction to island ecosystems of murid rodents, which negatively impacted endemic island fauna through habitat degradation and resource competition (Shiels et al., 2014). The Iron Age introduction of the black rat, a native of Asia, to the eastern African coast has been tracked using a combination of peptide mass fingerprinting, aDNA, and dental morphology. This study showed that the black rat was introduced to eastern Africa by the mid-first millennium CE (Prendergast et al., 2017). ZooMS has also been used to trace the arrival of murid rodents to the Cayman Islands following human colonization and to shed further light on their impact on endemic fauna (Harvey et al., 2019a).

Peptide mass fingerprinting is also increasingly being used to directly track the spread of a broad range of domesticated species, including in eastern Africa (Culley et al., 2021; Janzen et al., 2021), southern Africa (Le Meillour et al., 2020; Coutu et al., 2021) and central and eastern Asia (Taylor et al., 2018, 2020). Species-specific palaeoproteomic identification of milk proteins in human dental calculus (Wilkin et al., 2020, 2021; Bleasdale et al., 2021) has further contributed to this effort and is particularly useful in regions where zooarchaeological evidence is lacking for taphonomic or cultural reasons. Studying the introduction of domesticated species is critical to conservation efforts as many have played a significant role in transforming local ecosystems (Boivin et al., 2016; Hofman and Rick, 2018). The introduction of herd animals, for example, has shaped the formation of open landscapes (Ventresca Miller et al., 2020) as well as soil enriched hotspots (Marshall et al., 2018), and in many of the world's islands, domesticated and commensal species introduced by early colonizing populations have had a significant impact on endemic plant and animal populations (Boivin et al., 2016). Proteomic and other biochemical analyses of coprolites from a Neolithic settlement on the Orkney Islands have provided further evidence of such trends, revealing the consumption of local micromammals by domestic dogs (Romaniuk et al., 2020).

IDENTIFYING ILLICITLY TRADED MATERIAL

The international trade in wildlife and wildlife-derived products includes a considerable number of illicitly traded materials originating from endangered species (Galimberti et al., 2015). Many specimens that are illicitly traded are heavily processed or consist only of fragmentary remains (Jacobs and Baker, 2018) and are thus difficult to identify based on morphology alone. DNA-based methods are already being used to identify wildlife products (Eaton et al., 2010; Galimberti et al., 2015; Jacobs and Baker, 2018). However, proteomic approaches offer a quicker and cheaper means to identify these specimens and thus have the potential to aid the identification of illegally traded materials in resource and funding-limited contexts.

The application of ZooMS on collagen-based materials such as bone and ivory (Coutu et al., 2016; Coutu and Damgaard, 2019) has the potential to provide taxonomic identification of illicitly traded material. There is also emerging interest in using peptide mass fingerprinting of keratinous materials (Solazzo et al., 2011, 2013), such as hair, horn, and baleen, to identify illicit trade, such as of rhinoceros horn powder (Price et al., 2018a) or contraband fiber (Azémard et al., 2021). In South America, for example, wild camelids are endangered by poaching and black-market sale of their fibers, and proteomic analysis can be used to help identify the origin of confiscated animal fibers particularly when DNA is degraded because of taphonomic and diagenetic processes (Azémard et al., 2021). At the same time, peptide markers still require validation, diagnostic peptides are infrequently detected, and issues of hybridization challenge proteomic identification of domestic camelid species in particular. Yet, diagnostic peptides, when present, appear to enable absolute identification of vicuña fiber. As producers of the finest camelid fiber, vicuña was poached almost to extinction before receiving protection; however, they still suffer from illegal hunting and sale, making them a species of significant conservation interest.

PRIORITIZING SPECIES FOR CONSERVATION

Palaeoproteomic techniques also hold significant potential to help resolve phylogenetic relationships between extant and extinct species and are particularly beneficial when aDNA is not preserved. Analysis of 1.77-million-year-old protein sequences has been used to assess phylogenetic relationships between extant and extinct rhinoceroses, for example, revealing a close relationship between the extinct *Coelodonta* and *Stephanorhinus* and extant *Dicerorhinus* (Welker et al., 2017; Cappellini et al., 2019). Similarly, the phylogeny of recently extinct endemic South American ungulates has been resolved using palaeoproteomics (Welker et al., 2015a). Palaeoproteomic analyses of ancient collagen have also been used to generate phylogenetic reconstructions of several species of extinct West Indies island-shrews. This research revealed the presence of a number of distinct clades and species in this biodiversity hotspot, with interpopulation variability perhaps attributable to sexual dimorphism, providing new insights into the evolution and biogeography of these extinct species (Buckley et al., 2020). By improving our understanding of the origin, evolution, and distribution of this extinct shrew lineage, proteomic sequencing helps improve estimates of past species richness, which has been obscured by the magnitude of recent extinctions in the Caribbean (Buckley et al., 2020). Such data help improve understanding of faunal community structure in the West Indies today, broadly contributing to regional conservation management strategies.

Although the application of palaeoproteomics for phylogenetic reconstruction is a nascent area of research, its potential application in conservation assessments deserves further attention moving forward especially in biogeographic regions where aDNA is poorly preserved. Estimates of phylogenetic diversity are often used to prioritize species for conservation, with preservation of species diversity, genetic variation, and unique evolutionary histories as the ultimate goal (Rolland et al., 2012; Pellens and Grandcolas, 2016; Upham et al., 2019). Phylogenetic reconstructions of modern and extinct taxa can also help resolve past dispersal and extinction events that have shaped biotic communities today (Lamsdell et al., 2017), estimate biogeographical ranges of past lineages (Lawing and Matzke, 2014), and identify possible replacements for extinct species in rewilding projects (Svenning et al., 2016).

THE FUTURE OF CONSERVATION PALAEOPROTEOMICS

The modern biodiversity crisis is one of the most pressing challenges of the Anthropocene. As researchers increasingly recognize the importance of information about the past to studying, understanding, and conserving biodiversity today, a novel suite of methods is being brought to bear on resolving long-standing as well as emerging questions in biodiversity research. We demonstrate that the key among the newest

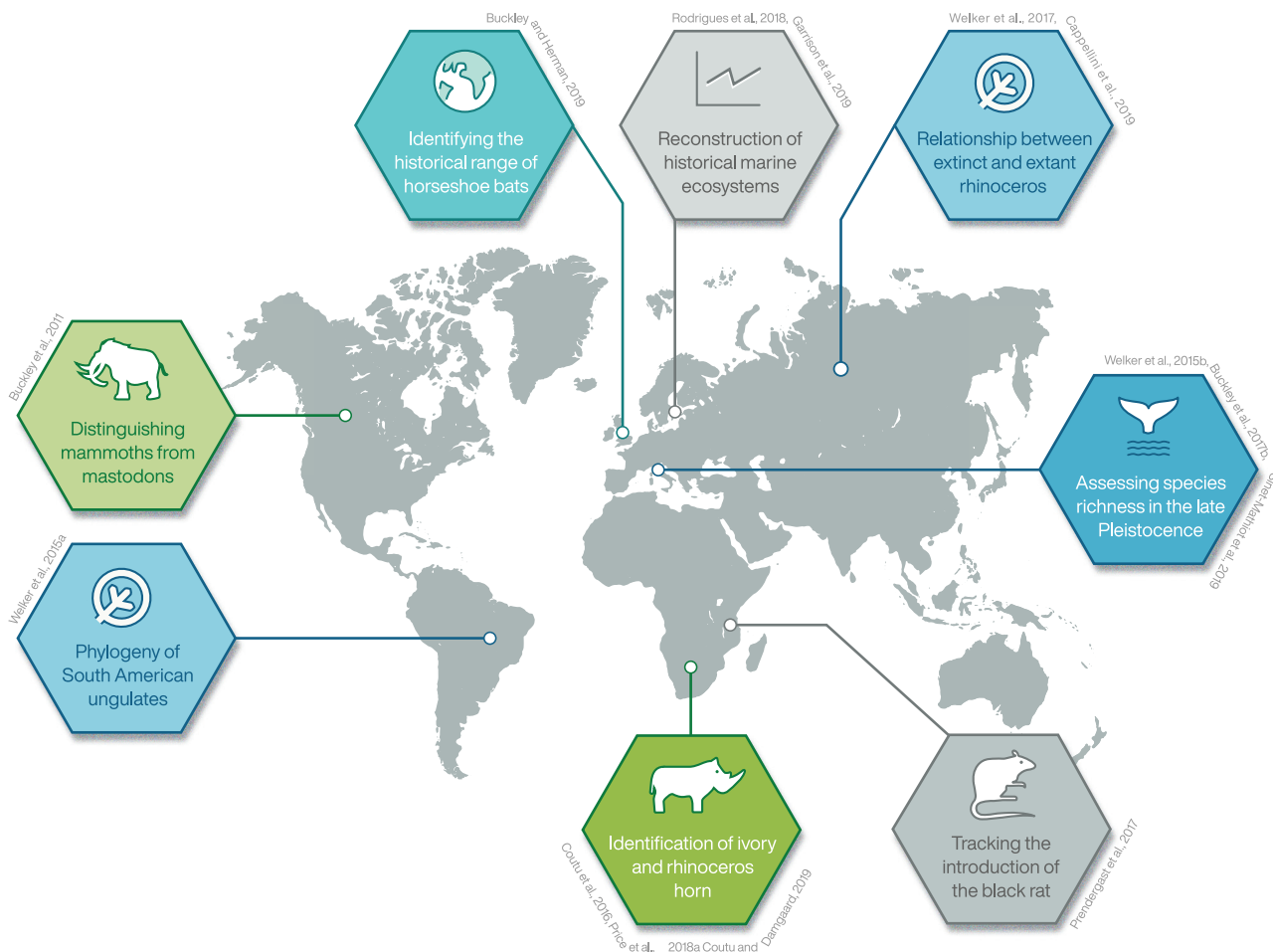


Figure 3. Case studies highlighting the potential of palaeoproteomics techniques to address key conservation requirements.

and most cutting-edge tools is palaeoproteomics, a method that draws on the long-term preservation of certain proteins and the taxonomic information contained within them. Our review demonstrates that although there are still challenges that need to be addressed, palaeoproteomics has the potential to contribute to improved recovery of a range of different types of data useful to conservation efforts (Figure 3). Although palaeoproteomics has only been minimally instrumentalized in biodiversity, conservation, restoration, and rewilding research to date, numerous studies already point to its significant potential to address issues ranging from establishing baselines and assessing range shifts to tracking the spread of introduced species.

At the same time, the application of palaeoproteomics for conservation purposes does not come without caveats. Protein-based methods are still at an early stage of development, and more work is needed to address the challenges of contamination and ancient protein authentication, particularly in shotgun proteomics studies (Hendy et al., 2018; Cleland et al., 2021). Further work is also needed to gain a better understanding of the degradation pathways of ancient proteins and the circumstances in which they preserve in the archaeological record (Van Doorn et al., 2012; Demarchi et al., 2016; Cleland et al., 2021). This research will provide important clarification as to how applicable palaeoproteomics will be in different regions of the world and for different time periods.

Beyond this, limitations to current reference libraries are also significant. The incompleteness of reference collections significantly shapes the taxonomic diversity recovered in palaeoproteomics studies. The majority of reference markers developed for ZooMS to date belongs to medium- to large-sized Eurasian mammals. Although recent years have seen the expansion of the reference library to include a larger variety of

ecologically interesting taxa such as micromammals (Buckley and Herman, 2019; Harvey et al., 2019a; Buckley et al., 2020), fish (Richter et al., 2011, 2020; Harvey et al., 2018), amphibians (Buckley and Cheylan, 2020), and reptiles (Harvey et al., 2019b), significant development of databases is still required. The lack of reference sequences at genomic or transcriptomic level for many species of interest further contributes to the paucity of available reference datasets (Sakalauskaite et al., 2020). To maximize the potential of palaeoproteomics for conservation research, more investment will be needed in developing the peptide markers and protein sequence data that are critical references for ancient protein studies.

On the other hand, the increasing number of reference data for taxa that are difficult to identify morphologically, such as micromammals, fish, birds, reptiles, and amphibians also represents critical progress. These taxa are important environmental indicators, and their more accurate and widespread identification offers significant potential to enable improved reconstruction of past ecosystems and associated assessments of recovery potential. Further growth of these reference datasets will no doubt pay significant dividends. Even with an expanded database, however, ancient protein studies will still be limited by the variability of protein sequences between taxa. This is specifically problematic for ZooMS studies, because COL1 is a functionally constrained protein with a slow rate of evolutionary change. In practice, this means that taxonomic resolution with ZooMS is often limited to family-level or genus-level resolution.

At a broader level, another key challenge is that it will take time and effort to integrate palaeoproteomics into existing conservation and restoration frameworks. Although zooarchaeology and paleontology have had decades to develop methods and metrics suitable to addressing conservation agendas, palaeoproteomics is only beginning to consider how its methods are suitable for this purpose. Accordingly, one of the major investments required in the near future will be the development of approaches that will enable incorporation of ZooMS data into the already well-established framework for faunal metrics that exists in zooarchaeology. Quantitative measures such as number of identified specimens (NISP), minimum number of elements (MNE), and minimum number of individuals (MNI) have been developed to address the issue of multiple bone fragments from the same individual, providing information critical to reconstructions of biodiversity changes through time. As ZooMS moves away from screening hundreds to thousands of fragmentary bones to a more holistic approach that is well-incorporated within existing zooarchaeological frameworks, such quantitative measures will need to be redefined.

Despite these initial caveats, the last decade has seen many exciting developments that will improve the applicability of ZooMS and other proteomics-based methods to a wider range of ecological and conservation questions in the future. Researchers have recently established a more standardized nomenclature system for ZooMS peptide markers (Brown et al., 2021a), for example. Development of open-source software for the automated examination of ZooMS spectral data, which would significantly decrease the time required for manual data analysis while simultaneously providing a way for researchers with only limited training to analyze ZooMS data is also underway (Gu and Buckley, 2018; Hickinbotham et al., 2020). In addition, new initiatives are in motion to develop minimally destructive screening methods, such as Fourier transform infrared spectroscopy (FTIR) and amino acid racemization, to assess the degree of molecular degradation of fossil material before paleoproteomic analysis (Pothier Bouchard et al., 2019; Kontopoulos et al., 2020; Presslee et al., 2021). The rapid identification of well-preserved specimens allows for the development of more suitable and sustainable sampling strategies that promote sustainable study of a fossil record that is not unlimited (Pálsdóttir et al., 2019). Finally, at a more practical level, the growing investment in new laboratories and palaeoproteomics facilities and the steadily rising number of trained ZooMS and palaeoproteomics researchers reflect the tremendous potential of ancient protein research and will increasingly allow a broader range of palaeoproteomics applications as the method becomes established in archaeology and paleontology.

Of course, the addition of biomolecular techniques to study the fossil record does not resolve its inherently biased nature as a result of site-specific formation histories and preservation conditions (Wolverton and Lyman, 2012), and palaeoproteomics is not a panacea for modern conservation efforts. Instead, it is the application of biomolecular approaches like palaeoproteomics in concert with more established methods like paleobiology, zooarchaeology, and paleontology that provides a powerful new conservation tool, with the potential to significantly expand our knowledge and understanding of ecosystems in the past as well as the ecology of both extinct and extant species (Evans et al., 2016; Faurby and Araújo, 2018; Mychajliw et al., 2020). As numerous examples cited here demonstrate, interdisciplinary studies that combine

palaeoproteomics with additional biomolecular methods such as stable isotope or aDNA analysis also offer significant potential. Combined palaeoproteomic and stable isotope methods have, for example, been used to investigate the dietary behavior of extinct giant tortoises on Mauritius (Van der Sluis et al., 2014) and the historical ivory trade in eastern Africa (Coutu et al., 2016), whereas other approaches have combined palaeoproteomics and aDNA (Biard et al., 2017; Prendergast et al., 2017; Taylor et al., 2018). We accordingly highlight the need for an interdisciplinary approach, involving close collaboration and communication between all relevant fields from the start of a project onward.

As archaeology increasingly reorients from a strict focus on the past to a wider remit that includes addressing the major challenges in the Anthropocene (Boivin and Crowther, 2021), palaeoproteomics is poised to become a critical tool in the discipline's toolbox. We emphasize the wide suite of questions this method is suited to address and the increasing contribution it can make as part of interdisciplinary investigations. Palaeoproteomics is not just a research tool but also offers a cheaper and in some cases more accessible approach to addressing certain practical conservation challenges, including the identification of illicitly traded wildlife material. We suggest that with sufficient investment and development, palaeoproteomics will become an important tool in historically-informed conservation research and practice in the coming decade, clarifying long-term trends and helping support a new phase of close collaboration between archaeological and biodiversity research initiatives.

LIMITATIONS OF THE STUDY

This review argues that palaeoproteomics has significant potential to inform conservation, restoration, and rewilding strategies. Studies that directly support this argument are not yet abundant. The purpose of this review is to raise awareness concerning how the analysis of ancient proteins can be leveraged to examine biodiversity and environmental changes, to encourage further research in this area.

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AUTHOR CONTRIBUTIONS

Conceptualization, C.P. and N.B.; Writing – Original Draft: C.P. and N.B.; Writing – Review & Editing, C.P., K.K.R., J.C.S., and N.B.; Supervision: N.B.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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