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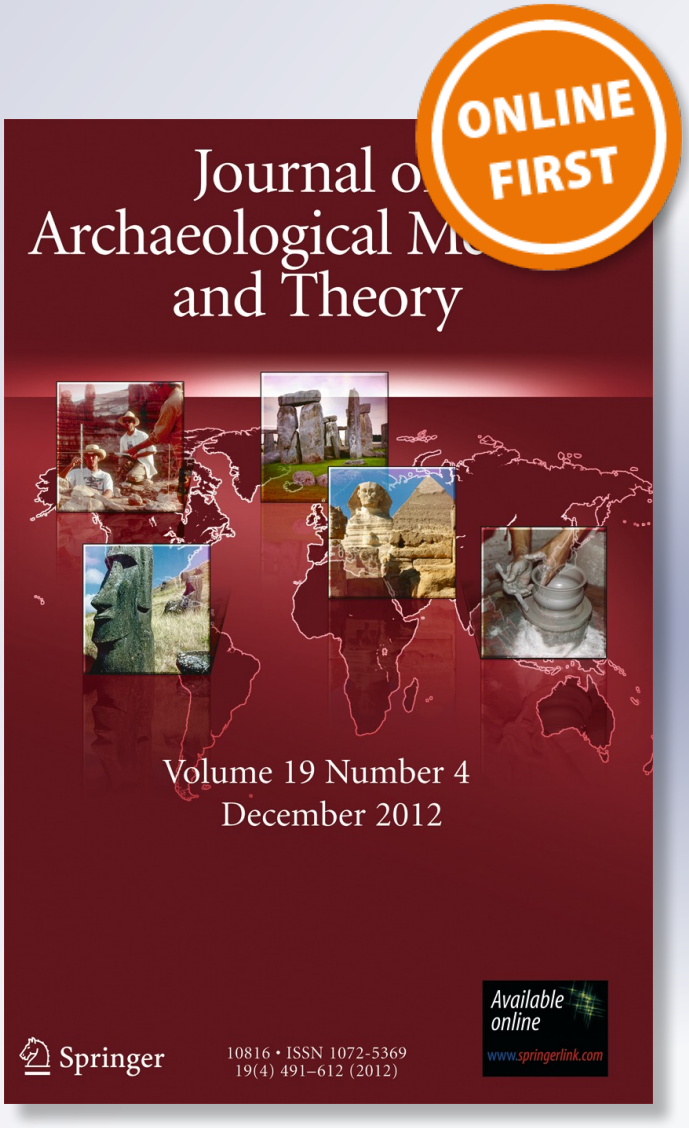

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A Late Holocene Population Bottleneck in California Tule Elk (*Cervus elaphus nannodes*): Provisional Support from Ancient DNA

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Abstract Zooarchaeological analyses have suggested a possible case of late Holocene resource depression in California tule elk (*Cervus elaphus nannodes*). We develop and conduct a preliminary independent test of this here based on trends in genetic diversity derived from ancient DNA extracted from archaeological elk bone. Mitochondrial DNA sequence data from 24 tule elk temporally dispersed across the late Holocene deposits of the Emeryville Shellmound, California, provide provisional support for a decline in genetic diversity and a population bottleneck beginning about 1600 B.P. Final confirmation of this pattern must await complete replication of the sequences. Stable isotope analyses of the elk bone provide a record of change in the terrestrial environment across the period of deposition and no suggestion that climate change may have played a role in an elk population decline. The analysis has implications for our understanding of change in human behavior and biology during late Holocene of central California, the methodology of resource depression analyses, and the conservation biology of tule elk.

Keywords Tule elk · Late Holocene · Ancient DNA · Resource depression · Climate change

Introduction

The extremely high densities of large game in California during the early historic period (early 1800s) astonished explorers, and their accounts of ungulate densities are

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routinely taken as benchmarks for the state's original or pristine zoological condition. Interpretations of California's indigenous peoples also have been deeply conditioned by these descriptions and the apparent abundance of the natural food supply. The indigenous harvesting strategies that some suggest may have promoted these faunal abundances have also been proposed as models for the management of wilderness areas and national parks today (e.g., Anderson 2005; Blackburn and Anderson 1993; but see also Berkes 2004; Berkes *et al*. 1995 for complexities on the role of traditional ecological knowledge in resource conservation). Many of these perceptions have been challenged recently by research grounded in behavioral ecology. That work suggests that times were anything but easy in many native California contexts and that resource stress brought on by late Holocene prey depressions and/or severe climatic disruptions provided the primary catalyst for a host of other changes in human behavior and lifeways. These include changes in technology and settlement patterns; increasing territoriality, violence, and warfare; increasing mortality, morbidity, and reduced adult stature; and perhaps even changes involving gender-differentiated reproductive effort, work organization, and fertility (e.g., Bartelink 2006; Raab and Jones 2004; Broughton *et al*. 2010). Importantly, there could be justification from this perspective to reverse the logic underlying proposals to implement indigenous harvesting strategies in wilderness management contexts. One suggestion is that insofar as native hunting had maintained low densities of ungulates in North American landscapes for millennia, "natural" environments should be managed to that end today (Kay 1994, 2002, 2007).

A secure documentation of either stability of ungulate populations or significant declines through time would thus be directly relevant to these far-reaching issues. The primary lines of evidence that have been used to this end so far include standard zooarchaeological data such as trends in the relative frequencies of identified animal bones from dated archaeological sites (e.g., prey abundance indices). For instance, the relative frequencies of elk (*Cervus elaphus*) bones decline over the last 2,000 years compared to smaller terrestrial mammalian prey in San Francisco Bay area sites; signals of harvest pressure in the age structure of exploited artiodactyl populations have been documented as well (Broughton 1999, 2002). Similar patterns have also been documented from a wide range of other economically attractive species of marine invertebrates, fishes, birds, and mammals (e.g., Braje 2010; Braje *et al*. 2007; Broughton *et al*. 2007; 2010; Erlandson and Rick 2010; Grayson 2001; Hildebrandt and Jones 1992, 2002; Porcasi *et al*. 2000; Rick 2011). These analyses suggest that historic period reports of large game abundances may stem from earlier—sixteenth and seventeenth century—European disease-based declines in aboriginal human populations and subsequent protohistoric period large game rebounds (e.g., Broughton 2002; Broughton *et al*. 2010).

Still, these archaeological measures provide only indices of past prey-encounter rates and the relationship between the latter and actual population sizes is clearly complex. We have no theory to guide us in estimating what a decline from say 75 % elk bones in one sample to 5 % in another means in terms of the underlying elk population size. It is quite possible, for example, that ever diminishing numbers of elk bones through time may not be reflecting broad-scale population declines, but rather more localized movements of elk herds away from areas with densely settled human populations. Taphonomic and quantification issues are, of course, always at play in

analyses that attempt to estimate trends in prey population sizes from archaeofaunal data (see Lyman 1994a, b; 2008). More refined tests capable of measuring population-level trends in prehistoric prey population sizes thus seem warranted—and since genetic diversity varies sensitively with population size (see review in de Bruyn *et al*. 2011), tracking trends in genetic diversity from archaeological faunal remains can provide a means of doing just that. In this paper, we develop and conduct such a test based on trends in genetic diversity derived from the mitochondrial DNA preserved in a late Holocene sample of tule elk (*Cervus elaphus nannodes*) from the Emeryville Shellmound, a large residential locality located on the eastern shore of San Francisco Bay. In addition to providing a novel independent test of the tule elk depression question in central California, our study has methodological implications for documenting the extent and more specific causes of resource depression that have been increasingly suggested from zooarchaeologial analyses the world over. These data will also be useful in guiding management strategies that rely increasingly on modern analyses of genetic diversity. For tule elk, considerable genetic analysis of extant populations has been recently conducted, but these studies have been hampered by the lack of historical genetic data from which to compare and assess current patterns. The analysis may thus contribute to the applied zooarchaeology that Lyman (e.g., 1988; 1994a, b; 1996; 2011; Lyman and Cannon 2004) has promoted for decades.

Tule Elk Demographic History

Three native subspecies of elk have long been recognized to occur in California based on morphological differences: tule elk, Roosevelt elk (*Cervus elaphus roosevelti*) and Rocky Mountain elk (*Cervus elaphus nelsoni*). Subspecific status for each has more recently been supported by genetic analyses (e.g., Polziehn *et al*. 1998, 2000; Polziehn and Strobeck 2002; Meredith *et al*. 2007). In California, the distribution of Roosevelt elk is confined to the thickly forested regions of the northwest, while Rocky Mountain elk occur in limited numbers within a narrow swath of the extreme northeastern part of the state. Tule elk were far more widespread, occupying much of California's extensive lower elevation oak woodland and perennial grassland habitats including the vast Central Valley, the San Francisco Bay area, and adjacent coastal hills and valleys. With their large size, impressive antlers, graceful stature, and extremely high densities, tule elk commanded the attention of eighteenth and nineteenth century explorers and settlers. Some herds were reported to contain over 3,000 head (Preston 1998:279). While population estimates are necessarily quite crude, McCullough and colleagues (McCullough 1969; McCullough *et al*. 1996) suggest as many as half a million animals lived in California prior to major Euro-American settlement.

The 1849 Gold Rush brought "a virtual tidal wave of human immigration" to California and an onslaught of unrestricted market hunting directly ensued (McCullough *et al*. 1996:375). By 1875, only 25 years later, the entire population of tule elk had crashed to as few as a single pair of animals. With the help of private land owners and full protection granted in 1873, the population grew slowly to 28 individuals by 1895. Modest growth during the twentieth century brought the population to 500 animals by 1971. Numerous relocation and conservation efforts since 1974 have raised the current total to about 3,800 individuals distributed across 22 disjunct herds scattered

across their historic-period range (Williams *et al*. 2004; California Fish and Game 2011). Conservation management plans for tule elk have increasingly been informed by genetic analyses that have revealed, among other patterns, extremely low levels of genetic diversity in current herds that is consistent with, and interpreted as, a direct result of the nineteenth and twentieth century population bottleneck (e.g., Williams *et al*. 2004; Meredith *et al*. 2007; Cronin *et al*. 2009). However, the question remains "how low is low" (Williams *et al*. 2004:118) since no pre-nineteenth century genetic diversity baselines are currently available for tule elk. The potential impact of earlier pre-Columbian hunting-based bottlenecks on current levels of genetic diversity has also not been entertained in these studies, but as noted above, has been recently suggested from analyses of zooarchaeological data.

Specifically, a prehistoric population bottleneck in tule elk has been proposed on the basis of patterns in the relative abundances of their bones derived from dated archaeological faunas. Reconstructing population trends from archaeological abundance data are founded on logic from foraging theory models, especially the prey model (see Stephens and Krebs 1986). As has been discussed in detail elsewhere, the model predicts that the relative frequency with which prehistoric foragers selected high- and low-ranked prey within a resource patch can provide an index of the encounter rate of high-ranked prey. Hence, decreasing frequencies of high-ranked prey species should be a measure of declines in the encounter rate and at least the local density of the species in the surrounding environment over the time the fauna accumulated (Bayham 1979, 1982; Broughton *et al*. 2011).

Empirical data demonstrate that for many classes of animal prey that are singly handled by human consumers, post-encounter return rates (i.e., prey ranks) are closely scaled to prey body mass (see Broughton *et al*. 2011 for a recent review). Recent research on modern hunter–gatherers further underscores the overriding significance that hunters attach to prey size. Many hunters ignore small game, even when pursuing them would increase their overall caloric returns (e.g., Hawkes 1991; Hawkes *et al*. 1991). Clearly, smaller-sized prey move into and out of the set of targeted prey for human hunters, but large prey are invariably included.

Since the prey model predicts that the highest-ranked prey types should be attacked whenever they are encountered, large-sized species should be the most susceptible to hunting-based depressions. This feature is exacerbated by the fact that large species also tend to exhibit delayed sexual maturity, slower growth rates, longer lifespans, and lower intrinsic rates of increase (e.g., Winterhalder and Lu 1997). As long as assumptions of the prey model are met, declining relative abundances based on abundance indices of those taxa should signal reductions in their encounter rates. Such data do not reveal, however, the specific cause or causes for those reductions. Climatic deteriorations, local movement of animals out of contexts heavily populated by human predators, or hunting-based regional population-level declines may all be at work.

The most detailed data sets bearing on late Holocene trends in archaeological elk remains are derived from the San Francisco Bay area (Fig. 1). Figures 2, 3, and 4 show the changing abundances of elk specimens compared to all other terrestrial vertebrates across: (a) 18 dated components from sites distributed across the San Francisco Bay shoreline, (b) a tight cluster of sites located in the Coyote Hills area of the southeast bay, and (c) the occupational history of the Emeryville Shellmound (data from Broughton 1994, 1999). To maintain consistency with previously

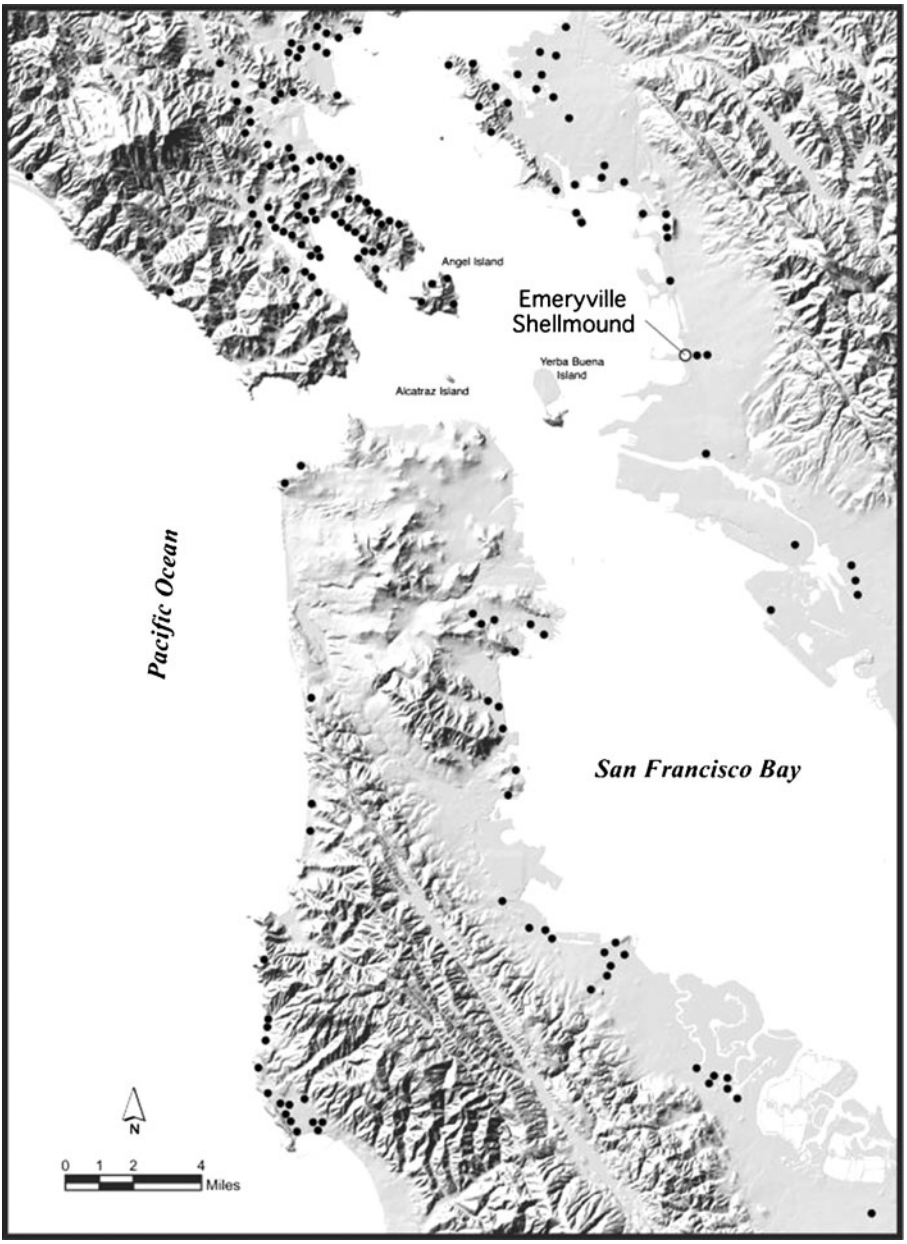

Fig. 1 Map of San Francisco Bay area showing location of the Emeryville Shellmound and other shellmound sites

published literature in this context, uncalibrated radiocarbon years before present is the timescale used throughout. Using Cochran's Chi-square test of linear trends that takes the underlying sample sizes into account (Cannon 2001), each case exhibits significant linear declines in the relative abundance of elk compared to other terrestrial mammals (San Francisco Bay region, $X^2_{trend}=75.67$, $P<.001$; Coyote Hills,

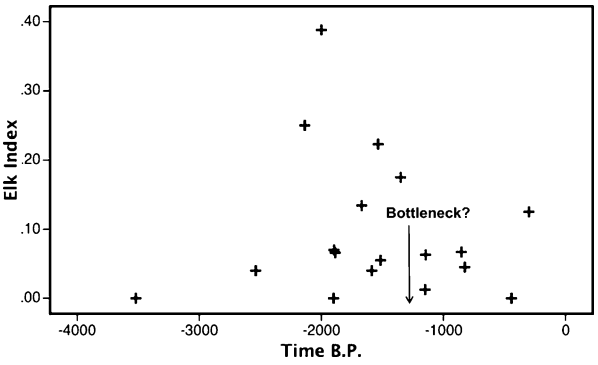

Fig. 2 Distribution of the Elk Index (Σ[NISP Elk]/ΣNISP [Terrestrial Mammals]) across 18 dated components from sites distributed across the San Francisco Bay shoreline (total NISP=9,229). (Does not include potentially intrusive rodents and lagomorphs)

X^2_{trend}=102.63, P<.001; Emeryville X^2_{trend}=484.8, P<.001). In each context, substantial declines are evident between about 1600 and 1200 B.P. These patterns may suggest that San Francisco Bay area tule elk experienced a substantial prehistoric population bottleneck over this time. Alternatively, the diminishing numbers of elk may not be reflecting broad population declines, but the movement of elk herds out of the densely settled bayshore context. And although these apparent linear declines do not appear to correlate with existing reconstructions of paleoenvironmental change (e.g., Broughton 1999; Ingram *et al*. 1996; McGann 2008; Malamud-Roam *et al*. 2007; Goman *et al*. 2008), we examine that possibility in more detail below based on analyses of the stable isotope chemistry of a sample of Emeryville elk bones.

In this context, we emphasize that the relatively high, possibly even rising, frequencies of elk at the beginning of the San Francisco Bay sequence between 2500 and 2000 B.P. (see especially Fig. 4) are consistent with the hypothesis developed elsewhere (Broughton and Bayham 2003; Byers and Broughton 2004; Broughton *et al*. 2008) that substantial expansions of artiodactyl populations occurred as climate ameliorated at the beginning of the late Holocene, between roughly 4500 and 2500 B.P. (e.g., Anderson and Smith 1994; Benson *et al*. 2002). Thus, prior to the proposed late Holocene anthropogenic depressions, elk populations may have in fact been on the rise in this setting. Since genetic diversity can also reflect increases in population size, such analyses may eventually inform on the nature (e.g., growing, stationary) of the hypothetical pre-bottleneck elk population.

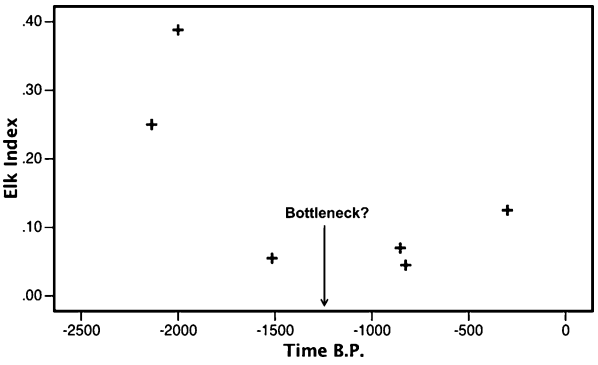

Fig. 3 Distribution of the Elk Index (Σ[NISP Elk]/ ΣNISP [Terrestrial Mammals]) across a tight cluster of sites located in the Coyote Hills area of the southeast bay (total NISP= 2,168). (Does not include potentially intrusive rodents and lagomorphs)

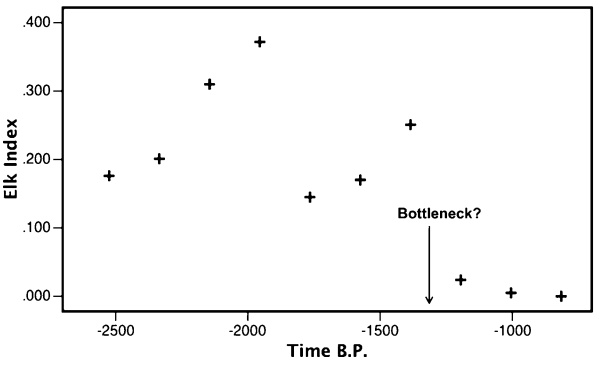

Fig. 4 Distribution of the Elk Index (Σ[NISP Elk]/ΣNISP [Terrestrial Mammals]) across the occupational history of the Emeryville Shellmound (total NISP=6,032). (Does not include potentially intrusive rodents and lagomorphs)

Genetic Variation and Population Bottlenecks

Genetic variation within a population can be measured in a variety of ways and tends to be high in large populations and low in small ones: population declines or bottlenecks are thus signaled by declines in genetic variation (e.g., Beck 2009; Frankham 1996; Glenn *et al*. 1999; Hoelzel *et al*. 2002; Rogers 1995; de Bruyn *et al*. 2011; Hadly *et al*. 1998). There are many examples showing that genetic variation responds to experimental manipulations of population size (Frankham 1996; Montgomery *et al*. 2000) and historical changes in population size have also produced the expected changes in genetic variation in a number of vertebrate taxa (e.g., Glenn *et al*. 1999; Hoelzel *et al*. 2002; Larson *et al*. 2002; Leonard 2008; Weber *et al*. 2004). Some of these studies have focused attention on the genetic effect of historically documented over-hunting on several taxa such as in elephant seals (Hoelzel *et al*. 2002), fur seals (Weber *et al*. 2004), sea otters (Larson *et al*. 2002), and whooping cranes (Glenn *et al*. 1999) among others. Finally, the relationship between genetic variation and population size, coupled with established ancient DNA methods has been used successfully to reconstruct prehistoric population trends in Hawaiian geese (Paxinos *et al*. 2002), brown bears (Calvignac *et al*. 2008; Barnes *et al*. 2002; Leonard *et al*. 2000), cave bears (Hofreiter *et al*. 2002; Bon *et al*. 2011), steppe bison (Shapiro *et al*. 2004), grey wolves (Pilot *et al*. 2010), musk ox (Campos *et al*. 2010), southern elephant seals (de Bruyn *et al*. 2011), and caribou (Kuhn *et al*. 2010); we use it here to monitor changes in the size of the San Francisco Bay elk population across the late Holocene.

A Prehistoric Bottleneck in San Francisco Bay Tule Elk: Hypotheses and Methods

Specific Hypotheses

There are a variety of conceivable research designs that could be implemented to test the general hypothesis that prehistoric hunters caused a population bottleneck in California tule elk. Diachronic trends in genetic variation derived from multiple geographic contexts across the state spanning the past 10,000 years into the historic period would, of course, be ideal. Unfortunately, elk remains are typically uncommon

in California archaeological deposits, except for the San Francisco Bay area (see Broughton 1994, 1999; Hildebrandt and Jones 1992). And even here, the vast majority of elk specimens are derived from a single site: the Emeryville Shellmound. That site thus provides the most substantial, well-documented archaeological elk sequence that exists in California. Fortunately, the site spans the period over which the hypothesized bottleneck occurred—indeed, that hypothesis is based in part on the pattern of elk abundances derived from Emeryville (Fig. 4).

Focusing on a single archaeological context also has decided advantages in the extraction and amplification of ancient DNA. Subtle differences in depositional and taphonomic context affect polymerase chain reaction (PCR; see below) optimization processes. Slight variation in sediment chemistry, pH, and moisture, for example, all affect the efficiency of any PCR amplification protocol. Successful and consistent amplification of ancient DNA requires considerable effort to find the most effective ratios of PCR reaction reagents and these optimized protocols change from one archaeological context to another. For all these reasons, we have chosen to focus initially on elk specimens recovered from the Emeryville Shellmound. Future, more comprehensive tests will involve specimens from other sites. Our focus on this locality does, however, have a variety of implications for our research design that relate to the high mobility of elk. These are addressed in the following more specific hypotheses that pertain to temporal patterns in elk genetic variation derived from the Emeryville locality.

Hypothesis A The tule elk inhabiting the San Francisco Bay area and represented at Emeryville were not isolated from the larger population of tule elk that occupied other regions of California (i.e., the Central Valley, southern Coast Range). The decline in elk numbers at Emeryville represents a microcosm for a subspecies-wide population decline. This hypothesis predicts a decline through time in genetic variation of the Emeryville elk.

Hypothesis B The elk remains recovered from the Emeryville Shellmound were derived from a San Francisco Bay area sub-population of tule elk that was effectively isolated from the larger population in other regions due to population fragmentation. The decline in elk numbers registered at Emeryville reflects a reduction in the population size of elk in this region and this reduction was not offset by immigration from the outside. This hypothesis also predicts a decline in genetic variation for the Emeryville elk.

Hypothesis C Tule elk are highly mobile on a large scale. Even if the elk population declines around Emeryville, that population will continue to receive some immigrants from the larger surrounding region. If the larger region experienced no population decline, then the genetic variation will not decline in the Emeryville elk.

Hypothesis D The San Francisco Bay area tule elk population did not decline. The elk merely moved away from the densely populated bayshore context near Emeryville to other regions where they were more difficult to harvest. This hypothesis also predicts no decline in genetic variation.

A significant decline in genetic variation through time at Emeryville would thus imply a population decline either for the entire tule elk population (Hypothesis A), or

for an isolated San Francisco Bay area population (Hypothesis B). The formation of isolated sub-populations would, of course, imply population fragmentation or the extirpation of elk in the intervening areas. Distinguishing between Hypothesis A and B would, however, require additional genetic patterns derived from elk obtained from localities outside the San Francisco Bay area. Conversely, a failure to document a decline in genetic variation across the Emeryville sequence would suggest that the diminishing archaeological abundance of elk was caused by highly localized population declines or merely by behavioral adjustments of the animals and no declines at all. A decline or lack of decline in genetic variation from the Emeryville elk would thus provide a test of the main essence of the general elk bottleneck hypothesis.

Finally, we can define still another hypothesis:

Hypothesis E The tule elk population of the San Francisco Bay area was divided into several smaller herds, which were largely isolated. Reduction in the size of each herd would have two effects: it would reduce variation within each herd and increase variation between them.

This last hypothesis (E) seems least plausible given the high mobility of elk and is one that would require data from several Bay area sites; we do not test it here. Further hypotheses could also be developed that involve the analysis of genetic variation from protohistoric and/or historic-period elk samples insofar as sufficient samples from these contexts could be gathered.

Genetic diversity might also change for other reasons: a change in the mutation rate or the sweep to fixation of a favored allele. The first factor changes diversity only slowly and is therefore not a problem here. The possibility of a selective sweep, on the other hand, is real. Fortunately, such sweeps are rare (see above), and we would be unlikely to observe one during the two millennia represented at Emeryville.

The Tule Elk Sample from Emeryville

The Emeryville Shellmound was the largest of almost 500 shellmounds that lined the San Francisco Bay shoreline (Fig. 1). The mound measured roughly 100×300 m and extended to a depth of 10 m. During the early twentieth century, the mound was excavated on three occasions (by Max Uhle, Nels Nelson, and Egbert Schenck), each time in a different location. In these projects, most of the sediments were excavated stratigraphically and sieved with coarse-mesh screens; collectively, ten primary strata were revealed during this work. Fourteen radiocarbon dates have now been derived from charcoal and bone collagen materials distributed from the top to the bottom of the mound and bracket the deposition of the site between 2600 and 700 radiocarbon years B.P. There are no chrono-stratigraphic inconsistencies in the dates; namely, within each excavation the oldest dates are from the lowest strata, whereas the youngest dates are from the highest ones. Following Broughton (1999), this series of dates was used to establish the time span of deposition for the ten primary strata; on average, each stratum took about 200 years to accumulate. These excavations produced a total sample of 808 elk bones, among over 24,000 identified vertebrate specimens (Broughton 1999, 2004; see Wake 2003 for a summary of additional elk materials recovered from a final excavation in 1999). Formal subspecies level

taxonomic identifications were not attempted for these specimens, although the small size of the elements is consistent with tule elk, the only subspecies known to have occupied the San Francisco Bay area during the Holocene.

Since we do not wish to use the bones from the same individual animals in our calculations of genetic diversity, we took great care in identifying from the total sample of the Emeryville elk bones (NISP=808), those specimens that must have been derived from different elk. Specifically, we used the major well-defined strata from the three different projects as aggregation units prior to calculating minimum numbers of individuals (MNI). Within each aggregation unit, our determinations took into account the ontogenetic age, size, and side of the represented elements (see Lyman 2008 for discussion); a total elk MNI of 53 was established with this approach. Based on their stratigraphic placement and associated dating, the specimens were then assigned radiocarbon date ranges and midpoint values. Based on the evident decline in elk numbers in San Francisco Bay archaeological contexts as early as 1600 B.P., we then assigned specimens to hypothetical pre-bottleneck (i.e., pre-~1600 B.P.) and post-bottleneck (post-~1600 B.P.) groups. These analyses resulted in elk MNI values of 33 and 20 from the pre-bottleneck and post-bottleneck periods, respectively. From this sample, we selected 43 individuals for DNA analysis. We acknowledge that any number of cutoff dates between 1600 and 1200 B.P. would be reasonable here to split the samples into pre- and post-bottleneck periods—our decision to use 1600 B.P. was to ensure adequate samples falling in the post-bottleneck period as elk specimens are generally uncommon in deposits dating after 1200 B.P. at Emeryville. Future analyses with larger samples could allow for different comparisons (e.g., pre- and post-1500 B.P., pre- and post-1400 B.P., etc.) to identify more precisely the timing of changes in genetic diversity.

To provide a barometer of terrestrial climate change across the period these samples were deposited, the carbon, oxygen, and nitrogen isotopic composition was analyzed from a subset (n=16) of these specimens that produced ancient DNA sequences. These specimens were selected to obtain a balanced representation in both the pre- and post-bottleneck time periods.

Ancient Tule Elk DNA: Extraction, Amplification, Sequencing, and Statistical Analysis

DNA Extraction

Between 0.2 and 0.67 g of bone were removed from each specimen and surface decontaminated by soaking in 10 % bleach for 10 min and thoroughly rinsing with sterile water. The bone fragments were then dried overnight at room temperature and mechanically powdered with a stainless steel mortar and pestle. The powdered bone was incubated overnight at 56 ° C with constant agitation in 5 ml of digestion buffer consisting of 0.5 M EDTA, pH 8.0, and 250 μg/ml proteinase K. Digested bone powder was centrifuged for 5 min and 500 μl of the supernatant was added to 1.5 ml Dehybernation Solution A (MP Biomedicals) and 400 μl Ancient DNA Glassmilk (MP Biomedicals), then incubated 3 h at room temperature with constant agitation. DNA was then extracted using a GENECLEAN® for Ancient DNA Kit (MP Biomedicals) following the manufacturer's instructions. Between 4 and 7 specimens

were extracted at a time and a negative extraction control was included in each extraction batch.

PCR Amplification and Sequencing

PCR primers were designed with Primer 3 (Rozen and Shaletsky 2000) from the *C. e. nannodes* TULE457 mitochondrial D-loop complete sequence (GenBank Accession No. AF016976.1). Primers Cen_L676 (5′-AAATCGCCCACTCCTTGTAA-3′) and Cen_R847 (5′-GTCCCGCTACAATTCATGCT-3′) were selected to target a 172 base pair (bp) fragment of the mitochondrial D-loop including 132 bp of nonpriming sequence. A BLAST search of the NCBI nucleotide database with each primer was performed to ensure that these primers would preferentially amplify all sub-species of North American elk DNA and not potentially contaminating human DNA. There is a point mutation common to North American elk sequences at a single position on the forward primer (Cen_L676) and another single point mutation on the reverse primer (Cen_R847). In both cases, these mutations are singular and are not found on the first or final position and are unlikely to have biased our results.

PCR amplification was performed in 25 μl reaction volumes containing 2.0–5.0 μl ancient DNA extract, 0.5 μM of each primer, 200 μM dNTPs, 2.0 mM MgCl, 2× BSA, 1× GeneAmp® PCR Gold Buffer (Applied Biosystems), and 2 U AmpliTaq Gold® DNA Polymerase (Applied Biosystems). A negative PCR control was included in each amplification batch by substituting 2.0–5.0 μl water in the place of ancient DNA extract. After an initial polymerase activation step (95 °C, 5 min), 45 cycles of amplification (95 °C, 45 s; 45 °C, 45 s; 72 °C, 45 s) was followed by a final extension step (72 °C, 5 min) in an Applied Biosystems Veriti® thermal cycler. Seven microliters of the final PCR product was loaded onto a 3 % agarose gel stained with SYBR® Green (Applied Biosystems) and an appropriate size standard. The remaining PCR product of successfully amplified ancient DNA extracts was cleaned with an UltraClean PCR Clean-up Kit (MO BIO) following the manufacturer's instructions and was submitted for bidirectional sequencing at the University of Utah Health Sciences Center Core Sequencing Facility.

DNA Sequence Authentication and Contamination Controls

Contamination of PCR reactions by exogenous DNA templates is one of the most serious problems confronting ancient DNA research (Gilbert *et al*. 2005a, 2006; Kaestle and Horsburgh 2002; O'Rourke *et al*. 2000; Pääbo *et al*. 2004; Willerslev and Cooper 2005; Yang and Watt 2005). The ancient DNA laboratory in the Department of Anthropology at the University of Utah maintains a series of protocols designed to minimize the potential for contamination in ancient DNA research. DNA extractions and PCR set-up is conducted in a dedicated ancient DNA clean room with positive pressure HEPA-filtered ventilation and integrated UV lights. Inside the clean room, individual bench-top enclosures are used and surfaces are cleaned with a bleach solution before and after each use. Equipment, tubes, and most reagents are UV cross-linked prior to use. To monitor for potential contamination, including the possibility of cross-contamination of reactions, multiple negative controls are included at every step. These negative controls are processed in exactly the same manner as

are those tubes that contain DNA template and are carried through the entire amplification process.

In addition to PCR contamination, ancient DNA sequences can be compromised by a number of complications including post-mortem DNA damage (Gilbert *et al*. 2005b, 2003; Hofreiter *et al*. 2001a, b; Pääbo *et al*. 2004; Willerslev and Cooper 2005) and nuclear insertions (Bensasson *et al*. 2001; Martin 2003; Mourier *et al*. 2001; Willerslev and Cooper 2005). We used a rigorous DNA sequence authentication protocol appropriate to the risk of this project generating faulty sequences (e.g., Gilbert *et al*. 2005b). To ensure that sequences made taxonomic sense, each acquired sequence fragment was used to search the NCBI nucleotide database using the BLAST search tool. The local alignments generated by this search were then used to guide manual trimming of low-quality bases from raw sequence files. Quality trimmed forward and reverse sequences were then aligned to the *C. e. nannodes* TULE457 mitochondrial D-loop complete sequence (GenBank Accession No. AF016976.1) and combined to generate the sequences used in subsequent analysis.

To further allow an evaluation of possible post-mortem degradation (see Hofreiter *et al*. 2001b; Willerslev and Cooper 2005), we acquired two or three replicated sequences for 15 specimens (Table 1). Replicate sequences were obtained from independent PCR amplifications of existing DNA extractions following the amplification and sequencing protocols outlined above. These sequence replicates were then used to classify each tule elk sequence as either confirmed or provisional. Confirmed sequences are those where we observed two or more identical sequences or where three independently replicated sequences for a given specimen could be used to infer a consensus sequence. Provisional sequences are those for which we have not yet been able to obtain replicate sequences from independent PCR amplifications or for specimens for which we have only been able to acquire two independent sequences that differ from one another.

Statistical Assessment of Genetic Diversity

We estimated a number of population genetic parameters from these DNA sequences using DnaSP Version 5.10.01 (Librado and Rozas 2009) including: the number of haplotypes, haplotype diversity, the number of polymorphic (segregating) sites (S), nucleotide diversity (π), and theta (per site) from S. We also used DnaSP to estimate Tajima's D and Fu's F_s. A haplotype is a unique combination of genetic markers present in a chromosome (Hartl and Clark 1997:57) and here each unique DNA sequence is defined as a distinct haplotype. Haplotypes are distinct from haplogroups, the discussion of which is common in much of the anthropological genetics literature. Haplogroups are groups of similar genetic markers that share a common ancestor and can be used to describe genetically related populations such as mitochondrial haplogroups A, B, C, D, and X found in native human populations throughout the Americas. Description of haplotype variation within a population is a useful measure of genetic diversity whereas definition and description of haplogroups facilitate population affinity studies. Haplotype diversity is a measure of the distribution of unique sequences (i.e., haplotypes) in a population and is conceptually similar to ecological diversity (evenness) measurements of heterogeneity that are familiar to many archaeologists. We expect that both the number of haplotypes and the distribution of

Table 1 Tule elk specimens from the Emeryville Shellmound with partial mitochondrial D-loop sequences

Specimen no.	Independent PCRs	Haplotype[a]	Period	Provenience	Element	Ontogenetic age	^{14}C years B.P. (approx.)
30	3	A	Post-B.	Uhle, Stratum 1	Tibia shaft	Adult	720–910
59	3	A	Post-B.	Uhle, Stratum 2	L humerus shaft	Young adult–adult	910–1100
29	3	A	Post-B.	Uhle, Stratum 4	L distal humerus	Adult	1290–1480
38	3	A	Post-B.	Uhle, Stratum 4	L prox. radius	Neonate	1290–1480
58	3	A	Post-B.	Uhle, Stratum 4	L distal tibia	Younger subadult	1290–1480
41	3	A	Post-B.	Uhle, Stratum 5	L ulna shaft	Younger subadult	1480–1670
55	3	A	Post-B.	Uhle, Stratum 5	R scapula	Subadult	1480–1670
35	3	A	Pre-B.	Uhle, Stratum 7	Thor. vertebrae	Neonate	1860–2050
36	1	$_{p}$A	Pre-B.	Trench[b], Level 1	L distal tibia	Neonate	1860–2050
47	1	$_{p}$B	Pre-B.	Trench, Level 1	R distal tibia	Older subadult	1860–2050
50	1	$_{p}$C	Pre-B.	Trench, Level 1	R distal tibia	Younger subadult	1860–2050
09	3	A	Pre-B.	Uhle, Stratum 8	L prox. radius	Adult	2050–2240
28	1	$_{p}$D	Pre-B.	Trench, Level 2	R distal femur	Adult	2050–2240
46	3	A	Pre-B.	Trench, Level 2	R prox. femur	Younger subadult	2050–2240
32	2	$_{p}$A	Pre-B.	Trench, Level 3	L prox. ulna	Adult	2240–2430
49	1	$_{p}$A	Pre-B.	Uhle, Stratum 9	R distal tibia	Younger subadult	2240–2430
07	1	$_{p}$E	Pre-B.	Trench, Level 4	L prox. humerus	Subadult	2430–2620
14	2	$_{p}$F	Pre-B.	Nelson, Stratum 11	R prox. femur	Younger subadult	2430–2620
33	1	$_{p}$G	Pre-B.	Nelson, Stratum 11	R prox. femur	Subadult	2430–2620
42	3	A	Pre-B.	Nelson, Stratum 11	R rib	Neonate	2430–2620
51	3	A	Pre-B.	Trench, Level 4	R ilium	Neonate	2430–2620
53	2	A	Pre-B.	Trench, Level 4	L distal humerus	Younger subadult	2430–2620

Table 1 (continued)

Specimen no.	Independent PCRs	Haplotype[a]	Period	Provenience	Element	Ontogenetic age	^{14}C years B.P. (approx.)
Sp54	1	$_{p}$H	Pre-B.	Uhle, Stratum 10	L prox. femur	Younger subadult	2430–2620
Sp61	1	$_{p}$A	Pre-B.	Uhle, Stratum 10	R scapula	Younger subadult	2430–2620

Post-B. post-bottleneck, *Pre-B*. pre-bottleneck

[a] The subscript "p" indicates a non-replicated or "provisional" sequence

[b] The "Trench" provenience units are from the 1924 Schecnk excavation

haplotypes across samples will be substantially reduced after ~1600 B.P., the hypothesized time of substantial population decline. We also expect to see a reduction in genotypic diversity in elk populations after 1600 B.P., if the bottleneck hypothesis is correct. Where gene diversity statistics like number of distinct haplotypes and haplotype diversity examine patterns of change in whole sequences, nucleotide diversity statistics examine patterns of change from individual nucleotide substitutions in DNA sequences. The number of segregating sites (S) is simply a count of the number of individual nucleotide positions in a collection of aligned DNA sequences that contain a substitution. The number of segregating sites (S) is conceptually similar to many estimates of ecological richness (e.g., numbers of taxa). Nucleotide diversity (π), also called mean pairwise difference, is the average number of polymorphic nucleotide sites between each pair of sequences in a collection of aligned DNA sequences. Theta (per site) from S (θ_S) is an expression of the number of segregating sites (S) that is normalized by both the length and number of DNA sequences in a sample.

Estimates of nucleotide diversity can be affected by natural selection, and changes in population size and structure, among other factors, and several statistical indices have been devised to measure these effects. Tajima's D and Fu's F_s provide estimates of the effect of natural selection at loci that are argued to be selectively neutral. At selectively neutral loci, in populations of constant size, estimates of theta from π and from S are expected to be roughly equal and the ratio between estimates of theta from pi and estimates of theta from S is approximately zero. Significant positive departures from zero suggest that natural selection is affecting variation at a given locus, while significant negative departures suggest that a population has been growing or has experienced directional selection (e.g., genetic hitchhiking, selective sweeps, etc.). We estimate Tajima's D and Fu's F_s in an effort to better contextualize the diversity statistics that we obtain for pre-bottleneck and post-bottleneck sequences.

Ancient DNA Results

We have performed DNA extractions on 43 specimens to date and from this sample have obtained 24 (56 %) high quality sequences that include 132 base pairs (bp) of nonpriming sequence from the mitochondrial control region (Table 1; Appendix 1).

Thirteen of these sequences have been replicated and confirmed; the remaining 11 have not yet been confirmed and are considered provisional. These 24 tule elk sequences are from specimens that span the entire Emeryville temporal sequence with 17 falling within the hypothesized pre-bottleneck period (pre-1600 B.P.) and 7 falling in the post-bottleneck period (post-1600 B.P.). Genetic diversity summary statistics for the complete collection of 24 confirmed and provisional sequences, as well as for hypothesized pre-bottleneck and post-bottleneck populations, are presented in Table 2.

From these 24 DNA sequences, we found 12 polymorphic sites that collectively define 8 distinct haplotypes (Haplotype A–Haplotype H). All 13 replicated and confirmed sequences are Haplotype A and all 12 segregating sites are transitions that are nearly equally distributed between purine–purine mutations (n=5) and pyrimidine–pyrimidine mutations (n=7). The replicated sequences are nearly equally divided between the pre- and post-bottleneck groups. We emphasize that all 13 sequences that have been replicated and confirmed are identical and thus no temporal trend in genetic diversity is apparent with this authenticated subset of the sample. Considering the entire data set, however, patterns of genetic diversity between the pre-bottleneck and post-bottleneck DNA sequences are striking. Eight unique DNA haplotypes are represented in this Emeryville sample and all of them are found in the pre-bottleneck collection. Among the post-bottleneck specimens, however, there is only a single haplotype (Haplotype A), and this sequence is also found in the pre-bottleneck sample. This apparent reduction in genetic diversity is statistically significant (X^2= 17.00, df=7, P=0.017) and is reflected by measures of haplotype diversity that estimate the probability that any two randomly chosen sequences represent different haplotypes (Nei 1987). While post-bottleneck specimens are identical and show no diversity, pre-bottleneck sequences, in contrast, are considerably more diverse (H=0.669±0.129) and are more diverse than the Emeryville elk sequence collection as a whole (H=0.507±0.125). We note here that the seven post-bottleneck individuals were distributed across nearly a 1,000-year time period, derived from four distinct stratigraphic units and thus could not be sampling a single closely related family unit.

The possible loss of genetic diversity between pre-bottleneck and post-bottleneck tule elk sequences from the Emeryville shellmound also resulted in a significant loss of genotypic diversity (Table 2). All twelve polymorphic sites from the 24 sequences reported here are found among the pre-bottleneck specimens. By contrast, the seven DNA sequences from post-bottleneck specimens are monomorphic and show no variation. Still, genetic variation in natural populations is influenced by a host of factors, so we estimated several additional indices of polymorphism. Nucleotide diversity (π) is the average number of differences between all pairs of sequences in the population sample (Hartl and Clark 1997). Post-bottleneck sequences exhibit no variation while nucleotide diversity for pre-bottleneck sequences (π=0.011±0.003) is greater than the same statistic for the collection as a whole (π=0.008±0.003). Theta (θ) is a well-known population genetic parameter that provides a direct molecular estimate of population size and/or mutation rate (θ=4Nu; where 2N = the number of genes in a population and u = mutation rate; Hartl and Clark 1997). Here, we estimate theta from the number of segregating sites (θ_S; Watterson 1975). Again, post-bottleneck sequences exhibit no variation, while pre-bottleneck sequences are more

Table 2 Summary of genetic diversity in tule elk from the Emeryville Shellmound

Sample	*n*	No. of polymorphic sites, *S*	No. of haplotypes, h	Haplotype diversity, H (S.D.)	Nucleotide diversity, π (S.D.)	Theta (per site) from *S*, θ_S (S.D.)	Tajima's *D*	Fu's F_s
All sequences	24	12	8	0.507 (0.125)	0.008 (0.003)	0.024 (0.010)	−2.282	−4.179
Pre-bottleneck	17	12	8	0.669 (0.129)	0.011 (0.003)	0.027 (0.012)	−2.151	−3.731
Post-bottleneck	7	0	1	0.000	0.000	0.000	[a]	[a]

[a] An estimate could not be made by DnaSp because there are no polymorphic sites in these sequences

diverse (θ_S=0.027±0.012), though less dramatically, than the Emeryville tule elk sequences as a whole (θ_S=0.024±0.010) when theta (per site) is estimated.

We also estimate the long-term stability of the tule elk population from our DNA sequence data (Table 2). Tajima's *D* is a statistic developed to determine whether a locus is selectively neutral (Tajima 1989) and is sensitive to a number of demographic processes and can be used to evaluate whether a population has recently experienced population growth or decline (Hartl and Clark 1997; Rogers *et al*. 1996). Negative values of Tajima's *D* are suggestive of previous population growth, and most notably, pre-bottleneck sequences are negative and deviate significantly from zero (D=−2.151, P<0.05). Like Tajima's *D*, Fu's F_s was developed to evaluate whether a locus is selectively neutral, similarly, it is sensitive to a number of demographic processes and can too be used to evaluate recent trends in population history (Fu 1997). The DNA sequences from pre-bottleneck specimens also suggest that this population was growing (F_s=−3.731).

Further work to replicate and confirm the 11 provisional sequences reported here will help to verify that the novel haplotypes, all derived from the earlier pre-bottleneck sample, reflect genetic variation and are not simply mis-incorporations that are the product of post-mortem DNA damage. The latter seems less likely, however, given the excellent collagen preservation in these materials (see below and Table 3), their relatively young absolute age and narrow age range (2600 to 700 B.P.), and the fact that extensive post-mortem damage of this sort has only been documented with much older material—mammoth, cave bear, bison, and Neandertal sequences~25,000–65,000 years in age (e.g., Gilbert *et al*. 2005b; Grigorenko *et al*. 2009; Hofreiter *et al*. 2001b).

Climate Change and the Tule Elk Population Decline

Tule Elk and San Francisco Bay Paleoenvironments

While our general hypothesis suggests that tule elk experienced a population bottleneck due to human hunting pressure, late Holocene climate change could also have a played a role. Indeed, to what degree ungulate herds are structured from the top down, by predation, or from the bottom up, by range conditions and resource limits, is an issue of general interest in ecology and wildlife management (Estes 1996; Kay 1998; Testa 2004) and one that is directly relevant to our analysis here.

Empirical research on modern tule elk populations in California indicates that elk are sensitive to variation in climate, especially effective precipitation. Although the relationship between climatic variables and tule elk reproduction and survivorship is clearly complex, hot and dry climates, or droughts, appear to have substantial negative effects on elk herds (Howell *et al*. 2002; McCullough 1969; McCullough *et al*. 1996) independent of hunting pressure. If the apparent population decline in tule elk documented from both archaeological relative abundance and genetic diversity data were driven by climate-based reductions in range quality, then paleoclimatic data should indicate enhanced drought conditions in the San Francisco Bay area during the post-bottleneck period. And insofar as the Emeryville relative abundance and genetic data reflect a pan tule elk population decline, climate data from across their California

Table 3 Stable isotope values, atomic C/N, collagen yield, and temporal range for pre- and post-bottleneck tule elk from the Emeryville Shellmound

Specimen no.	Cat. no.	Age at death	$\delta^{13}C$ ‰$_{PDB}$ collagen	$\delta^{15}N$ ‰$_{AIR}$ collagen	Collagen Wt%, C	Collagen Wt%, N	C/N ratio	Atomic C/N	Wt% collagen	$\delta^{13}C$‰$_{PDB}$ apatite	$\delta^{18}O$‰$_{PDB}$ apatite	^{14}C years B.P. (approx.)
Post-bottleneck												
30	H16147	Adult	−20.3	5.3	44.8	15.3	2.9	3.4	13.9	−11.1	−4.8	720–910
29	H12-1440	Adult	−19.9	5.8	42.9	15.0	2.9	3.3	13.1	−11.8	−4.5	910–1100
59	HA5884	Young adult/adult	−20.8	5.6	42.5	15.3	2.8	3.2	11.6	−10.5	−4.8	910–1100
38	H12-1375	Neonate	−21.4	5.6	40.8	14.7	2.8	3.2	15.2	−13.4	−5.4	1290–1480
58	HA8983	Younger subadult	−20.8	6.3	40.3	14.6	2.8	3.2	9.6	−10.9	−5.1	1290–1480
41	HA1012	Younger subadult	−21.3	5.8	40.1	14.4	2.8	3.3	8.9	−11.6	−5.4	1480–1670
55	HA9564	Subadult	−20.4	6.8	42.8	15.4	2.8	3.2	12.8	−12.5	−4.8	1480–1670
Pre-Bottleneck												
35	HA10976	Neonate								−13.0	−6.3	1860–2050
36	H7707	Neonate	−21.7	4.9	39.2	14.0	2.8	3.3	12.3	−14.1	−6.0	1860–2050
47	HA9564	Older subadult	−20.6	5.7	38.7	14.0	2.8	3.2	6.5	−11.9	−4.8	1860–2050
9	HA1826	Adult	−20.0	4.9	25.6	9.2	2.8	3.2	4.7	−10.4	−4.6	2050–2240
21	H33501	Adult	−20.7	5.3	41.2	14.6	2.8	3.3	6.9	−11.9	−4.5	2240–2430
7	H7304	Subadult	−20.6	5.4	39.2	14.8	2.6	3.1	7.7	−11.6	−5.4	2430–2620
14	H1-9843	Younger subadult	−20.9	5.1	41.2	14.7	2.8	3.3	3.0	−11.2	−5.1	2430–2620
11	H1-9842	Adult	−20.7	5.8	37.7	14.5	2.6	3.0	3.0	−10.2	−4.7	2430–2620
33	H1-9843	Subadult	−20.8	5.1	39.3	14.4	2.7	3.2	3.6	−10.9	−5.0	2430–2620

range should also document droughted conditions during the post-bottleneck period. However, since our analysis is focused on tule elk from Emeryville, we focus on climatic reconstructions for San Francisco Bay area.

Over the last several decades, a wealth of late Holocene paleoenvironmental information has been generated from San Francisco Bay proxy records, especially those derived from marsh and estuarine sediments (e.g., Ingram *et al*. 1996; McGann 2008; Malamud-Roam *et al*. 2007; Malamud-Roam and Ingram 2004; Goman *et al*. 2008). Much of this work has been geared towards understanding variation in regional moisture history as reflected by variation in San Francisco Bay salinity levels. Since San Francisco Bay receives runoff from the vast Sacramento–San Joaquin watershed that covers 40 % of the state of California and variation in freshwater inflow influences salinity levels of the estuary, periods during the past characterized by enhanced salinity reflect more arid conditions over the regional watershed.

Reconstructions of San Francisco Bay salinity have been derived from analyses of diatom and foraminiferal taxonomic composition, $\delta^{18}O$, $\delta^{13}C$, and trace element ratios derived from various estuarine sediments, and bay vegetation pollen assemblages to reveal late Holocene trends in salinity and regional moisture history. Those data suggest that the time period represented by the Emeryville elk record (i.e., 2600 to 700 B.P.) was generally cool and moist compared to the middle Holocene and that relatively low amplitude shifts between warm and dry, and cool and moist conditions also occurred over this interval. Most noteworthy, many of these records suggest increasing regional aridity between about 1300 and 700 B.P., an interval that corresponds with both the Medieval Warm Period (or Medieval Climatic Anomaly; MCA) documented from a variety of records across western North America (e.g. Graham *et al*. 2007), and the hypothesized elk population decline. San Francisco Bay area microclimate is, however, known to differ from prevailing regional or interior conditions. For example, hotter temperatures in the Central Valley (~150 km to the east) are often associated with an increased draw of fog cover and lower temperatures within the San Francisco Bay region (Gilliam 2002; Patton 1956). Indeed, several late Holocene paleoclimatic records that reflect local variations in effective precipitation have been read to suggest the MCA in the San Francisco Bay may have been characterized by relatively cool and moist conditions (Starratt 2008; Adam 1975). Clearly, more refined records that reflect variation in local climate and terrestrial ecosystems will be required to evaluate the potential role that climate change may have played in the tule elk population decline and we provide the foundation and a preliminary test of an approach to do this here based on the stable carbon, oxygen, and nitrogen isotope chemistry of the tule elk specimens analyzed for ancient DNA.

An Isotope-Based Paleoclimatic Reconstruction

Several stress factors can affect plant $\delta^{13}C$ in addition to vegetation type, altering photosynthetic rates and/or stomatal conductance. The most pronounced of these is aridity. Plant stable carbon isotope values are negatively correlated with water availability given the inverse relationship between transpiration and stomatal conductance (see Ehleringer and Monson 1993 and Farquhar *et al*. 1989 for reviews). Although the effects of aridity on intraspecific $\delta^{13}C$ is limited by the range of conditions under which a particular species can grow, increases in water

use efficiency produce enrichment in plant $\delta^{13}C$ as marked as 2‰, more than an order of magnitude greater than analytical uncertainty. Such increases in C_3 lowland plant $\delta^{13}C$ values in particular are typically indicative of increasing aridity.

Nitrogen isotope signatures increase with each step up the food web and in temperate and semi-arid ecosystems, plant $\delta^{15}N$ commonly ranges from 3‰ to 6‰ (Coltrain and Leavitt 2002; Evans and Ehleringer 1994; Pate 1994), while desert ecosystems can produce plant values >12 ‰ (Schwarcz *et al*. 1999). Enrichment in plant $\delta^{15}N$ appears to co-vary with soil aridity based on the understanding that isotopically light or depleted ammonia gas, formed in soils by microbial action, is volatized in droughted settings enriching soil $\delta^{15}N$ values available for plant uptake. Enrichment is passed up the food web, initially documented in the nitrogen isotope chemistry of herbivores as primary consumers. Analysis of a single geographically constrained herbivore population effectively holds trophic level constant. Thus, significant variability in elk $\delta^{15}N$ should reflect variation in moisture driven soil $\delta^{15}N$ values and provide an indicator of climatic variability, tracking drought conditions severe enough to drive elk into decline (but see also Ugan and Coltrain 2011). Given the distance between Emeryville and the open coast, nitrogen values should not likely be influenced by sea spray (see Heaton 1987; Sealey *et al*. 1987).

Analysis of oxygen isotope values in vertebrate bone can be used to reconstruct trends in the temperature of precipitation and thus imbibed water with enriched $\delta^{18}O$ values reflecting warmer water sources (Levin *et al*. 2006; Sponheimer and Lee-Thorp 1999). Droughted conditions or increases in temperature should thus be reflected by enriched $\delta^{18}O$ values in the Emeryville tule elk bone.

Sixteen tule elk were analyzed for bone apatite $\delta^{13}C$ and $\delta^{18}O$ isotope signatures; we report collagen $\delta^{13}C$ and $\delta^{15}N$ values on 15 samples (Table 3). Methods followed Coltrain *et al*. (2007). Collagen yields were adequate to produce reliable stable carbon isotope values. Atomic C/N ratios were within the 2.9–3.6 range indicative of well preserved archaeological bone collagen (Ambrose 1990). These isotope data are in keeping with expected values for elk foraging in a temperate, C_3 lowland setting. Given the +5‰ offset between herbivore diets and bone collagen, our sample of tule elk foraged virtually entirely on C_3 vegetation, with a mean carbon isotope value of approximately −25‰.

Mean collagen $\delta^{13}C$ and $\delta^{15}N$ values for nine elk from the pre-bottleneck sample are −20.7±0.5‰ and 5.3±0.4‰, respectively (Table 4). Mean bone apatite $\delta^{13}C$ is −11.5±1.2‰. Seven elk from the post-bottleneck sample show mean collagen $\delta^{13}C$ and $\delta^{15}N$ values of −20.7±0.5‰ and 5.9±0.5‰, respectively, and a mean bone apatite $\delta^{13}C$ value of −11.7±1.0‰. The mean $\delta^{18}O$ value for samples from the pre-bottleneck sample is −5.2±0.6‰, whereas those from the post-bottleneck group are −5.0±0.3‰. Thus, mean carbon isotope values are virtually identical in pre- and post-bottleneck samples and apatite is within the range of analytical uncertainty. Mean $\delta^{18}O_{apt}$ values are also similar between groups. In sum, only $\delta^{15}N$ mean values suggest climatic change over the pre- to post-bottleneck transition. However, the study included two subadults (sp 55, 58) whose bone chemistry may have been biased by pre-weaning enrichment in $\delta^{15}N$ (Table 3). Both high $\delta^{15}N$ subadults are in the post-bottleneck population and their $\delta^{15}N$ values are outside the range of other samples. Not surprisingly the mean for post- *versus* pre-bottleneck $\delta^{15}N$ is significantly different (P=0.02, t=2.67, df=11) and nitrogen is the only isotope for

Table 4 Mean $\delta^{13}C$, $\delta^{15}N$, and $\delta^{18}O$ values for pre- and post-bottleneck periods and selected age at death categories for the Emeryville Shellmound tule elk

Sample	Age at death category	*n*	$\delta^{13}C$‰ Coll	$\delta^{15}N$‰ Coll	$\delta^{13}C$‰ Apt	$\delta^{18}O$‰ Apt
Post-bottleneck	All	7	−20.7±0.5	5.9±0.5	−11.7±1.0	−5.0±0.3
Pre-bottleneck	All	9	−20.7±0.5	5.3±0.4	−11.7±1.2	−5.2±0.6
Combined	All	16	−20.7±0.5	5.6±0.5	−11.7±1.1	−5.1±0.5
	Neonates only: Sp 35, 36, 38	3	−21.5±0.2	5.3±0.5	−13.5±0.5	−5.9±0.5
Post-bottleneck	Neonates deleted	6	−20.6±0.5	5.9±0.5	−11.4±0.7	−4.9±0.3
Pre-bottleneck	Neonates deleted	7	−20.6±0.3	5.3±0.4	−11.2±0.7	−4.9±0.3
Combined	Neonates deleted	13	−20.6±0.4	5.6±0.5	−11.3±0.7	−4.9±0.3
	Nursing (?) SA only: Sp 55, 58	2	−20.6±0.3	6.6±0.4	−11.7±1.1	−4.9±0.2
Post-bottleneck	Nursing (?) SA deleted	5		5.6±0.2		
Pre-bottleneck	Nursing (?) SA deleted	9		5.3±0.4		
Combined	Nursing (?) SA deleted	14		5.4±0.3		

SA subadult

which between-group mean differences are significant. When these subadults are removed from the post-bottleneck population, the difference between groups is not significant (P=0.1, t=1.85, df=9). We do not advocate removing these data from the study but note that the significant difference between pre- and post-bottleneck $\delta^{15}N$ is a function of enrichment apparent in two subadult individuals that may have still been nursing or were recent weanlings at the time of death. (Elk calves typically wean by 4 to 6 months of age [McCullough 1969]). Thus, it is not clear that enrichment in post-bottleneck $\delta^{15}N$ can be attributed to increasing aridity.

The study also includes three late-term fetal or neonate samples (sp 35, 36, 38) whose collagen and apatite $\delta^{13}C$ values fall outside the range of other individuals and whose mean $\delta^{18}O$ value is nearly a per mil more negative (Table 3). When fetal/neonate isotope values ($\delta^{13}C_{coll}$=−21.5±0.2‰, $\delta^{13}C_{apt}$=−13.7±0.5‰; Table 4) are deleted, the relationship between pre- and post-bottleneck isotope mean values is essentially unchanged, although absolute means with the exception of nitrogen are depleted by 0.1–0.4 ‰. Slight adjustments to carbon and oxygen values do not affect the outcome of the study but correct for depletion coincident with fetal/neonate carbon and oxygen isotope chemistry, which indicates the importance of maternally derived lipids as an energy source.

Conclusion

Population genetic statistics estimated from 24 ancient tule elk DNA sequences are consistent with a hypothesized late Holocene (1600–1200 B.P.) population bottleneck inferred previously from patterns in traditional zooarchaeological indices. Statistical estimates of haplotype diversity and estimates of genotypic diversity all implicate a reduction in genetic diversity across the hypothesized tule elk population bottleneck. We caution, however, that only 12 of the 24 sequences have been replicated and confirmed and there is no variation in the sequences from this authenticated sample. In addition, no meaningful temporal trends

were apparent in the stable carbon, nitrogen, and oxygen values derived from the Emeryville elk specimens. As these values should reflect local change in the San Francisco Bay terrestrial environments, the available data would be inconsistent with a climate-based cause for a population decline, should one be confirmed with additional analysis. Planned analyses of both ancient DNA and isotope chemistry with additional samples including high resolution dating of the bone samples are clearly required to provide a more robust evaluation of variation in the late Holocene elk population.

Insofar as the trends documented here can be replicated with larger samples, the evidence of the decline in genetic diversity would be consistent with two of our specific hypotheses relating to the magnitude of the decline in elk: Hypothesis A, a population decline in the entire population of California tule elk, and Hypothesis B, a population decline in a San Francisco Bay sub-population of tule elk that was effectively isolated from the larger population in other regions. Although the latter directly implies a population bottleneck for only an isolated San Francisco Bay elk herd, the very formation of such isolated sub-populations implicates population fragmentation or the extirpation of elk in the intervening areas. As noted above, choosing between these two hypotheses will require additional genetic data derived from elk obtained from localities outside the San Francisco Bay area and such work is now planned.

Whatever the case, a secure documentation of declining genetic diversity in California tule elk would corroborate previous archaeofaunal work in this setting that has suggested substantial late Holocene depressions from a wide range of large-sized vertebrate prey types, including tule elk. Further, such evidence would bolster the broader argument that declining foraging efficiencies and resource intensification was the driving force behind many of the changes evident in human behavior and biology across the late Holocene in central California (e.g., Bartelink 2006; Broughton *et al*. 2010; Raab and Jones 2004).

Since our general hypothesis for elk population declines was derived originally from standard zooarchaeological measures of resource depression derived from foraging theory, the confirmation of it based on independent ancient DNA analyses would clearly have theoretical and methodological implications. Most notably, such a test would confirm in this context that body-size based abundance indices and the prey model logic and assumptions upon which they are based are appropriate and allow accurate reconstructions of trends in past prey encounter rates. Although due consideration attending to quantification, sampling, and taphonomic issues germane to the application of abundance indices continue to be warranted of course, independent genetics-based support would give us greater confidence that abundance indices reflect trends in prey encounters.

We also emphasize that the general congruence we provisionally obtained here between abundance indices and genetic diversity data need not apply in other contexts and other taxa. As we noted above, depending on the life history and behavioral characteristics of the prey taxa harvested and factors related to the context of human foragers involved, meaningful prey declines resulting from behavioral or micro-habitat depression may be indicated by abundance indices but may not be reflecting population-level demographic trends and would thus be genetically undetectable. In other words, the genetic barometer may be insensitive to changes in prey populations that had significant effects on foraging efficiency and diet breadth of past human consumers. Both standard zooarchaeological measures of the latter and those based on ancient DNA thus clearly can play important roles in increasing the precision of our understanding of the relationship between past peoples and animal populations.

Finally, our analysis may have implications for both the phylogenetic significance and modern management of tule elk. Insofar as we can replicate several of our novel tule elk haplotypes, we would have information allowing a more detailed evaluation of the phylogenetic significance of tule elk relative to the other North American and Asian subspecies. This issue has not been fully resolved due to the virtual lack of modern genetic diversity in tule elk from which to conduct phylogenetic analyses. This low extant genetic diversity is commonly attributed to the historic period population bottleneck but may well have deeper roots.

Importantly, because genetic diversity is associated with the accumulation of deleterious alleles and increased risk of extinction, patterns of current and historical genetic diversity are now routinely used to inform specific management strategies for declining or threatened animal populations (Frankham *et al*. 2002)—such is the case with California tule elk. Drawing on microsatellite DNA variation from modern elk derived from several of the separate managed herds, current work has focused on predicting the persistence of genetic variation under different management and relocation strategies (Williams *et al*. 2004; Meredith *et al*. 2007). This work has documented, as noted above, very low levels of genetic variation in tule elk—more so than any other elk subspecies—and is viewed as consistent with, and invariably attributed to, the historic period population crash. Quantitative analyses providing estimates of future variation based on a range of management scenarios involving various relocation plans are based, however, on "surrogates for historical levels of variation in tule elk" which in this case is assumed to be similar to levels of genetic variation documented for Rocky Mountain elk. Williams *et al*. (2004:116), for example, note that:

> We do not know the levels of genetic variation in pristine tule elk herds and acknowledge that the validity of our interpretation depends on the appropriateness of variation in Rocky Mountain elk for historical, pre-bottleneck levels in tule elk. We think that our assumption that variation in the large, pristine, tule herds approached that of Rocky Mountain elk is reasonable…

Our analysis suggests that the demographic and genetic history of tule elk that forms the basis for specific management policies and practices for the modern herds may be in need of revision. It now seems likely that tule elk not only experienced a relatively brief (~100 years), but severe, historic period bottleneck but possibly a substantial protracted (~1,000 years) late Holocene one as well. Further work with tule elk ancient DNA will help clarify the late prehistoric population dynamics of this iconic mammal of the California landscape.

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Appendix 1. Sequence data for a portion of the mitochondrial D-loop from 24 tule elk (*Cervus elaphus nannodes*). Tule elk specimen numbers are given on the *left* and numbers on the *right* indicate nucleotide position number from the *Cervus elaphus nannodes* TULE457 mitochondrial D-loop complete sequence (GenBank Accession No. AF016976.1). *Hyphens* indicate alignment gaps and *asterisks* indicate polymorphic sites

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Sp7 Provisional      T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp9 Confirmed        T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp14 Provisional     T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T A [735]
Sp28 Provisional     T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp29 Confirmed       T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp30 Confirmed       T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp32 Provisional     T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp33 Provisional     T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
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Sp41 Confirmed       T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp42 Confirmed       T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp46 Confirmed       T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp47 Provisional     T A T A A G A C A T C T C G A T A G A C T A A T G A C T A A T C A G C C C A T G [735]
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Sp50 Provisional     T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T T A G C C C A T G [735]
Sp51 Confirmed       T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp53 Confirmed       T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
Sp54 Provisional     T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]
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Sp61 Provisional     T A T A A G A C A T C T C G A T G G A C T A A T G A C T A A T C A G C C C A T G [735]

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Sp7 Provisional      C T C A C A C A T A A C T G T G G T G T C A T A C A T T T G G T A T T T T T A A [775]
Sp9 Confirmed        C T C A C A C A T A A C T G T G G T G T C A T A C A T T T G G T A T T T T T A A [775]
Sp14 Provisional     C T C A C A C A T A A C T G T A A T G T C A T A C A T T T G G T A T T T T T A A [775]
Sp28 Provisional     C T C A T A C A T A A C T G T G G T G T C A T A C A T T T G G T A T T T T T A A [775]
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Sp59 Confirmed       C T C A C A C A T A A C T G T G G T G T C A T A C A T T T G G T A T T T T T A A [775]
Sp61 Provisional     C T C A C A C A T A A C T G T G G T G T C A T A C A T T T G G T A T T T T T A A [775]
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Sp7 Provisional     T T T T T G G G G G G A T G C T T G G A T T T A G C A A T G G C C G T C T G A G [815]
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Sp28 Provisional    T T T T T G G G G G G A T G C T T G G A C T C A G C A A T G G C C G T C T G A G [815]
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Sp59 Confirmed      T T T T T G G G G G G A T G C T T G G A C T C A G C A A T G G C C G T C T G A G [815]
Sp61 Provisional    T T T T T G G G G G G A T G C T T G G A C T C A G C A A T G G C C G T C T G A G [815]

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Sp14 Provisional    G C C C C G T C C C G G [827]
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Sp59 Confirmed      G C C C C G T C C C G G [827]
Sp61 Provisional    G C C C C G T C C C G G [827]
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