THE MOLECULAR GENETICS OF PREHISTORIC HUNTING: INFERRING PREY POPULATION HISTORIES WITH ANCIENT DNA

by

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ABSTRACT

Long-term prey population histories are fundamental to reconstructing spatial and temporal variation in human diet, hunting technology, capture strategies, and a host of other prehistoric hunting behaviors. Typically, such reconstructions have involved the use of taxonomic relative abundance indexes from bone counts as measures of population history, but such measures are subject to many complicating quantitative and taphonomic issues.

Fortunately, animal populations maintain a record of population history in the form of genetic diversity. By assessing temporal variation in the genetic diversity of populations, zooarchaeologists have access to this history. This dissertation describes research that was designed to use the genetic record of population history to develop long-term histories of two prey species and to compare these genetic diversity-derived histories with those drawn from relative abundance measures.

Taxonomic relative abundance measures from California's San Miguel Island suggest that Guadalupe fur seals maintained a large and stable population through the late Holocene, whereas the archaeological record of Tule elk in California's San Francisco Bay area suggests a late Holocene population bottleneck. To evaluate the genetic diversity implications of these two contexts, I obtained ancient DNA sequences from 39 Guadalupe fur seal specimens from four archaeological sites on San Miguel Island and from 24 Tule elk specimens from the San Francisco Bay area's Emeryville Shellmound.

In both cases trends in genetic diversity support inferences made from relative abundance data. Guadalupe fur seal sequences, aggregated into three late Holocene temporal periods, show considerable genetic diversity within each period and no differentiation between periods suggesting a large and stable population. Tule elk sequences were divided into two groups that span a hypothesized population bottleneck. Analysis of these aggregations shows considerable diversity among pre-bottleneck sequences but no diversity in post-bottleneck sequences. This result is surprising for Guadalupe fur seals whose life history characteristics suggest that their populations are rather susceptible to hunting pressure. One plausible reason for long-term stability of Guadalupe fur seal populations in the face of what was likely significant hunting pressure is the presence of population refugia from which migration sustained genetically diverse populations.

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CHAPTER 1

INTRODUCTION

A fundamental question for zooarchaeologists interested in making inferences about prehistoric hunting behavior is: how many animals were there? To understand spatial and temporal variation in diet, hunting technology, prey capture tactics, and a host of other prehistoric hunting behaviors, researchers need to have at least a good general sense of the long-term population histories of prey animals. Although simple in concept, answering this fundamental question is not exactly straightforward. One particularly productive approach has been the use of taxonomic relative abundance indexes as proxy measures of population history. Temporal variation in various taxonomic relative abundance measures has successfully been used to document long-term change in hunting behavior in a number of contexts, most notably along the coast of California (Broughton 1994, 1997, 1999, 2004; Broughton et al. 2010), in the Great Basin of western North America (Byers and Broughton 2004; Janetski 1997; Ugan 2005), and in the American Southwest (Broughton et al. 2010; Cannon 2000, 2003), among others.

While providing a rather successful research strategy for making inferences about long-term prey population histories, taxonomic relative abundance measures are subject to a host of potentially complicating variables that are common to archaeology. Animal remains are unlikely introduced into archaeological contexts in direct proportion to their abundance on the surrounding landscape. Carcass processing and transport from kill site

to campsite oftentimes resulted in incomplete skeletal representation at locations where animal remains were ultimately deposited. Butchery and meal preparation at camp similarly modified what was deposited. While in the ground, taphonomic processes like density mediated attrition further altered what would eventually be available for archaeological research. Site excavation strategies are rarely able to completely recover an assemblage and the typically highly fragmented nature of most faunal assemblages can complicate species-specific taxonomic identification. Given the host of common archaeological complications that can bias estimates of prey population history it is worthwhile to explore the possibility for independent measures.

Fortunately, animal populations maintain a record of population history written into their genome in the form of genetic diversity. By assessing temporal variation in the genetic diversity of populations, zooarchaeologists have access to this history. The chapters that follow describe a research program that was designed to use the genetic record of population history to develop long-term histories of two prehistorically important prey species, Guadalupe fur seals (*Arctocephalus townsendi*) and tule elk (*Cervus elaphus nannodes*), and to compare these genetic diversity-derived histories with those drawn from taxonomic relative abundance measures. To gain access to these genetic records, established protocols for the extraction, amplification, and sequencing of ancient DNA were used.

1.1 Organization of Dissertation

Chapter 2 was previously published in the journal California Archaeology (Beck 2009) and summarizes the theoretical framework for using genetic diversity to assess population history. This discussion is presented in the context of current archaeological

concern along coastal California with the specific role played by marine mammals in prehistoric subsistence economies. Inferences about the relevance of marine mammals to prehistoric foragers have generally been made by making appeals to taxonomic relative abundance indexes, and this chapter provides a critical review of the theoretical underpinnings of those indexes as well as their potential limitations. Next, the relationship between genetic diversity and population size is detailed. In short, current population genetics theory suggests that large populations are characterized by a large degree of genetic diversity while small populations exhibit much more limited genetic diversity. This relationship has been subject to empirical evaluation many times over and this chapter summarizes three such tests. The mathematically derived and empirically verified relationship between genetic diversity and population size provides the key archaeological implication: diachronic change in genetic diversity at certain loci reflects diachronic change in population size and by assessing temporal changes in genetic diversity from well-dated faunal assemblages, zooarchaeologists can infer long-term population histories. With this key implication established, Chapter 2 closes by summarizing a published case study where ancient DNA methods were used to assess the well-documented near-extirpation of northern elephant seals (*Mirounga angustirostris*) by early 20th century market hunters (Weber et al. 2000).

Chapter 3 provides the first of two case studies that use ancient DNA to evaluate late Holocene population histories of two prehistorically important prey species in divergent archaeological contexts. Variation in the archaeological abundance of marine mammal remains and the relative roles that human hunting and climate change may have played in structuring that variation has been debated among eastern Pacific archaeologists

for decades. Marine mammal assemblages from California's northern Channel Islands have figured prominently in this debate and this chapter explores the late Holocene population history of Guadalupe fur seals (*Arctocephalus townsendi*), one of the most frequently recovered marine mammals from Channel Islands archaeological sites. Several taxonomic relative abundance measures estimated from faunal assemblages recovered from four sites on San Miguel Island, the northernmost of the Channel Islands, suggests relative population stability throughout the late Holocene. This pattern stands in contrast to much of the regional archaeological record that suggests an increase in marine mammal use, reaching a crescendo at approximately 1500 B.P. In an effort to reconcile these conflicting patterns, the research summarized in this chapter examined temporal patterns of genetic diversity observed from ancient DNA sequences obtained from 39 Guadalupe fur seal specimens. Twenty-eight of these ancient sequences were fully replicated and confirmed, the remaining 11 sequences were considered provisional. Genetic diversity statistics estimated from these specimens exhibit considerable diversity across temporal periods. This long-term stability in genetic diversity suggests that Guadalupe fur seal populations from San Miguel Island were fairly substantial and remained stable for much of the late Holocene.

Chapter 4 was also previously published (Broughton et al. 2013) and summarizes a case study designed to evaluate a hypothesized prehistoric population bottleneck in California tule elk (*Cervus elaphus nannodes*) from the San Francisco Bay area. California's early explorers often made note of the apparent high densities of large game, observations that are often taken as benchmarks for the region's pristine ecological condition. Zooarchaeological analysis of several measures of prey encounter rates that

use taxonomic relative abundance, however, implicate significant variation during the late Holocene. Indeed, such relative taxonomic abundance measures suggest a case of resource depression in San Francisco Bay Area tule elk. The study described in this chapter again takes advantage of the relationship between genetic diversity and population size to investigate this hypothesized instance of resource depression. Fifty-three individual tule elk specimens were selected for analysis from faunal assemblages recovered during excavations of the Emeryville Shellmound located along the eastern margin of California's San Francisco Bay. From these individuals, we obtained 24 high quality DNA sequences that included 132 base pairs of nonpriming mitochondrial control region sequence. Thirteen of these sequences were fully replicated and confirmed while the remaining eleven are considered provisional. Genetic diversity statistics estimated for these sequences implicate a population bottleneck at about 1600 B.P. with considerable diversity among sequences that predate the bottleneck and an absence of diversity among post-bottleneck sequences. In addition to DNA sequences, stable isotope analysis of the elk bones provided a climate record of the late Holocene study period and yielded no evidence that climate change played a role in the inferred elk population decline.

1.2 Discussion

Estimation of prey population histories is an important but complex zooarchaeologial research goal and the investigations summarized in this dissertation were designed to explore the potential for ancient DNA studies to achieve this objective. In both case studies detailed in the chapters that follow, genetic diversity estimated from temporally aggregated prehistoric populations corroborates population history inferences drawn from zooarchaeological taxonomic relative abundance measures. Importantly,

these studies suggest that ancient DNA is an effective analytical tool for developing or independently evaluating prey population histories. Laboratory methods for extracting, amplifying, and obtaining DNA sequences from archaeological specimens, coupled with application of well-established theory from population genetics do present archeological researchers with a powerful research tool. Finally, ancient DNA study design provides a robust set of long-term biological and environmental data that can effectively inform important questions in conservation biology and historical ecology. Application of this research tool to archaeological specimens has great potential to transcend traditional disciplinary boundaries and can provide opportunities for archaeological researchers to contribute significantly to important research outside of that commonly pursued by archaeologists.

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CHAPTER 2

THE MOLECULAR GENETICS OF PREY CHOICE: USING ANCIENT DNA TO INFER PREHISTORIC POPULATION HISTORIES[1]

Zooarchaeologists interested in the complex relationships between prehistoric hunters and their chosen prey frequently work to infer detailed prey population histories. The chain of inference necessary to proceed from an observed faunal assemblage to an estimate of population history is, however, routinely affected by a suite of complicating variables. Accordingly, the development of methods to produce such histories has proven to be challenging. Nonetheless, methods to infer temporal trends in prey population histories are increasingly in demand as evinced by a number of current debates in California archaeology.

The archaeological record of late Holocene California suggests considerable use of relatively high-cost resources and a general decline in overall foraging efficiency when efficiency is measured by changes in the relative abundance of high return and low return resources. Increasing reliance on acorns that are expensive to process (Basgall 1987),

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increased use of small fishes in the northern Sacramento Valley (Broughton 1994a), and occupation of high elevation sites in the White Mountains of eastern California (Bettinger 1991; Broughton and Grayson 1993) have all been suggested as examples of declining foraging efficiency and resource intensification. The zooarchaeological implications of resource intensification have been explored most completely by Broughton (1994b, 1997, 1999, 2002, 2004a) using a large faunal assemblage recovered from the Emeryville Shellmound found on the eastern margin of the San Francisco Bay. Evidence in support of the expected temporal declines in foraging efficiency associated with resource intensification come from examination of patterns in age composition, damage morphology and bone processing, and body part representation; however, the best evidence for resource intensification comes from inferences suggesting declines in prey encounter rates drawn from taxonomic relative abundance indexes.

Second, archaeologists interested in subsistence change in California have inferred a marked increase in foraging efficiency during the Middle Holocene when efficiency is measured as an increase in the relative abundance of large versus small game. This apparent increase in foraging efficiency occurs at a time of inferred population growth and seems to contradict simple expectations of the prey choice model from optimal foraging theory that expect, all other things equal, decreasing foraging efficiency in contexts with increasing forager population size. Recently, two opposing explanations for this Middle Holocene foraging pattern have been proposed. The first is termed Prestige Hunting and is predicated on the apparent divergent fitness related goals of men and women (Hildebrandt and McGuire 2002, 2003; McGuire and Hildebrandt 2005). The second reconsiders the simple foraging efficiency expectation of the prey

choice model in the context of inferred increases in overall large game abundance resulting from Middle Holocene climate change (Broughton and Bayham 2003; Byers and Broughton 2004).

Finally, California archaeologists continue to examine the effects of prehistoric foraging practices on the distribution and population dynamics of targeted prey species (Rick and Erlandson 2009). The effect of prehistoric hunting on prey population dynamics has been perhaps best explored among coastal California's marine mammals. In an influential set of papers, Hildebrandt and Jones (1992; Jones and Hildebrandt 1995; Jones et al. 2004) suggest that prehistoric hunting pressure dramatically affected marine mammal populations in two critical ways. First, they argue that hunting pressure forced large bodied, migratory marine mammals from onshore haul-outs and rookeries to more distant off shore locations. Subsequently, the increased cost to prehistoric foragers associated with pursuit of these animals encouraged hunting of smaller bodied, resident marine mammals drastically affecting their populations. Additional research suggests that the patterns inferred by Hildebrandt and Jones exhibit considerable spatial and temporal variability (Lyman 1989, 1995, 2003). Moreover, changes in sea surface temperature have been suggested as key to understanding prehistoric marine mammal hunting patterns (Arnold 1992; Colten 1995; Colten and Arnold 1998). Porcasi et al. (2000), using a well dated marine mammal assemblage from the southern Santa Barbara Channel Islands, evaluate these hypotheses and do not find clear, unambiguous support for the falsification of any one model. Patterns of marine mammal use on the California coast appear to be the result of a number of complex processes. Understanding the role of prehistoric foragers in structuring ecosystems is vital to conservation biology and recognition of the

potential for zooarchaeological data to inform such issues is emerging (Broughton 2004b; Kay and Simmons 2002; Lauwerier and Plug 2004; Lyman 1996, 2006; Lyman and Cannon 2004; Murray 2008; Steadman 1995).

The nexus of variables that influence relationships between prehistoric hunters and their prey makes the development of explanatory models a daunting proposal. For more than two decades California archaeologists have made significant progress in understanding these relationships by using simple foraging models that explore interactions between a few select variables. The three research themes outlined here all make extensive use of such models from Human Behavioral Ecology and each unambiguously point to the need for detailed prey population histories. Fortunately, a record of population history is maintained within the genome of each prey animal and researchers are now poised to access this information using established ancient DNA laboratory methods coupled with powerful theory from population genetics. This article explores the potential for molecular zooarchaeology to develop critical population histories for prehistoric prey taxa.

2.1 Inferring Demographic History: Taxonomic Relative Abundance

A central issue to archaeologists exploring prehistoric subsistence systems is the rubric of decisions surrounding the selection of specific prey items from the suite of available resources. A nexus of variables including available extraction technology, prey demographic and behavioral characteristics, and prey abundance and distribution across space and through time all conspire to affect prey choice decisions. To gain a full understanding of those decisions, researchers must estimate prehistoric prey abundance.

Taxonomic relative abundance measures have a long history in zooarchaeological scholarship as a useful method for summarizing and comparing faunal assemblages. Beginning in earnest during the early 1980s, taxonomic relative abundance indexes have increasingly been used to develop inferences about diachronic changes in prehistoric prey population size. The rationale for using taxonomic relative abundance as a proxy measure of prey population size is grounded in the prey choice model of optimal foraging theory (Stephens and Krebs 1986) and was first outlined by Bayham (1979, 1982).

The prey choice model was developed to address the following question: which prey items, given a suite of available resources, is a forager expected to pursue when encountered? According to the model, individual prey items are only pursued when encountered if the return for attacking the item does not fall below the average return for all available resources. The prey choice model assumes all potential prey items are randomly distributed, are encountered sequentially, and that time spent searching for prey is mutually exclusive from time spent processing acquired prey. In practice, all potential prey items are ranked by post-encounter profitability with the general expectation that the highest ranking items will always be taken when encountered and lower ranking items taken or ignored depending on the expected rate of encounter with higher ranked items. The key implication of this model for taxonomic relative abundance indexes as proxy measures for prey population size is that prey items are not taken based upon their abundance in any given foraging context, but are taken or not depending on the expected rate of encounter with higher profitability prey. It follows that an index measuring the abundance of a high ranking prey item relative to a lower ranking prey item is an estimate of the encounter rate with the higher ranking item. If encounter rate is a function of

population size, then changes in encounter rates with high ranking prey indicates changes in the population size of that taxon. This rationale has been employed by a number of scholars in a myriad of contexts to develop inferences regarding foraging efficiency (Bayham 1979, 1982; Broughton 1994a, 1994b, 1997; Cannon 2003; Janetski 1997; Nagaoka 2002b) and resource depression (Butler 2000, 2004; Cannon 2000; Nagaoka 2002a; Ugan 2005).

In spite of its many successes, taxonomic relative abundance measures are subject to a number of biasing agents common to archaeological and paleontological research. Even in the best of recovery contexts, any archaeological faunal assemblage is an incomplete reflection of the original burial assemblage that is only a subset of the actual animal population. Some elements survive burial while others are destroyed through bone density mediated attrition (Lyman 1984, 1994). Excavation methods affect which bones are actually discovered during recovery (Cannon 1999). Moreover, not all recovered bones can be taxonomically identified (Driver 1991; Gobalet 2001). Finally, recent research has questioned the methods and assumptions commonly employed to estimate prey rank in applications of the prey choice model (Bird et al. 2009). Careful zooarchaeological analysis can and oftentimes does minimize the effects of these biasing agents; nonetheless, independent means of estimating prey population size histories are clearly warranted.

2.2 Inferring Demographic History: The Population Size – Genetic Diversity Relationship

Genetic diversity in natural populations is affected by a variety of genetic and demographic processes (Amos and Harwood 1998). Diversity increases in populations

through mutation and the in-migration of individuals from other populations. Diversity is lost through natural selection, random genetic drift, and out-migration of individuals from a population. The social structure of a population may affect diversity, and a host of additional factors structure patterns of genetic variation. When a locus is selectively neutral, that is, it does not affect the phenotype of an individual, then patterns of change are best understood within the framework of Kimura's neutral theory (Kimura 1983). Hartl and Clark (1997:316) argue that the neutral theory, "models the fate of mutations that are so nearly selectively neutral in their effects that their fate is determined largely through random genetic drift." In practice, the neutral theory makes strong predictions about the relationship between genetic diversity and the size of a population.

At selectively neutral loci, genetic variation is primarily conditioned by two factors: mutation and genetic drift. Variation is introduced into a population by mutation and is removed by genetic drift. For any given population size, an equilibrium is reached between the subtractive forces of genetic drift and the additive forces of mutation. This theoretically derived relationship is described by:

$$\widehat{H} = \frac{4Nu}{1+4Nu} \qquad 2.1$$

where $2N$ is the number of genes in a population and u is the mutation rate given as the number of substitutions per site per generation (Gillespie 1998:28). (Note that referring to the number of genes in a population by the term $2N$ is standard population genetics notation and in the above equation $2Nu + 2Nu = 4Nu$.) If genetic mutation rates remain relatively constant across generations, then the equilibrium heterozygosity derived from theory is a product of population size. For example, assuming a mutation rate of 5.25 X 10^{-7} substitutions/site/generation that was estimated from extant southern elephant seal

(*Mirounga leona*) populations (Slade et al. 1998), a population of 2*N* = 50000 genes has an expected equilibrium heterozygosity of $\widehat{H} = .0499$. A population of 2*N* = 5000 has an expected equilibrium heterozygosity of $\widehat{H} = .0052$, and a population of 2*N* = 500 gives an equilibrium heterozygosity of $\widehat{H} = .0005$. As the size of a population decreases, so too does the expected genetic diversity within that population. This relationship showing the decline in expected genetic diversity with declining population size is shown in Figure 2.1.

The theoretically derived relationship between population size and genetic diversity has been subject to a number of empirical tests. Among the earliest of these tests was an evaluation conducted by Nei and Graur (1984). They accumulated published data from 77 species that had estimated heterozygosities of 20 or more protein loci and for which either a reliable population size was known or could be easily estimated. These data yielded a highly significant correlation (Nei and Graur 1984:82) and provided substantial support for the hypothesized relationship between genetic diversity and population size. This result is even more surprising given that the measures of heterozygosity used by Nei and Graur were derived from allozyme loci and it seems intuitively unlikely that these loci that code for enzyme variants are genuinely selectively neutral.

Frankham (1996) provides a second empirical evaluation of the expected relationship between heterozygosity and population size. In this paper, Frankham develops a series of hypotheses which should be true if the genetic variation-population size model is correct. Again, data are taken from previously published sources and come from 387 populations of 24 species including such disparate organisms as meadow sage

(*Salvia pratensis*), the common fruit fly (*Drosophila melanogaster*), and bighorn sheep (*Ovis canadensis*) (Frankham 1996:1503). The data marshaled by Frankham are consistent with several related hypotheses and yield support for the relationship between diversity and population size. These data, for example, find that measures of genetic diversity are reduced in small, insular populations relative to large populations of the same taxon. Further, Frankham's data find that populations which live in small, constrained habitats have less diversity than populations with larger ranges.

A third empirical test of the relationship between diversity and population size modeled by neutral theory comes from Montgomery et al. (2000). These researchers sought to test the expected relationship by experimentally manipulating the sizes of several *Drosophila melanogaster* populations. Twenty-three populations with sizes of 25, 50, 100, 250, and 500 individuals were raised for 50 generations. Genetic diversity was examined at seven allozyme loci, chromosome II inversions, and morphological mutations. These data showed that diversity was lost at a greater rate in smaller populations than in larger populations.

Kimura's neutral theory suggests a strong relationship between genetic diversity and population size that has been empirically verified by several researchers. It follows that diachronic change in genetic diversity at selectively neutral loci reflects diachronic change in the size of a population. By assessing temporal changes in genetic diversity from well dated faunal assemblages, zooarchaeologists can begin to infer prehistoric prey population histories independent of the complications of taxonomic relative abundance indexes.

2.3 Assessing Genetic Diversity with Ancient DNA

The northern elephant seal (*Mirounga angustirostris*) currently ranges throughout the North Pacific, from Baja, Mexico, northward to the Gulf of Alaska and Aleutian Islands. Northern elephant seals were hunted extensively for their oil during the middle of the 19th century. Indeed, at one point it was believed that this species of seal had been hunted to extinction. Hoelzel et al. (1993:443) recount a report from a late 19th century hunting party which encountered a small group of eight seals on Guadalupe Island off the central Baja coast, and proceeded to kill seven of the animals. At the turn of the century a single population of about 20 seals was found on Guadalupe Island. Legislative control of northern elephant seal hunting by both Mexican and the United States governments in the early 1920s allowed this species to expand in numbers and recent estimates suggest that more than 150,000 animals exist (The Marine Mammal Center 2002 [http://www.tmmc.org]).

The northern elephant seal was the first animal of concern for conservationists that was studied with molecular methods to determine the genetic effects of such a severe, historically documented population bottleneck. In the early 1970s, Bonnell and Selander (1974) examined protein variation in blood samples of 159 seals from five different rookeries. These researchers conducted starch-gel electrophoresis analysis of 21 proteins presumptively encoded by 24 gene loci (Bonnell and Selander 1974:908) and found no variation. These results were compared with allozyme diversity for southern elephant seals (*Mirounga leonine*) and with comparable data from 22 additional vertebrate taxa (Bonnell and Selander 1974:908). Southern elephant seals were subject to considerable 19th century hunting but not to the degree of its northern counterpart and

historical records do not suggest the population crisis evident for northern elephant seals. This is reflected by southern elephant seal protein diversity. In one study cited by Bonnell and Selander, five polymorphisms among 18 proteins in 42 individuals were found. The 22 additional vertebrate taxa were selected to provide a general vertebrate comparison and exhibited between 10 to 20 % polymorphic loci. These comparisons suggest that northern elephant seals are severely depauperate with respect to protein diversity when compared with a closely related taxa and also when compared with a general vertebrate sample.

Significant advances during the 1980s and early 1990s in molecular genetics methods (i.e., PCR) allowed researchers access to genetic information at the level of individual DNA molecules. With such technical advances in hand, researchers began to directly assay DNA variability in a number of animals such as the northern elephant seal. Rus Hoelzel and colleagues (Hoelzel et al. 1993) examined post-bottleneck genetic diversity of the northern elephant seal with both DNA and allozyme data from blood samples of 67 seals from two locations. These researchers evaluated allozyme diversity for 41 polymorphic protein loci and DNA variation from sequences of both the mitochondrial control region and the mitochondrial 16S RNA gene. Again, no allozyme diversity was observed. Sequences of the control region showed three polymorphic sites and two distinct haplotypes. Mean pairwise differences among the 16S RNA gene sequences showed markedly decreased diversity for northern elephant seals when compared with pairwise differences of the same gene from several southern elephant seals.

These studies by Bonnell and Selander and by Hoelzel and colleagues were able

to demonstrate a reduction in genetic diversity resulting from a historically documented population bottleneck by comparing the diversity of modern, living animals from a taxon that experienced a bottleneck with taxa that had not. These studies were not, however, able to directly assess the severity of the northern elephant seal bottleneck event because they did not compare pre-bottleneck genetic diversity with post-bottleneck diversity of the affected population. A direct assessment of the effects of a population bottleneck can only be obtained with diversity data that both predates and postdates the bottleneck event. This is the utility of ancient DNA.

In the late 1990s, a research group (Weber et al. 2000) undertook efforts to estimate pre-bottleneck genetic diversity of northern elephant seals by assessing genetic diversity with ancient DNA. Diana Weber and colleagues examined genetic diversity in these seals by sequencing a 300 base pair segment of the mitochondrial control region. These researchers were able to genotype this segment from 11 bones and from 111 tissue samples. Bone samples ranged in age from ~1000 B.P. to 30 B.P. and were obtained from California's San Miguel Island and San Nicolas Island; and from Guadalupe Island, Mexico. Specific dates for these bone samples were not provided. Tissue samples were taken from museum skins collected by C. H. Townsend in 1892 and blood samples were taken from seals living on the Channel Islands of southern California. These bone specimens, tissue, and blood samples cover pre-bottleneck, circa-bottleneck, and post-bottleneck periods. Weber and colleagues found only two distinct mitochondrial control region haplotypes in post-bottleneck specimens, supporting the observations made by Hoelzel et al. (1993). Circa-bottleneck samples likewise only exhibited two haplotypes. In contrast, the five bones from pre-bottleneck contexts exhibited four distinct

mitochondrial control region haplotypes. Of five pre-bottleneck specimens, four exhibited distinct mitochondrial DNA haplotypes. Even with a very small pre-bottleneck sample, using ancient DNA, these researchers were able to demonstrate a substantial loss of genetic diversity across a historically documented population bottleneck event. The dramatic decline in northern elephant seal population size as a result of 19th century hunting is clearly written in the genetic diversity of seals examined by Weber.

The northern elephant seal, hunted nearly to extinction during the late 19th century and with an amazing 20th century rebound, provides an example of conservation genetics at work and demonstrates the utility of ancient DNA to addressing issues of changing population size. Despite the impressive return in numbers of the northern elephant seal, this species maintains excessively low levels of genetic diversity. Importantly, the research summarized here clearly demonstrates the potential utility of ancient DNA techniques to examining diachronic population history.

2.4 Conclusion

Some of the earliest problem oriented research to use ancient DNA focused on anthropological issues (e.g., Hagelberg and Clegg 1993) and it continues to play a significant role in biological anthropology. Still, ancient DNA research has not figured prominently in zooarchaeology despite its clear potential to address relevant issues. Only recently have the possibilities of molecular zooarchaeology begun to be explored (e.g., Barnes et al. 1998; Barnes et al. 2006; Cannon and Yang 2006; Matisoo-Smith and Allen 2001; Matisoo-Smith and Robins 2004; Moss et al. 2006; Nicholls et al. 2003; Speller et al. 2005; Yang et al. 2004; Yang et al. 2005). Here, I have outlined a number of current issues in California prehistory that might be well served by a molecular zooarchaeology

approach. Research questions that require detailed prey population histories are particularly well suited to this methodology that more directly estimates prey population size and is not affected by common zooarchaeological taphonomy issues.

It is important to note that the molecular methods for estimating prehistoric prey population size outlined here provide a measure of changing population size only and do not explain why such changes occur. Research in the biological sciences using ancient DNA has documented declines in genetic diversity for many species for which historic records suggest substantial population declines (see Leonard 2008: Table 1); but with respect to efforts to infer population size changes in the absence of such historic documentation, this method remains largely underutilized. Still, the utility of this approach to documenting changes in population sizes is well documented (see Leonard 2008).

In applying this ancient DNA approach to estimating prey population size, several sampling issues need to be carefully considered. Faunal assemblages need to be well characterized with confident taxonomic and ancillary (e.g., specimen side and age) data. These data are important to avoid selecting two specimens from the same individual for molecular assay. Moreover, the number of individuals sampled needs to be sufficiently large so as to allow confidence in inferences of population size change or stasis. As with all statistical sample size questions, in ancient DNA research there is no magic sample size number. The size of an adequate sample will depend largely on the amount of variation initially present in a population, the expected degree of change, and the distribution of variation in each population. Finally, to facilitate meaningful aggregation of specimens into temporally defined populations, assemblages must come from well

dated contexts or dates from individual specimens must be obtained.

Population genetic theory provides a detailed description of the relationship between population size and genetic diversity that has been supported through a number of independent empirical studies. Current ancient DNA laboratory methods provide an instrument for directly examining diachronic trends in genetic diversity. Together, these tools provide a powerful mechanism for evaluating prehistoric prey population histories independent of many problems common to traditional archaeological research. The emerging field of molecular zooarchaeology holds the promise to contribute significantly to our collective understanding of the prehistory of California.

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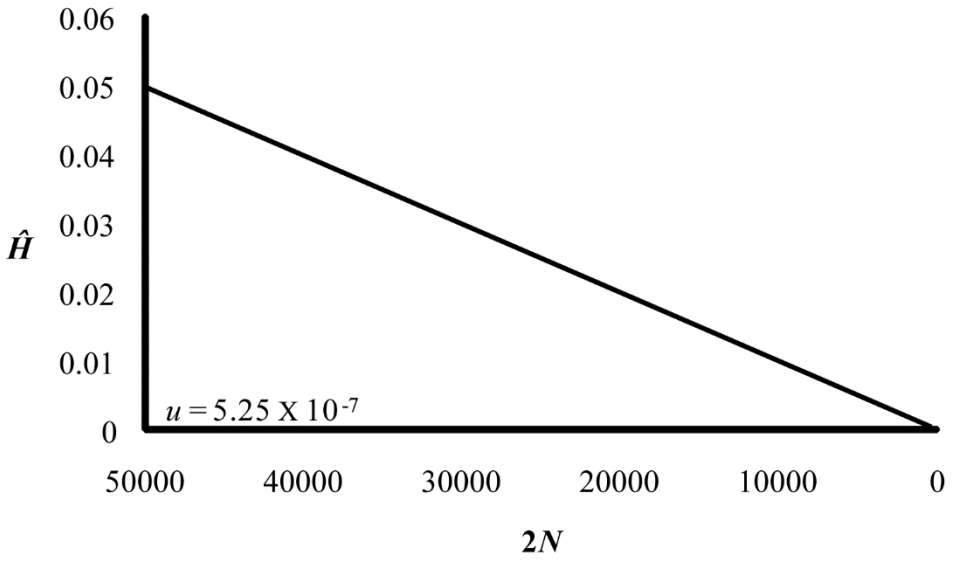

Figure 2.1. The relationship between genetic diversity and population size where Ĥ is the equilibrium heterozygosity for 2*N* genes in a population with a mutation rate $u = 5.25$ X 10-7.

CHAPTER 3

ANCIENT DNA EVIDENCE FOR LATE HOLOCENE POPULATION STABILITY OF GUADALUPE FUR SEALS, SAN MIGUEL ISLAND, CALIFORNIA

3.1 Introduction

Holocene variation in the archaeological abundance of marine mammal remains and the relative roles that human hunting and climatic change may have played in structuring that variation has been debated among eastern Pacific archaeologists for decades. Far reaching implications for related changes in human behavior and lifeways have also been derived from patterns in pinniped abundances including the development of ocean-going watercraft technology and the emergence of social and economic complexity (Ames 1994; Butler and Campbell 2004; Colten and Arnold 1998; Hildebrandt and Jones 1992, 2002; Jones and Hildebrandt 1995; Moss and Losey 2011; Rick 2007). While some analysts have focused on the relative importance of pinnipeds and other marine mammals relative to other major subsistence resource classes (e.g., terrestrial mammals, fish, plant resources, shellfish), others have focused on temporal trends among different marine mammal taxa.

In an initial exploration, for example, Hildebrandt and Jones (1992, 2002; Jones and Hildebrandt 1995; Jones et al. 2004) examined temporal patterns in several marine mammal faunal assemblages that indicated a temporal shift from the use of relatively

easy to obtain large-sized, migratory taxa that breed in terrestrial contexts during initial occupations to more costly aquatic breeders during the later Holocene. These scholars suggest that the marine mammal hunting pattern described effected a prehistoric "tragedy of the commons" involving overexploitation of the high-ranking migratory breeders with concomitant use of more costly and smaller-sized resident marine mammal resources. Lyman (1995, 2003) questions the empirical support and universal effects of such hunting and suggests instead considerable spatial and temporal variation in observed patterns.

Concerning variation in the overall importance of marine mammals, relative to other resource classes, Colten and Arnold (1998) suggest that observed variation in marine mammal representation from archaeological assemblages reflects climate-driven variation in general marine productivity. Examining a detailed climate record for coastal southern California and employing the relationship between climate and marine productivity, these researchers infer a diachronic population history for marine mammals that is compared with available faunal assemblages from Channel Island archaeological sites and find that marine mammal abundance varies little over the long-term but does decrease significantly during a period of warmer ocean temperatures between about 800 – 650 B.P. Though finding congruence between their inferred marine mammal population history and observed variation in archaeological faunal assemblages, recent fine-scale paleoclimate research (Kennett and Kennett 2000; Kennett and Ingram 1995) has brought to question the climate record initially used by Colten and Arnold.

Marine mammal assemblages from California's northern Channel Islands have figured prominently in this debate. Available evidence summarized for the northern

Channel Islands in general by Kennett (2005) and for San Miguel Island specifically by Rick (2007) suggests that marine mammals—especially Guadalupe fur seals (*Arctocephalus townsendi*)—were consistently harvested during most of the early and middle Holocene but their abundance relative to all other faunal resources peaked during the late Holocene at roughly 1,500 years ago. A number of alternative explanations for the observed pattern on San Miguel Island have been offered and Kennett (2005:222-223) cogently summarizes these arguments. First, early and Middle Holocene climate conditions may have prohibited the establishment of island rookeries. Second, extensive evidence for early and Middle Holocene marine mammal use may have been largely restricted to the western end of San Miguel Island, where these animals are primarily found today, and these sites might have been inundated during post-Pleistocene sea level changes. Third, late Pleistocene and early Holocene aboriginal marine mammal use may have limited later populations to offshore rocks and smaller islands where the cost of pursuit may have outweighed the potential benefits. Finally, early occupation of the islands may have simply been focused on areas far removed from extant marine mammal population centers with high associated transport costs. Clearly, these potential explanations need not be mutually exclusive and each may have contributed to the observed patterns of marine mammal use on San Miguel Island. Equally clear is the need for detailed population histories to begin to sort-out the relative contributions of each factor on observed archaeological patterns.

Zooarchaeological methods to estimate prey population histories often employ one or more measures of taxonomic relative abundance (Bayham 1979; Broughton 1994a, b, 1999, 2004; Broughton et al. 2010; Butler 2000; Butler and Campbell 2004;

Byers and Broughton 2004; Byers, et al. 2005; Cannon 2000, 2003; Ugan 2005). Such measures are, however, subject to many of the biases common to archaeological research such as: incomplete archaeological recovery of specimens, post-depositional taphonomic processes, and unidentified or misidentified specimens.

Given the significance of accurate long-term population histories to zooarchaeological inferences, it is important to explore methods that facilitate independent evaluation of archaeological patterns identified using relative abundance measures. Fortunately, a record of population history is maintained by an organism's genome. Genetic diversity in natural populations is affected by a host of variables including migration, geographic population structure, selective mating, and natural selection, among countless others. At selectively neutral loci that are invisible, or nearly so, to the effects of selection, genetic diversity is influenced most extensively by population size and genetic mutation rate. This relationship forms the backbone of Kimura's neutral theory of molecular evolution (Kimura 1983) and has been subject to extensive empirical tests (Frankham 1996; Montgomery et al. 2000; Nei and Graur 1984). In effect, we should expect to see greater genetic diversity in large populations and less diversity in small populations. It follows that diachronic change in genetic diversity at selectively neutral loci reflects diachronic change in the size of a population. By assessing temporal changes in genetic diversity from well dated faunal assemblages, zooarchaeologists can infer prehistoric prey population histories independent of the complications of taxonomic relative abundance indexes (Beck 2009; Broughton et al. 2013; de Bruyn et al. 2011).

Here, we take advantage of the well-supported relationship between genetic

diversity and population size to estimate diachronic change in the late Holocene population history of the most abundant and important marine mammal resource recovered from archaeological sites on San Miguel Island— Guadalupe fur seal (*Arctocephalus townsendi*). Temporal patterns of genetic diversity observed from ancient DNA sequences obtained from 39 total fur seal DNA sequences and 28 fully replicated and confirmed sequences indicate considerable genetic variation that is consistent throughout the late Holocene. This long-term stability in genetic diversity suggests that Guadalupe fur seal populations from San Miguel Island were fairly substantial and remained stable for much of the late Holocene.

3.2 Late Holocene Guadalupe Fur Seal Population History

Guadalupe fur seals are the most abundant of seven (non-cetacean) marine mammals commonly identified at archaeological sites in the northern Channel Islands region (Table 3.1). Historically, the geographic range of Guadalupe fur seals extended from the Revillagigedo Islands, Mexico northward to the San Francisco Bay area of California (Belcher and Lee 2002). It is likely, however, that their prehistoric range was considerably greater as specimens have been identified by Etnier (2002) at the Ozette site in northern Washington. Although many pinniped species are seasonally migratory, Guadalupe fur seals show strong site fidelity and are year-round residents at landings (Peterson et al. 1968). Guadalupe fur seals eat a variety of bony fish and squid are also a common component of their diets. Foraging trips are made to open ocean and can be just a few days to weeks in duration (Gallo-Reynoso et al. 2008). Such foraging trips can take an individual to feeding grounds immediately adjacent to landings or can range as far as 2,000 – 3,000 kilometers (Gallo-Reynoso et al. 2008).

Guadalupe fur seals are sexually dimorphic with males averaging 160 – 170kg and females 40 – 50 kg, and are polygynous with a single territorial male maintaining a harem of roughly 6 females (Belcher and Lee 2002). Wickens and York (1997:258) note that the birthing season for all fur seal species is during the summer months, typically between May and early August. Observations made by Peterson et al. (1968), however, place Guadalupe fur seal pupping during the months of May, June, and July. Females typically give birth to a single pup that is nursed for roughly 9 – 11 months. Guadalupe fur seal females mate shortly postpartum and give birth to a new pup during the next birthing season.

Many species of marine mammals were heavily impacted by fur trade hunting in the 19th and early 20th centuries including Guadalupe fur seals. Numbers were so severely diminished that no sightings of this species were reported from 1892 until 1926 when a population of no more than 50 individuals was found (Belcher and Lee 2002:4). Hubbs (1956) reports that one of the two fishermen who found this small population in 1926 returned in 1928 and killed most of the herd. As expected, the near extinction of Guadalupe fur seals dramatically affected genetic diversity. To assess the genetic consequences of historic hunting, Weber and colleagues (Weber et al. 2004) compiled mtDNA control region sequences for 32 modern seals to compare with 26 homologous sequences obtained from archaeologically recovered Guadalupe fur seals from California's Point Mugu shellmound and from San Nicolas Island. Their analysis demonstrated a considerable loss of genetic diversity in Guadalupe fur seals across the historically documented population bottleneck caused by 19th century fur sealing. Comparisons between pre-bottleneck and post-bottleneck populations indicated

significant losses in number of unique haplotypes (pre-bottleneck = 25; post-bottleneck = 7) as well as substantial reductions in the proportion of variable sites (p_{pre} = 0.282; p_{post} = 0.057), haplotype diversity (h_{pre} = 0.997±0.012; h_{post} = 0.798±0.038), and nucleotide diversity (π_{pre} = 0.055±0.004; π_{post} = 0.025±0.003) (Weber et al. 2004:151).

The regional prehistoric demographic history of Guadalupe fur seals can be estimated from available zooarchaeological relative abundance data. The rationale for using such data to reconstruct population histories is drawn from the prey choice model (Stephens and Krebs 1986) and has been used extensively by zooarchaeologists (Bayham 1979, 1982; Broughton 1994a, b, 1997; Cannon 2003; Janetski 1997; Nagaoka 2002). In short, the relative frequency of a highly ranked prey item in an assemblage is expected to more-or-less track the rate at which a forager encounters that prey item, and encounter rate is expected to be a proxy measure for overall abundance and regional population size. Because Guadalupe fur seals are a large, highly valued prey item, they are expected to have been pursued whenever encountered and their frequency in an archaeological assemblage should thus closely track population size. As such, Guadalupe fur seal relative abundance histories from late Holocene Channel Island archaeological sites should provide an ordinal index of their regional demographic history.

Archaeological investigations on California's San Miguel Island have identified nearly 700 sites that reflect use of the island from the terminal Pleistocene, through the Holocene to historic times (Kennett 2005; Rick 2007). In this study, we use Guadalupe fur seal assemblages recovered through archaeological excavation at four sites on California's San Miguel Island (Figure 3.1): CA-SMI-1, CA-SMI-525, CA-SMI-528, and CA-SMI-602. Marine mammal taxonomic abundance data from these sites are first used

to establish a local Guadalupe fur seal population history and then this history is more fully assessed using several of these specimens through genetic analysis of ancient DNA.

Site CA-SMI-1 is an inland village site located on an elongated knoll overlooking Cuyler Harbor on the northeastern coast of the island (Erlandson 1991). Available radiocarbon dates suggest that this is a multicomponent site with occupation during the middle Holocene and the middle-late Holocene transition (Kennett 1998, 2005; Rick 2007). Site CA-SMI-525 is a dense shell midden located on the western edge of San Miguel Island (Rick 2007). Radiocarbon dating at this site indicates that this is a multicomponent site with occupation during the early and later late Holocene (Kennett 1998). Site CA-SMI-528 is a large shell midden located along the crest of an eroding dune immediately northeast of the Point Bennett pinniped rookery on the southeastern margin of the island (Rick 2007; Walker et al. 2000). Radiocarbon dates from this site suggest both middle and late Holocene occupation (Kennett 1998). Site CA-SMI-602 is a large shell midden with a residential structure that is likely part of a larger village complex (Kennett 2005; Rick 2007; Walker et al. 2000). This site is located in the midst of the Point Bennett pinniped rookery found on the southeastern margin of the island. The radiocarbon record from this site suggests a single late Holocene occupation that extended to early historic times.

The marine mammal assemblages from these four sites include 331 specimens identifiable to or below the family level that could be assigned to one of three broad temporal periods (Table 3.2). These periods are: 1) early (6770 – 1800 cal B.P.), 2) middle (1800 – 1200 cal B.P.), and 3) late (1000 – 400 cal B.P.). Although the earliest of these periods extends back well into the Middle Holocene, the specimens considered here

are late Holocene in age and are from contexts that date to no more than about 4000 cal B.P. Specimens were then characterized as either a migratory breeder or a resident breeder following Hildebrandt and Jones (1992). To estimate the population history of Guadalupe fur seals from these assemblages, three separate relative abundance indexes were derived. The first, Guadalupe fur seal (GFS) index considers the relative abundance of Guadalupe fur seals to all identifiable marine mammals. Guadalupe fur seals are characterized as a migratory breeder by Hildebrandt and Jones (1992) and the next, the migratory breeder (MB) index, calculates the relative abundance of migratory breeders relevant to all migratory and resident breeders. The last, the Guadalupe fur seal as migratory breeder (GFSMB) index considers only the relative abundance of Guadalupe fur seals relevant to migratory breeders only.

Temporal variation in any of these indexes is expected to reflect variation in a prehistoric forager's encounter rate with Guadalupe fur seals, and by inference provide a proxy measure of their overall abundance. Relative abundance indexes for each temporal period at each San Miguel Island site are given in (Table 3.3). Using Cochran's Chi-square test of linear trends, no significant linear trends are evident in the relative abundance of Guadalupe fur seals relative to all other marine mammals ($X^2_{trend} = 1.03$, $p = 0.31$) , migratory relative to resident breeders ($X^2_{trend} = 0.01$, $p = 0.95$), or Guadalupe fur seals relative to other migratory breeders ($X^2_{trend} = 0.86$, $p = 0.35$).

Taxonomic relative abundance measures designed to track temporal changes in Guadalupe fur seal population size from sites CA-SMI-1, CA-SMI-525, CA-SMI-528, and CA-SMI-602 do not find any appreciable variation across these three broad temporal periods and suggest that population size remained fairly constant across the late Holocene

on San Miguel Island. So while marine mammal resources may have varied in their importance relative to other resource classes, the available data do not suggest any temporal trends in the relative abundance among different marine mammal taxa. Insofar as these zooarchaeological relative abundance measures accurately reflect the late Holocene Guadalupe fur seal demographic history, it follows that genetic diversity data derived from samples of these materials should also show temporal stability.

3.3 Materials and Methods

3.3.1 Guadalupe Fur Seal Assemblages

Sixty-seven individual specimens from marine mammal assemblages recovered from CA-SMI-1, CA-SMI-525, CA-SMI-528, and CA-SMI-602 were selected for genetic analysis. Individual specimens were identified by grouping each site's Guadalupe fur seal assemblage by excavation unit and then by level/stratum and estimating the Minimum Number of Individuals (MNI) represented by each subdivided assemblage. MNI estimates were made by considering the specific elements represented and the side, sex, and ontogenetic age of each element.

3.3.2 DNA Extraction, Amplification, and Sequencing

Approximately 0.2 g – 0.7 g of bone was removed from each specimen and the surfaces of samples were decontaminated by soaking in 10% bleach for 10 – 15 minutes followed by thorough rinsing with ddH2O. Samples were then digested in 5 ml proteinase K buffer (0.5 M EDTA [pH 8.0], 250 μg / ml proteinase K) overnight at 56°C. Next, 0.5 ml of the digestion supernatant was added to 1.5 ml Dehybernation Solution A (MP Biomedicals) and 0.4 ml Ancient DNA GLASSMILK (MP Biomedicals) and incubated for 3 hours at room temperature. DNA was then extracted and cleaned using a

GENECLEAN for Ancient DNA kit (MP Biomedicals) following the manufacturers protocol.

Two microliters of extract was used as template in a 25 µl PCR reaction with 2 U AmpliTaq Gold (Applied Biosystems), 1X GeneAmp PCR Gold Buffer (Applied Biosystems), 2X BSA, 2 mM MgCl, 200 µM each dNTP, and 0.2 µM each PCR primer. Primers targeted a 201 bp fragment on the 3' end of the 1107 bp mitochodiral D-loop (GFS-L87: 5' – CGTCGTGCATTAGTGGTTTG – 3' and GFS-R287: 5' – CGGAGCGAGAAGAGGTACAC – 3') and included 161 bp of nonpriming sequence. Primers for this project were designed using Primer3 and a Guadalupe fur seal sequence available on the NCBI GenBank database (GenBank # NC_008420; REGION: 15465..16571). An initial denaturation and enzyme activation for 5 minutes at 95°C was followed by 45 cycles of 95°C (45 seconds), 55°C (45 seconds), and 72°C (45 seconds) with a final extension at 72°C for 5 minutes. PCR products were then visualized on SYBR Safe DNA Gel Stain (Life Technologies) stained 2-3% agarose gel with an appropriate size standard. PCR products without evidence for contamination were cleaned with an UltraClean PCR Clean-Up Kit (Mo Bio) per manufacturer's protocol and then submitted for direct bidirectional sequencing to the University of Utah Core Sequencing Facility.

3.3.3 Contamination Control and Sequence Authentication

Contamination of PCR reactions by exogenous DNA templates is one of the most serious problems confronting aDNA researchers (Gilbert et al. 2005; Gilbert et al. 2006; Kaestle and Horsburgh 2002; O'Rourke et al. 2000; Paabo et al. 2004; Willerslev and Cooper 2005; Yang and Watt 2005). The aDNA laboratory in the Department of

Anthropology at the University of Utah maintains a series of protocols designed to minimize the potential for contamination in aDNA research. Wherever possible, each laboratory process (i.e., extraction, PCR set-up, post-PCR electrophoresis, etc.) is conducted in physically separated spaces that are cleaned before and after each use with a bleach solution and 70% ethanol. Equipment, tubes, and most reagents are UV cross-linked prior to use and extensive use is made of sterile, positive pressure, bench-top enclosures with HEPA-filtered air supplies and integrated UV cross-linkers during extraction and PCR set-up. 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, aDNA sequences can be compromised by a number of complications including postmortem DNA damage (Gilbert et al. 2005; Gilbert et al. 2003; Hofreiter et al. 2001; Paabo et al. 2004; Willerslev and Cooper 2005) and nuclear insertions (Bensasson et al. 2001; Martin 2003; Mourier et al. 2001; Willerslev and Cooper 2005). Accordingly, we used a rigorous DNA sequence authentication protocol appropriate to the risk of this project generating faulty sequences (Gilbert et al. 2005). This authentication protocol guarded against cross-over contamination between samples, and limited the possibility of including compromised sequences in subsequent analysis.

To ensure that sequences included in our analysis were indeed from Guadalupe fur seals and not another closely related marine mammal, raw nucleotide sequences were

evaluated using the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST). BLAST finds regions of sequence similarity by comparing nucleotide sequences to sequence databases and then computes the statistical significance of matches. Only those raw sequences that were most closely similar to Arctocephalus in the BLAST search were further considered.

Replication of all successfully sequenced specimens was attempted from independent amplifications conducted at different times and checked for complementarity. Specimens for which two or more independent sequence replicates were identical, or those for which three or more independent replicates could be used to infer a consensus sequence are considered "confirmed" sequences (n = 28). Successful replication of sequences was not possible for a number of specimens. These specimens for which no identical replications or for which no consensus sequence could be inferred are considered "provisional" sequences (n = 11).

3.3.4 Statistical Assessment of Genetic Diversity

To infer diachronic change in in the late Holocene population history of Guadalupe fur seals from San Miguel Island, we estimated several common population genetic parameters from these DNA sequences using DnaSP Version 5.10.01 (Librado and Rozas 2009) including the number of unique haplotypes, the number of polymorphic (segregating) sites (S), nucleotide diversity (π), and theta (per site) from S (θ_S). A haplotype is a unique combination of genetic markers present in a chromosome (Hartl and Clark 1997:57). For the haploid mitochondrial DNA sequences reported here, a haplotype is defined as a unique DNA sequence. The number of segregating sites (S) is simply a count of the number of nucleotide positions in a collection of aligned DNA

sequences at which one or more nucleotide substitutions can be found and is conceptually similar to many ecological measures of richness. Nucleotide diversity (π), also referred to as mean pairwise difference, is the average number of segregating sites between every possible pair of sequences in a collection of aligned DNA sequences. Theta (per site) from *S* (θ_S) is a description of the number of segregating sites in a collection of aligned DNA sequences that is normalized by both the length and number of sequences included in the analysis.

Nucleotide diversity can be affected by natural selection, as well as by changes in population size and structure. Accordingly, several population genetic statistics have been developed to measure these effects. Tajima's *D* provides an estimate of the effect of natural selection at loci, like mitochondrial loci, that are argued to be selectively neutral and was estimated for the sequences reported here in DnaSP as well. Significant positive departures from zero suggest that the locus in question has been subject to natural selection while significant negative departures from zero suggest that the population from which a collection of sequences was obtained has been growing or has experienced directional selection.

3.3.5 Radiocarbon Dating and Stable Isotopes

To better assign temporal affiliation for specimens from which we were able to obtain DNA sequences, we also acquired accelerator mass spectrometry (AMS) radiocarbon dates. Stable carbon and nitrogen isotope values were also derived from these specimens to allow an assessment of change in foraging ecology and marine environments. Bone collagen was prepared for AMS radiocarbon dating at either the Archaeological Stable Isotope Laboratory at the University of Utah and sent to National

Ocean Sciences Accelerator Mass Spectrometry Facility (NOSAMS) at the Woods Hole Oceanographic Institution for AMS dating, or was prepared at the Human Paleoecology and Isotope Geochemistry Lab at Penn State University and sent to the Keck Carbon Cycle AMS Facility (KCCAMS) at University of California, Irvine for AMS dating. (Specimen AT72 was too small for AMS radiocarbon dating). Radiocarbon ages were calibrated in CALIB 7.1 using the Marine 13 curve and applying a marine reservoir correction (ΔR) of 261 ± 21 years. AMS radiocarbon dates and stable isotope values are provided in Table 3.4.

Median calibrated radiocarbon ages obtained from these specimens range from 6650 cal B.P. to 540 cal B.P. and provide extensive coverage of the late Holocene. Indeed, aside from two specimens—one dating to 6650 and another to 3820— the collection spans the last 3000 years. To facilitate comparisons with the zooarchaeological relative abundance data and evaluate the evidence for diachronic variation in Guadalupe fur seal populations, specimens were assigned to one of three temporal periods: 1) early (6770 – 1800 cal B.P.), 2) middle (1800 – 1200 cal B.P.), and 3) late (1000 – 400 cal B.P.). The single specimen AMS dated to 6650 (AT13) was placed in the earliest time period, to which it most closely approaches, but falls outside the range of this period. Omitting the specimen from the analysis has no impact either way on the trends.

3.4 Ancient DNA Results

We have obtained high quality DNA sequences from 39 Guadalupe fur seal specimens, of which 28 have been fully replicated and are considered confirmed sequences. The remaining 11 have not been fully replicated and are considered provisional sequences (Table 3.5). All of these sequences are from specimens that date to

the late Holocene and all but two fall within the last 3000 years. Genetic diversity summary statistics for the complete collection of 39 sequences, as well as for each temporally defined analytic population, are presented in Table 3.6.

For the collection of sequences as a whole, we identified 38 polymorphic sites that collectively define 30 distinct haplotypes (Haplotype 1 – Haplotype 30). All 38 segregating sites are transitions that are nearly equally divided between purine-purine mutations (n = 21) and pyrimidine-pyrimidine mutations (n = 18). These haplotypes are not represented evenly across the San Miguel Island temporal sequence considered here. Early period sequences include eight haplotypes, middle period sequences include 18 haplotypes, and late period sequences include eight haplotypes.

Temporal stability in population size is reflected by statistics that estimate genotypic diversity. The number of polymorphic sites is not distributed evenly across all time periods (S_{early} = 22; S_{middle} = 34; S_{late} = 23), but these differences do not affect other measures of genotypic diversity. Nucleotide diversity (π) is the average number of differences between all pairs of sequences in the sample of sequences (Hartl and Clark 1997) and does not vary significantly between temporal periods (π_{early} = 0.050 ± 0.007; π_{middle} = 0.048 ± 0.004; π_{late} = 0.050 ± 0.006; Figure 3.2). Moreover, nucleotide diversity for each temporal period is comparable to that of the collection as a whole (π = 0.047 ± 0.003). Theta (θ) is a common population genetic parameter that provides a direct estimate of population size and/or mutation rate ($\theta = 4N\mu$; where $2N$ is the number of genes in a population and μ is mutation rate; (Hartl and Clark 1997). Again, this measure of genetic diversity is constant across all time periods (θ_{early} = 0.053 ± 0.025; θ_{middle} = 0.058 ± 0.021; θ_{late} = 0.055 ± 0.026; Figure 3.3) and each time period is indistinguishable

from the collection as a whole ($\theta = 0.056 \pm 0.018$). The absence of clear temporal trends in any of these measures of genotypic diversity suggests long-term population size stability.

Finally, we evaluate long-term stability of the Guadalupe fur seal populations represented by the DNA sequences reported here by estimating Tajima's *D* for each period. Tajima's *D* is a statistic that was developed to assess whether a locus of interest is selectively neutral or has experienced some form of natural selection. This statistic is sensitive to a number of demographic processes and can be used to evaluate whether a population has experienced recent population growth or decline (Hartl and Clark 1997; Rogers et al. 1996). Negative values of Tajima's *D* suggest recent population growth whereas values that are close to zero suggest population stability. All three temporal periods have negative values for Tajima's *D*; however, none of these statistics depart significantly from zero ($D_{early} = -0.277$, $p > 0.10$; $D_{middle} = -0.606$, $p > 0.10$; $D_{late} = -0.472$, $p > 0.10$). Tajima's *D* for the collection of sequences as a whole is also negative but similarly does not deviate significantly from zero ($D = -0.574$, $p > 0.10$). Collectively, these statistics suggest that these populations remained relatively stable throughout the time periods assessed.

Considering only those sequences that have been fully replicated and confirmed similarly suggests late Holocene population stability in San Miguel Island Guadalupe fur seal populations (Table 3.7). For confirmed sequences, 35 polymorphic sites define 22 distinct hapolotypes. Similar to the distribution of haplotypes for all sequences, confirmed sequences haplotypes are not evenly distributed and the greatest number of unique sequences are found among middle period specimens.

Estimates of genotypic diversity from confirmed sequences similarly show little variation. Nucleotide diversity estimate distributions for each time period almost completely overlap (π_{early} = 0.050 ± 0.010; π_{middle} = 0.050 ± 0.006; π_{late} = 0.050 ± 0.006), as do the distributions of each temporal period's estimate of Theta (θ_{early} = 0.051 ± 0.027; θ_{middle} = 0.057 ± 0.023; θ_{late} = 0.055 ± 0.026). Last, estimates of long-term population stability from confirmed sequences using Tajima's *D* also indicate no substantial changes throughout the late Holocene.

Although summary genetic diversity statistics all indicate that Guadalupe fur seal populations on San Miguel Island were stable during the late Holocene, a close look at the number of segregating sites (*S*) and Tajima's *D* both raise an important question about the power of the DNA sequence data described here to detect population growth. Notably, the number of segregating sites (*S*) is greatest during the time period surrounding 1500 B.P. Similarly, Tajima's *D* deviates from zero the most—albeit non-significant deviation—during this same period. Together, these observations may be indicative of population growth. To determine how well 39 mitochondrial DNA sequences of 161 bp can detect population growth, we conducted an extensive simulation study using SIMCOAL 2.1. For these simulations we consider four different population scenarios. The first, derived from our observed San Miguel Island Guadalupe fur seal genetic diversity estimates, simulates a population of $2N$ = 50,000. This baseline was then compared with simulated populations of $2N$ = 75,000, $2N$ = 100,000, and $2N$ = 500,000. For each population size scenario, SIMCOAL 2.1 was used to generate 100 sets of simulated DNA sequence data, each set containing 39 sequences of 161 bp. For each simulation we assumed a substitution rate of 5.25×10^{-7}. In this way, a total of 400

simulated DNA sequence data sets was generated. For each set of simulated data we estimated π and θ_S. We then compared each population growth scenario to the baseline scenario to determine how frequently population growth was detected.

As expected, the ability to detect population growth from 39 DNA sequences of 161 bp improved as the magnitude of change increased. Population growth was detected by significant differences in π in 20% of simulated sequence pairs when population size was increased from $2N$ = 50,000 to $2N$ = 75,000. Growth was detected by π in 29% of pairs when population was increased to $2N$ = 100,000; and in 96% of pairs when population was increased to $2N$ = 500,000. Similarly, population growth was detected by significant differences in θ_S in 32% of sequence pairs when population size was increased to $2N$ = 75,000; in 49% of pairs when population was increased to $2N$ = 100,000; and in 100% of sequence pairs when population size was increased to $2N$ = 500,000.

The simulation studies described here suggest that common genetic diversity summary statistics like π and θ_S are only moderately effective at detecting population growth in small samples of short DNA sequences and serve to highlight an important limitation of the data marshalled during the San Miguel Island Guadalupe fur seal research described here. In spite of these limitations, however, our data do not show significant variation through time and are consistent with locally derived taxonomic relative abundance indexes that suggest late Holocene population stability.

3.5 Late Holocene Climate Change on San Miguel Island

Holocene paleoclimate reconstructions for the Santa Barbara Channel Island region have been well established and suggest considerable centennial- and millennial-scale variation across this time period (Cole and Lui 1994; Heusser and Sirocko 1997;

Kennett and Ingram 1995; Pisias 1978). The Santa Barbara basin sits at the boundary of two major ocean current systems and is an area of intense interest for ocean scientists. As a result, numerous regional climate records have been established. Kennett and Ingram (1995) present one of the most detailed of these records derived from Hole 893A; a 200 meter core drilled as part of the Ocean Drilling Program and located northeast of San Miguel Island. This core provides a detailed climate record of the last 20,000 years inferred from oxygen isotope analysis of *Globigerina bulloides* and *Neogloboquadrina pachyderma*, foraminiferal plankton that, respectively, occupy near surface and deep-water habitats. Kennett and Kennett (2000) summarize the recent 3,000 years of that record, as well as additional proxy climate data, and discuss its archaeological implications for coastal Southern California.

Kennett and Kennett (2000:383) suggest three major climate periods from these data reflected in temporal variation in sea surface temperature (SST). From the period of approximately 3000 B.P. to 1500 B.P. water temperatures were relatively warm and stable followed by a period of the coldest and most unstable temperatures of the Holocene between 1500 B.P. and 650 B.P. Following 650 B.P., water temperatures were again relatively warm and stable, generally similar to current SST. Marine productivity, as reflected by periods of increased upwelling and inferred from oxygen isotope ratios of surface dwelling and deep-water dwelling foraminiferal plankton shows a similar, though not identical pattern of late Holocene variation (Kennett and Kennett 2000:384-385). The period between 1000 B.P. and 400 B.P. experienced the greatest degree of upwelling with warmer temperatures and less upwelling between 3000 B.P. and 1500 B.P., and then again after 400 B.P.

Research on the effects of short-term variation in SST suggests that such events can significantly affect overall marine productivity. Warmer SST and reduced upwelling associated with El Nino/Southern Oscillation (ENSO) events substantially influence nearshore fisheries and marine mammal mortality. During ENSO events, interrupted upwelling reduces food available to lactating marine mammals (DeLong and Melin 2000) and thereby hindering their capacity to adequately feed offspring. High mortality among marine mammal pups during periods of late Holocene climate instability may have affected their overall abundance, and could have reduced marine mammal encounter rates for late Holocene human hunters. This is unlikely to be the case here, however, as no appreciable changes in genetic diversity were detected and Guadalupe fur seal populations appear to have been stable through the late Holocene.

3.6 Summary and Discussion

In the Channel Islands region of southern California much available zooarchaeological evidence indicates a peak in marine mammal use by prehistoric foragers about 1,500 years ago, though considerable spatial and temporal variability is found regardless of whether comparisons are made between migratory and resident breeders or whether comparisons are made between marine mammal assemblages relative to other terrestrial or marine resource types. To begin to differentiate between the various explanations that have been posited for such variation in marine mammal assemblages, detailed high-resolution histories of population change are necessary. Genetic diversity statistics estimated from 39 total Guadalupe fur seal DNA sequences and 28 fully replicated and confirmed sequences indicate considerable genetic variation, however, no evidence suggests the degree of this variation changes throughout the late Holocene.

This long-term stability in genetic diversity suggests that Guadalupe fur seal populations from San Miguel Island were fairly substantial and remained stable across this time period.

Apparent temporal and spatial variability in the archaeological evidence for late Holocene marine mammal use along North America's Pacific coast has led Lyman (2003) to argue that marine mammals in this region were structured as a metapopulation—a, "set of geographically isolated and/or local populations of a species" (Brown and Lomolino 1998:70). Metapopulation models suggest that, with sufficient migration between local populations, genetic diversity of the metapopulation as a whole should withstand population size reductions and disturbance of local populations (Frank and Wissel 1998). Indeed, the potential for between-group migration of individuals to mitigate a loss of genetic diversity in the face of apparent population size reduction has been suggested for the closely related northern fur seal (Pinsky et al. 2010). These researchers conducted a rigorous Bayesian analysis of northern fur seal mitochondrial control region sequences from specimens that predate early 20th century market hunting of the species and from modern northern fur seal sequences. These pre-sealing and modern sequences collectively do not show a decline in genetic diversity through time and suggest that, "a high dispersal rate combined with the maintenance of a large refuge during the extreme disturbance experienced by this species provided genetic resilience…" (Pinsky et al. 2010:2425). The apparent long-term stability in genetic diversity of Guadalupe fur seals on San Miguel Island similarly suggests that these animals maintained a refuge of high abundance and that there was significant dispersal between local colonies.

Estimating long-term population histories for key prey species is a central concern for zooarchaeological researchers. Robust histories can facilitate strong inferences about prehistoric hunting behavioral variation and also facilitate more defensible arguments about the technological and social changes accompanying such variation. Importantly, both taxonomic relative abundance measures and diachronic trends in genetic diversity of Guadalupe fur seals show no clear temporal trends and collectively suggest long-term population stability. Moreover, the congruence of these two independent data sources attests to the utility of taxonomic relative abundance as a proxy measure for prey population history.

A similar result has been recently obtained in comparing tule elk zooarchaeological abundance data with ancient DNA evidence for late Holocene population trends (Broughton et al. 2013). In that case, however, both the relative abundance data and ancient DNA-derived genetic diversity suggest substantial late Holocene declines in the San Francisco Bay tule elk populations. Thus, in the first two cases that have paired zooarchaeological abundance and genetic diversity data, the two independent indices of past population size have been in agreement. The trends in both cases also have far reaching implications for understanding the related aspects of human behavior and lifeways.

Although continuity and long-term sustainability in pinniped hunting practices have been documented in several settings (Etnier 2007; Lyman 2003), the archaeological record of long-term marine mammal hunting along much of the eastern Pacific Coast indicates considerable spatial and temporal variability and both human and climatic impacts on larger migratory pinnipeds have been suggested to underlie this variability. In

these settings, such as in greater northern Channel Islands region, the declining availability of pinniped resources has been cited as a causal mechanism for both the adoption of open-ocean sea craft and in the development of economic and social complexity (Arnold 1992). For the adoption of robust ocean-going vessels, the suggestion has been that onshore hunting pressure reduced the numbers of available marine mammals and caused those that remained to seek refuge at more distant and more difficult to access offshore locations. The emergence of social and economic complexity is argued to have been at least partially facilitated by competition for depleted resource stocks. Arguments for technological change and the emergence of social complexity thus both appeal to inferences about regional reductions in the abundance of marine mammals. The taxonomic relative abundance and genetic data summarized here suggests, however, that neither can be attributed to long-term variation in the population size of Guadalupe fur seals as caused by either human hunting pressure or climate change. Further genetic analyses with Guadalupe fur seals from other contexts in the region, and with other pinniped taxa, should help clarify the degree to which the pattern documented here is a more general one.

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Table 3.1. Common Santa Barbara Channel Islands region marine mammals.

		Breeding classification	**Adult male body size**	**Adult female body size**
Guadalupe fur seal	*Arctocephalus townsendi*	Migratory	160 – 170 kg	40 – 50 kg
Northern fur seal	*Callorhinus ursinus*	Migratory	175 – 275 kg	30 – 50 kg
Harbor seal	*Phoca vitulina*	Resident	140 kg	140 kg
Northern elephant seal	*Mirounga angustirostris*	Migratory	2,000 kg	600 kg
California sea lion	*Zalophus californianus*	Migratory	390 kg	110 kg
Steller sea lion	*Eumetopias jubatus*	Migratory	1120 kg	350 kg
Sea otter	*Enydra lutris*	Resident	40 – 45 kg	16 – 27 kg

Table 3.2. Number of identified marine mammal specimens from late Holocene San Miguel Island assemblages.

	CA-SMI-1 Early	CA-SMI-525 Early	CA-SMI-525 Middle	CA-SMI-525 Late	CA-SMI-528 Early	CA-SMI-528 Middle	CA-SMI-528 Late	CA-SMI-602 Late	Total
Migratory Breeders									
Guadalupe fur seal	2	26	36	1	5	67	1	26	164
Northern fur seal	—	—	7	—	—	12	—	12	31
Northern elephant seal	—	—	—	—	1	—	—	4	5
Stellar sea lion	—	—	1	—	1	12	—	—	14
California sea lion	—	2	1	—	6	6	—	8	23
Otaridae	—	5	6	—	—	12	—	3	26
Resident Breeders									
Sea otter	1	7	5	—	4	24	—	14	55
Harbor seal	—	2	4	—	—	4	—	3	13
Total	3	42	60	1	17	137	1	70	331

Table 3.3. Relative abundance index values for late Holocene San Miguel Island marine mammal assemblages.

	GFS Index			MB Index			GFSMB Index		
	Early	**Middle**	**Late**	**Early**	**Middle**	**Late**	**Early**	**Middle**	**Late**
CA-SMI-1	0.67	—	—	0.67	—	—	1.00	—	—
CA-SMI-525	0.62	0.60	—	0.79	0.85	—	0.79	0.71	—
CA-SMI-528	0.29	0.49	0.37	0.76	0.80	—	0.38	0.61	—
CA-SMI-602	—	—	—	—	—	0.76	—	—	0.49

Table 3.4. AMS radiocarbon dates and stable isotope values for San Miguel Island Guadalupe fur seal specimens.

Sample Name	Lab No.	Sample Provenience	fraction Modern	fMod Error	^{14}C Age (BP)	Age Error	cal ^{14}C Age B.P. (2 sigma)	d^{13}C ‰ (Collagen v. PDB)	d^{15}N ‰ (v. Air)	Atomic C:N
AT04	ACRF 2534	CA-SMI-1, Unit 360:18-24cm	0.599	0.0022	4120	30	3690-3930	-13.1	22.7	3.3
AT05	ACRF 2535	CA-SMI-1, Unit 583:12-18cm	0.6148	0.0023	3910	30	3450-3660	-13.9	23.2	3.2
AT13	ACRF 2536	CA-SMI-1, Unit 824:12-18cm	0.447	0.0019	6470	35	6540-6770	-13.5	20.1	3.4
AT15	ACRF 2537	CA-SMI-525,Unit 1-B:195cm	0.7102	0.0031	2750	35	2030-2290	-12.7	18.7	3.2
AT16	ACRF 2538	CA-SMI-525, Unit 1-D:110-120cm	0.7626	0.0029	2180	30	1360-1560	-13.0	18.7	3.2
AT17	UCIAMS 119367	CA-SMI-525, Unit 1-D:110-120cm	0.7543	0.0014	2265	15	1500-1670	-13.5	18.7	3.2
AT18	UCIAMS 119368	CA-SMI-525, Unit 1-D:140-150cm	0.7440	0.0011	2375	15	1600-1790	-12.8	18.6	3.2
AT19	UCIAMS 119369	CA-SMI-525, Unit 1-D:165-170cm	0.7273	0.0016	2560	20	1820-1990	-13.5	17.2	3.2
AT22	UCIAMS 119370	CA-SMI-525, Unit 2:0-20cm	0.8211	0.0015	1585	15	790-930	-12.7	19.4	3.2
AT24	ACRF 2539	CA-SMI-525, Unit Profile D	0.7603	0.0022	2200	25	1390-1580	-12.7	18.5	3.2
AT26	UCIAMS 119371	CA-SMI-525, Unit Profile D:18-19cm	0.7326	0.0013	2500	15	1760-1930	-13.0	18.2	3.2
AT29	ACRF 2540	CA-SMI-525, Unit Profile D:20-40cm	0.7299	0.002	2530	20	1800-1970	-12.9	19.1	3.2
AT30	UCIAMS 119372	CA-SMI-525, Unit Profile D:20-40cm	0.7553	0.0014	2255	15	1480-1650	-12.8	18.5	3.1
AT31	UCIAMS 119373	CA-SMI-525, Unit Profile D:40-60 (70)cm	0.7311	0.0012	2515	15	1780-1940	-13.7	18.3	3.2
AT34	UCIAMS 119374	CA-SMI-525, Unit Profile D:40-60 (70)cm	0.7434	0.0012	2380	15	1610-1800	-13.6	18.4	3.1
AT38	UCIAMS 119375	CA-SMI-528, Unit 1:10-20cm	0.7744	0.0013	2055	15	1280-1400	-13.0	18.7	3.1
AT39	ACRF 2541	CA-SMI-528, Unit 1:10-20cm	0.7796	0.0025	2000	25	1230-1360	-13.5	18.4	3.2
AT40	UCIAMS 119376	CA-SMI-528, Unit 1:10-20cm	0.7718	0.0015	2080	20	1280-1440	-13.6	20.7	3.1
AT41	UCIAMS 119377	CA-SMI-528, Unit 1:20-30cm	0.7702	0.0014	2095	15	1300-1470	-13.7	19.1	3.1
AT42	UCIAMS 119378	CA-SMI-528, Unit 1:20-30cm	0.7770	0.0013	2025	15	1260-1370	-12.8	18.4	3.1
AT45	ACRF 2542	CA-SMI-528, Unit 1:30-40cm	0.7673	0.0032	2130	35	1320-1520	-12.7	18.8	3.3
AT47	ACRF 2543	CA-SMI-528, Unit 1:40-50cm	0.7678	0.0021	2120	20	1320-1500	-13.2	18.5	3.2
AT48	UCIAMS 119379	CA-SMI-528, Unit 1:50-60cm	0.7637	0.0013	2165	15	1370-1530	-12.8	18.5	3.1
AT49	UCIAMS 119380	CA-SMI-528, Unit 2:10-20cm	0.7734	0.0014	2065	15	1280-1410	-13.1	18.1	3.1
AT50	UCIAMS 119381	CA-SMI-528, Unit 2:10-20cm	0.7766	0.0014	2030	15	1260-1370	-13.9	19.0	3.1

Table 3.4 (cont.).

Sample Name	Lab No.	Sample Provenience	fraction Modern	fMod Error	^{14}C Age (BP)	Age Error	cal ^{14}C Age B.P. (2 sigma)	d^{13}C ‰ (Collagen v. PDB)	d^{15}N ‰ (v. Air)	Atomic C:N
AT52	ACRF 2544	CA-SMI-528, Unit 2:20-30cm	0.7718	0.0021	2080	20	1280-1440	-12.5	18.6	3.2
AT53	UCIAMS 119382	CA-SMI-528, Unit 2:20-30cm	0.7681	0.0013	2120	15	1320-1500	-13.5	22.3	3.1
AT54	UCIAMS 119383	CA-SMI-528, Unit 2:20-30cm	0.8604	0.0017	1210	20	500-620	-13.6	18.4	3.1
AT55	UCIAMS 119384	CA-SMI-528, Unit 2:20-30cm	0.7738	0.0017	2060	20	1270-1410	-13.2	18.2	3.1
AT56	UCIAMS 119385	CA-SMI-528, Unit 2:30-40cm	0.7725	0.0013	2075	15	1280-1420	-12.8	18.7	3.2
AT57	UCIAMS 119386	CA-SMI-528, Unit 2:30-40cm	0.7666	0.0016	2135	20	1340-1510	-13.6	22.6	3.2
AT61	ACRF 2545	CA-SMI-528, Unit 2:50-60cm	0.7682	0.0021	2120	20	1320-1500	-12.8	18.7	3.2
AT62	UCIAMS 119387	CA-SMI-528, Unit 2:50-60cm	0.7624	0.0013	2180	15	1380-1540	-13.5	20.5	3.2
AT66	UCIAMS 119388	CA-SMI-602, Unit 3:0-50cm	0.8626	0.0016	1185	15	480-600	-13.3	19.4	3.2
AT67	ACRF 2546	CA-SMI-602, Unit 5:0-10cm	0.8641	0.0023	1170	20	470-570	-13.2	19.2	3.2
AT70	UCIAMS 119389	CA-SMI-602, Unit 5:10-20cm	0.8603	0.0019	1210	20	500-620	-14.2	23.3	3.2
AT71	UCIAMS 119390	CA-SMI-602, Unit 5:20-30cm	0.8597	0.0015	1215	15	500-620	-13.4	18.7	3.2
AT78	UCIAMS 119391	CA-SMI-602, Unit 5:40-50cm	0.8615	0.0019	1200	20	490-610	-13.8	18.2	3.2

Table 3.5. Sequenced San Miguel Island Guadalupe fur seal specimens.

Specimen no.	Sample Provenience	Element	Age	Sex	Authentication Status	Haplotype
AT04	CA-SMI-1, Unit 360:18-24cm	L Humerus	Immature		Confirmed	Hap. 2
AT05	CA-SMI-1, Unit 583:12-18cm	L Femur	Pup		Confirmed	Hap. 3
AT13	CA-SMI-1, Unit 824:12-18cm	R Proximal Mandible			Provisional	Hap. 1
AT15	CA-SMI-525, Unit 1-B:195cm	Humerus		F	Provisional	Hap. 4
AT16	CA-SMI-525, Unit 1-D:110-120cm	Humerus	Adult	F	Confirmed	Hap. 10
AT17	CA-SMI-525, Unit 1-D:110-120cm	Humerus	Immature	F	Confirmed	Hap. 11
AT18	CA-SMI-525, Unit 1-D:140-150cm	Mandible	Adult	F	Confirmed	Hap. 10
AT19	CA-SMI-525, Unit 1-D:165-170cm	Femur	Adult	F	Confirmed	Hap. 5
AT22	CA-SMI-525, Unit 2:0-20cm	L Mandible	Adult/Imm.	M	Confirmed	Hap. 7
AT24	CA-SMI-525, Profile D	Femur	Immature	F	Provisional	Hap. 13
AT26	CA-SMI-525, Profile D:18-19cm	L Mandible	Adult	F	Confirmed	Hap. 8
AT29	CA-SMI-525, Profile D:20-40cm	L Femur	Adult	F	Provisional	Hap. 6
AT30	CA-SMI-525, Unit Profile D:20-40cm	L Femur	Adult	F	Provisional	Hap. 12
AT31	CA-SMI-525, Unit Profile D:40-60 (70)cm	R Distal Femur	Immature		Confirmed	Hap. 7
AT34	CA-SMI-525, Unit Profile D:40-60 (70)cm	R Femur	Pup		Confirmed	Hap. 9
AT38	CA-SMI-528, Unit 1:10-20cm	L Mandible	Adult	F	Provisional	Hap. 10
AT39	CA-SMI-528, Unit 1:10-20cm	L Mandible	Immature	F	Confirmed	Hap. 21
AT40	CA-SMI-528, Unit 1:10-20cm	L Mandible	Pup		Confirmed	Hap. 20
AT41	CA-SMI-528, Unit 1:20-30cm	L Femur	Immature		Provisional	Hap. 8
AT42	CA-SMI-528, Unit 1:20-30cm	L Femur	Immature		Confirmed	Hap. 25
AT45	CA-SMI-528, Unit 1:30-40cm	L Femur	Immature		Confirmed	Hap. 17
AT47	CA-SMI-528, Unit 1,40-50cm	L Mandible	Adult	F	Confirmed	Hap. 18
AT48	CA-SMI-528, Unit 1:50-60cm	L Mandible	Adult	F	Provisional	Hap. 15
AT49	CA-SMI-528, Unit 2:10-20cm	L Humerus	Imm./Adult	F	Confirmed	Hap. 23
AT50	CA-SMI-528, Unit 2:10-20cm	L Humerus	Immature		Provisional	Hap. 24
AT52	CA-SMI-528, Unit 2:20-30cm	L Humerus	Immature		Confirmed	Hap. 21

Table 3.5 (cont.).

Specimen no.	Sample Provenience	Element	Age	Sex	Authentication Status	Haplotype
AT53	CA-SMI-528, Unit 2:20-30cm	L Humerus	Immature		Confirmed	Hap. 16
AT54	CA-SMI-528, Unit 2:20-30cm	L Humerus	Immature		Confirmed	Hap. 27
AT55	CA-SMI-528, Unit 2:20-30cm	L Humerus	Juvenile		Confirmed	Hap. 21
AT56	CA-SMI-528, Unit 2:30-40cm	R Mandible	Adult	F	Provisional	Hap. 22
AT57	CA-SMI-528, Unit 2:30-40cm	R Mandible	Immature	F	Confirmed	Hap. 16
AT61	CA-SMI-528, Unit 2:50-60cm	R Humerus	Adult	F	Confirmed	Hap. 19
AT62	CA-SMI-528, Unit 2:50-60cm	R Humerus	Immature		Provisional	Hap. 14
AT66	CA-SMI-602, Unit 3:0-50cm	L Femur	Pup		Confirmed	Hap. 30
AT67	CA-SMI-602, Unit 5:0-10cm	L Humerus	Adult/Imm.	F	Confirmed	Hap. 2
AT70	CA-SMI-602, Unit 5:10-20cm	L Femur	Pup		Confirmed	Hap. 28
AT71	CA-SMI-602, Unit 5:20-30cm	L Mandible	Adult	F	Confirmed	Hap. 10
AT72	CA-SMI-602, Unit 5:20-30cm	L Mandible	Adult	F	Confirmed	Hap. 26
AT78	CA-SMI-602, Unit 5:40-50cm	L Humerus	Immature		Confirmed	Hap. 29

Table 3.6. Genetic diversity summary statistics of all San Miguel Island Guadalupe fur seal sequences.

	n	**No. of polymorphic sites, *S***	**No. of haplotypes, *h***	**Nucleotide diversity, π (S.D.)**	**Theta (per site) from *S*, ϑ_S (S.D.)**	**Tajima's D**
All Sequences	39	38	30	0.047 (0.003)	0.056 (0.018)	-0.574, p > 0.10
Early	8	22	8	0.050 (0.007)	0.053 (0.025)	-0.277, p > 0.10
Middle	23	34	18	0.048 (0.004)	0.058 (0.021)	-0.606, p > 0.10
Late	8	23	8	0.050 (0.006)	0.055 (0.026)	-0.472, p > 0.10

Table 3.7. Genetic diversity summary statistics of confirmed San Miguel Island Guadalupe fur seal sequences.

	n	**No. of polymorphic sites, *S***	**No. of haplotypes, *h***	**Nucleotide diversity, π (S.D.)**	**Theta (per site) from *S*, ϑ_S (S.D.)**	**Tajima's *D***
All Sequences	28	35	22	0.049 (0.004)	0.056 (0.020)	-0.482, p > 0.10
Early	5	17	5	0.050 (0.010)	0.051 (0.027)	-0.144, p > 0.10
Middle	15	30	11	0.050 (0.006)	0.057 (0.023)	-0.525, p > 0.10
Late	8	23	8	0.050 (0.006)	0.055 (0.026)	-0.472, p > 0.10

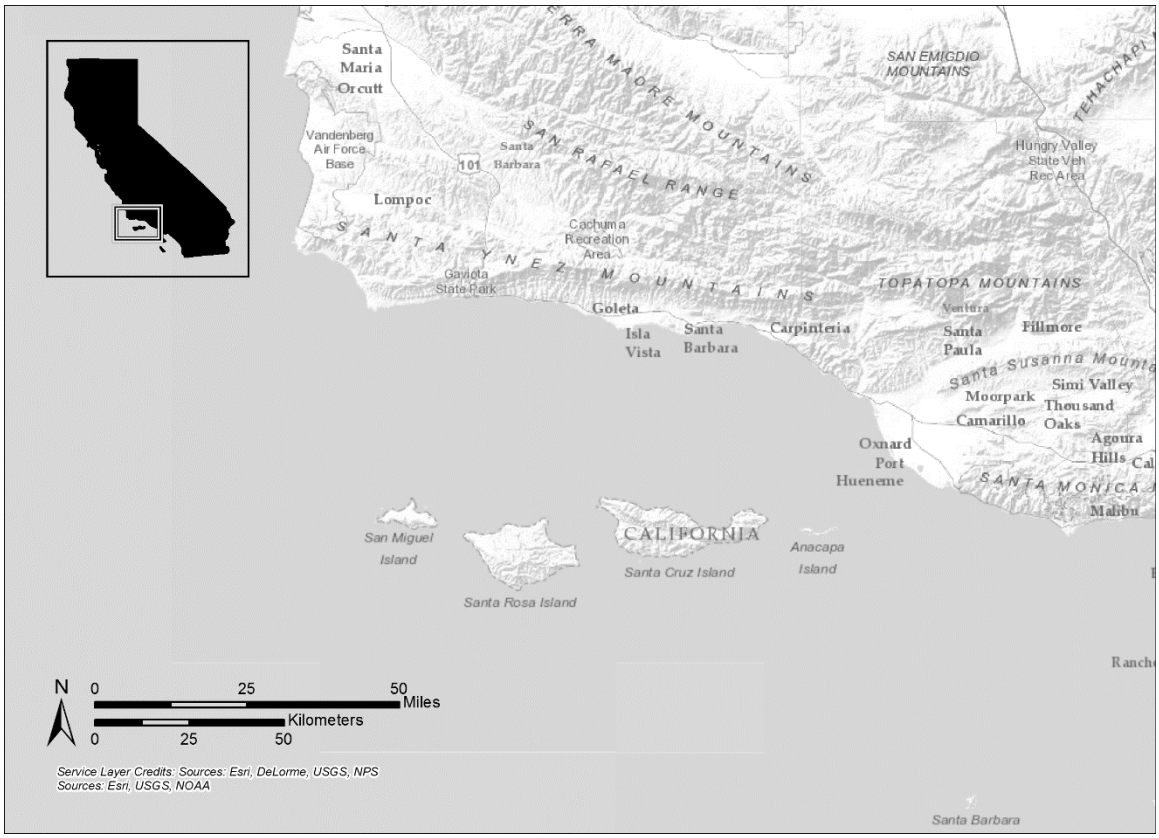

Figure 3.1. Map showing location of San Miguel Island.

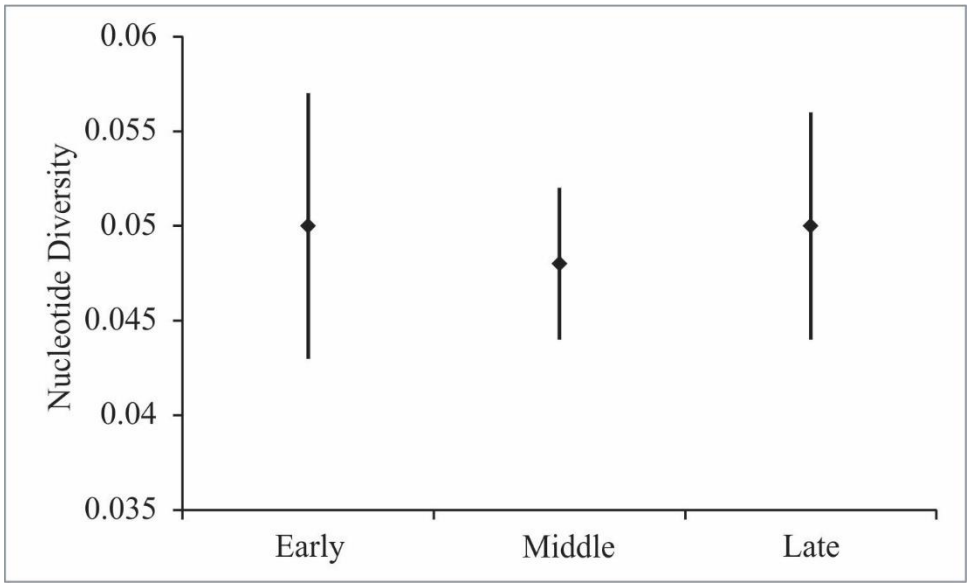

Figure 3.2. Distribution of nucleotide diversity (π) between temporal periods.

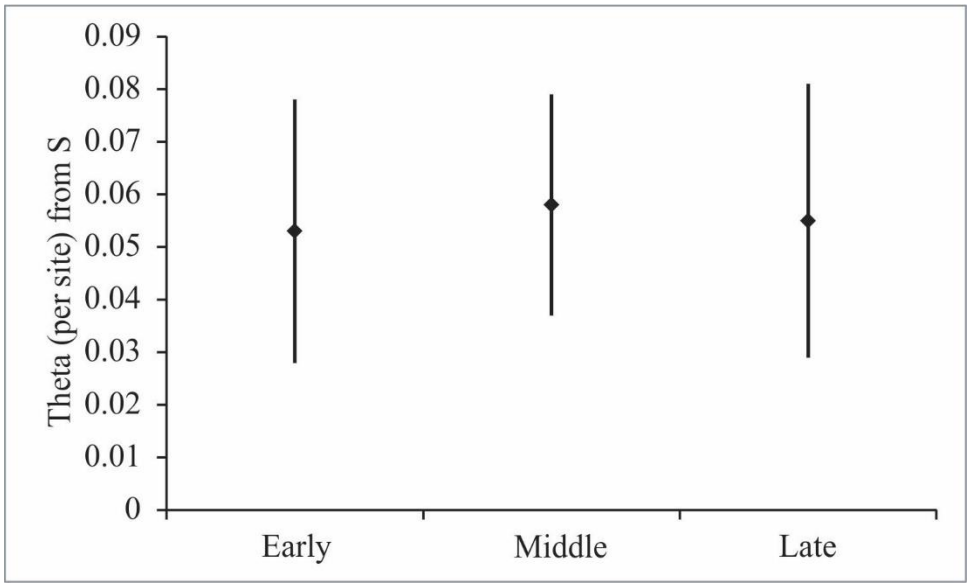

Figure 3.3. Distribution of theta (θ) between temporal periods.

CHAPTER 4

A LATE HOLOCENE POPULATION BOTTLENECK IN CALIFORNIA TULE ELK (*CERVUS ELAPHUS NANNODES*): PROVISIONAL SUPPORT FROM ANCIENT DNA[2]

4.1 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 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

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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; Broughton et al. 2010; Raab and Jones 2004). 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 —16th and 17th 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 1994, 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, 1994, 1996, 2011; Lyman and Cannon 2004) has promoted for decades.

4.2 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 (*C. e. roosevelti*) and Rocky Mountain elk (*C. e. nelsoni*). Subspecific status for each has more recently been supported by genetic analyses (e.g., Polziehn et al. 2000; Polziehn and Strobeck 2002; Merideth 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 18th and 19th 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 (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 20th 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 (California Fish and Game 2011; Williams et al. 2004). 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 19th and 20th century population bottleneck (e.g., Cronin et al. 2009; Meredith et al. 2007; Williams et al. 2004). However, the question remains "how low is low" (Williams et al. 2004:118) since no pre-19th 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 is 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, postencounter 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 (Figure 4.1). Figures 4.2 – 4.4 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 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, $X^2_{trend} = 102.63$, $P < .001$; 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. Although these apparent linear declines do not appear to correlate with existing reconstructions of paleoenvironmental change (e.g., Broughton 1999; Ingram et al. 1996; Malamud-Roam et

al. 2007; McGann 2008; 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 Figure 4.4) is 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 4,500 and 2,500 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.

4.3 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; de Bruyn et al. 2011; Frankham 1996; Glenn et al. 1999; Hadly et al. 1998; Hoelzel et al. 2002; Rogers 1995). 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 (Barnes et al. 2002; Calvignac et al. 2008; Leonard et al. 2000), cave bears (Bon et al. 2011; Hofreiter et al. 2002), 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.

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

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). 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 (Figure 4.4).

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 PCR (Polmerase chain reaction; 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 is that 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 is that the elk remains recovered from the Emeryville Shellmound

were derived from a San Francisco Bay area subpopulation 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 is that 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 is that 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 subpopulations 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.

4.5 The Tule Elk Sample from Emeryville

The Emeryville Shellmound was the largest of almost 500 shellmounds that lined the San Francisco Bay shoreline (Figure 4.1). The mound measured roughly 100 x 300 m and extended to a depth of 10 m. During the early 20th 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.

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

4.6.1 DNA Extraction

Between 0.2 and 0.67 grams of bone were removed from each specimen and surface decontaminated by soaking in 10% bleach for 10 minutes 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 0.5M EDTA, pH 8.0 and 250 µg/ml proteinase K. Digested bone powder was centrifuged for 5 minutes 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 hours 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.

4.6.2 PCR Amplification and Sequencing

PCR primers were designed with Primer 3 (Rozen and Shaletsky 2000) from the Cervus elaphus 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, 2X BSA, 1X GeneAmp® PCR Gold Buffer (Applied Biosystems), and 2U 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 were 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.

4.6.3 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; Gilbert et al. 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 postmortem DNA damage (Gilbert et al. 2005b; Gilbert et al. 2003; Hofreiter et al. 2001a; Hofreiter et al. 2001b; 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 *Cervus elaphus 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 postmortem degradation (see Hofreiter et al. 2001b; Willerslev and Cooper 2005), we acquired two or three replicated sequences for 15 specimens (Table 4.1). 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.

4.6.4 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 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.

4.7 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 4.1). 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 4.2.

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 ($H = 0.669 \pm 0.129$) and are more diverse than the Emeryville elk sequence collection as a whole ($H = 0.507 \pm 0.125$). We note here that the 7 post-bottleneck individuals were distributed across nearly a 1000 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 4.2). All 12 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 ($\pi = 0.011 \pm 0.003$) is greater than the same statistic for the collection as a whole ($\pi = 0.008 \pm 0.003$). Theta (θ) is a well-known population genetic parameter that provides a direct molecular estimate of population size and/or mutation rate ($\theta = 4Nu$; 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 diverse ($\theta_S = 0.027 \pm 0.012$), though less dramatically, than the Emeryville tule elk sequences as a whole ($\theta_S = 0.024 \pm 0.010$) when theta (per site) is estimated.

We also estimate the long-term stability of the tule elk population from our DNA sequence data (Table 4.2). 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 postmortem DNA damage. The latter seems less likely, however, given the excellent collagen preservation in these materials (see below), 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).

4.8 Climate Change and the Tule Elk Population Decline

4.8.1 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 was driven by climate-based reductions in range quality, than paleoclimatic data should indicate enhanced drought conditions in the San Francisco Bay area during the post-

bottleneck period. Insofar as the Emeryville relative abundance and genetic data reflect a pan tule elk population decline, climate data from across their California 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 (Adam 1975; Starratt 2008). 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.

4.8.2 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 Ehlringer 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 C3 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 3-6% (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 4.3). 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, C3 lowland setting. Given the +5 ‰ offset between herbivore diets and bone collagen, our sample of tule elk foraged virtually entirely on C3 vegetation, with a mean carbon isotope value of approximately -25 ‰.

Mean collagen $\delta^{13}C$ and $\delta^{15}N$ values for 9 elk from the pre-bottleneck sample are -20.7 ± 0.5 ‰ and 5.3 ± 0.4 ‰, respectively (Table 4.4). 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 is -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 preweaning enrichment in $\delta^{15}N$ (Table 4.3). 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 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 [McCullough1969]). 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 4.3). When fetal/neonate isotope values ($\delta^{13}C_{coll}$ = -21.5 ± 0.2 ‰, $\delta^{13}C_{apt}$ = -13.7 ± 0.5 ‰ [Table 4.4]) 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.

4.9 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 subpopulation 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 subpopulations 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 microhabitat

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 (Frankam 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 (Meredith et al. 2007; Williams et al. 2004). 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 year), but severe, historic period bottleneck but possibly a substantial protracted (~1000 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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Table 4.1. Tule elk specimens from the Emeryville Shellmound with partial mitochondrial D-loop sequences.

Specimen no.	Independent PCRs	Haplotype[a]	Period[b]	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[c], 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
54	1	$_{p}$H	Pre-B.	Uhle, Stratum 10	L prox. femur	Younger subadult	2430-2620
61	1	$_{p}$A	Pre-B.	Uhle, Stratum 10	R scapula	Younger subadult	2430-2620

[a]The subscript “p” indicates a nonreplicated or "provisional" sequence. [b]Post-B. = Post-bottleneck; Pre-B. = Pre-bottleneck. [c] The "Trench" provenience units are from the 1924 Schecnk excavation.

Table 4.2. 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	*	*

*an estimate could not be made by DnaSp because there are no polymorphic sites in these sequences

Table 4.3. 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 yrs 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	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

Table 4.3 (cont.).

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 yrs B.P. (approx.)
Pre-Bottleneck												
35	HA10976	neonate								-13	-6.3	1860-2050
36	H7707	neonate	-21.7	4.9	39.2	14	2.8	3.3	12.3	-14.1	-6	1860-2050
47	HA9564	older subadult	-20.6	5.7	38.7	14	2.8	3.2	6.5	-11.9	-4.8	1860-2050
9	HA1826	adult	-20	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	-11.2	-5.1	2430-2620
11	H1-9842	adult	-20.7	5.8	37.7	14.5	2.6	3	3	-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	2430-2620

Table 4.4. 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 (?)[a]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		

[a]SA =subadult

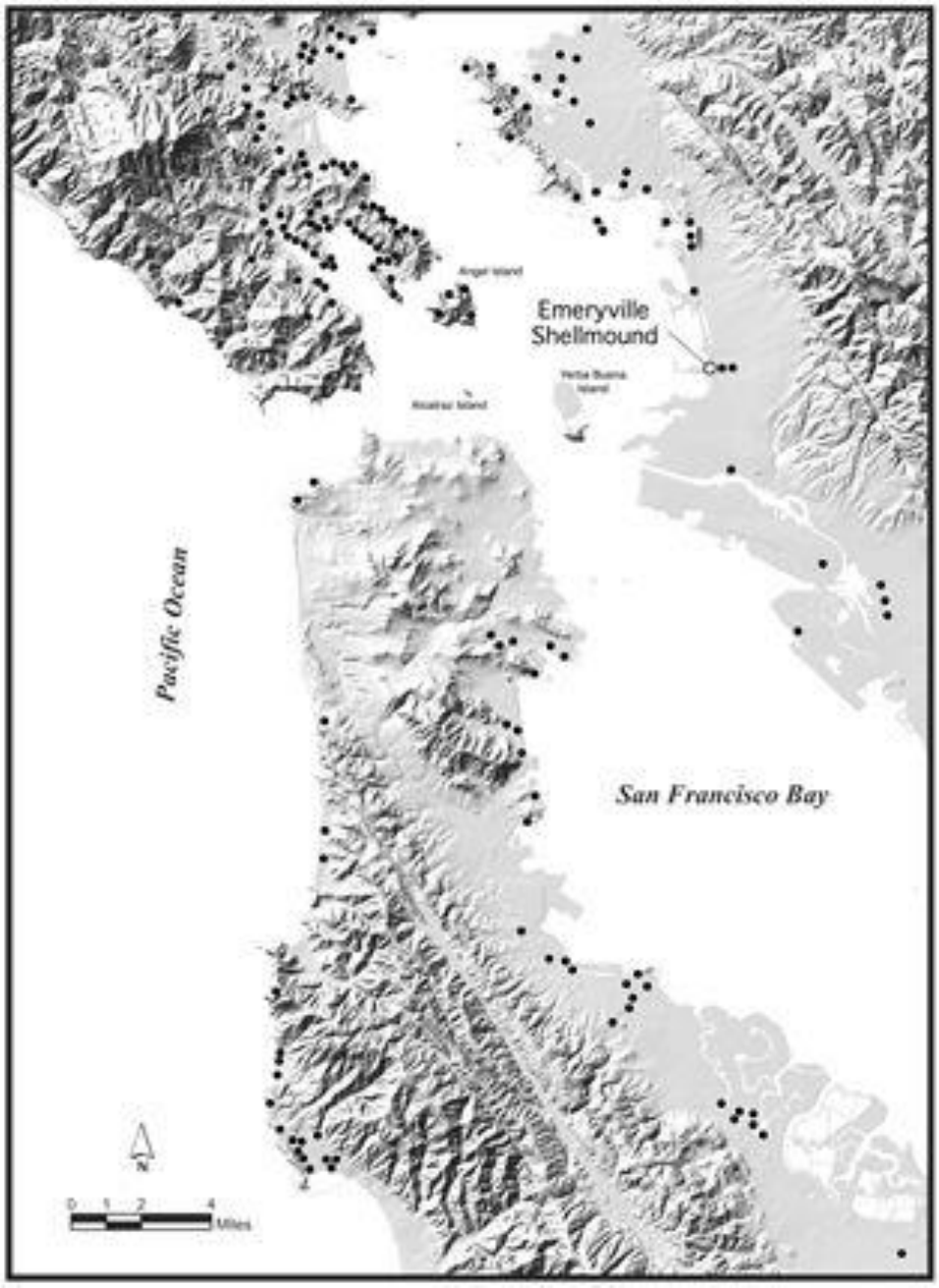

Figure 4.1. Map of San Francisco Bay area showing location of the Emeryville Shellmound and other shellmound sites.

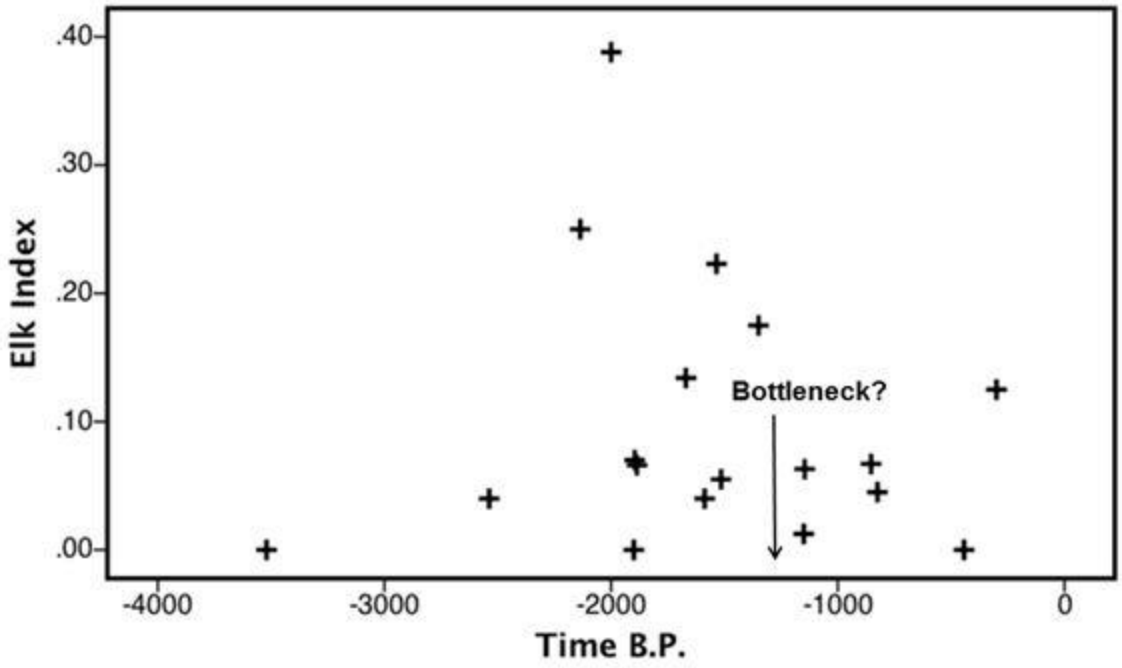

Figure 4.2. Distribution of the Elk Index (Σ [NISP Elk]/ Σ NISP [Terrestrial Mammals]) across 18 dated components from sites distributed across the San Francisco Bay shoreline (total NISP = 9,229). (Does not include potentially intrusive rodents and lagomorphs).

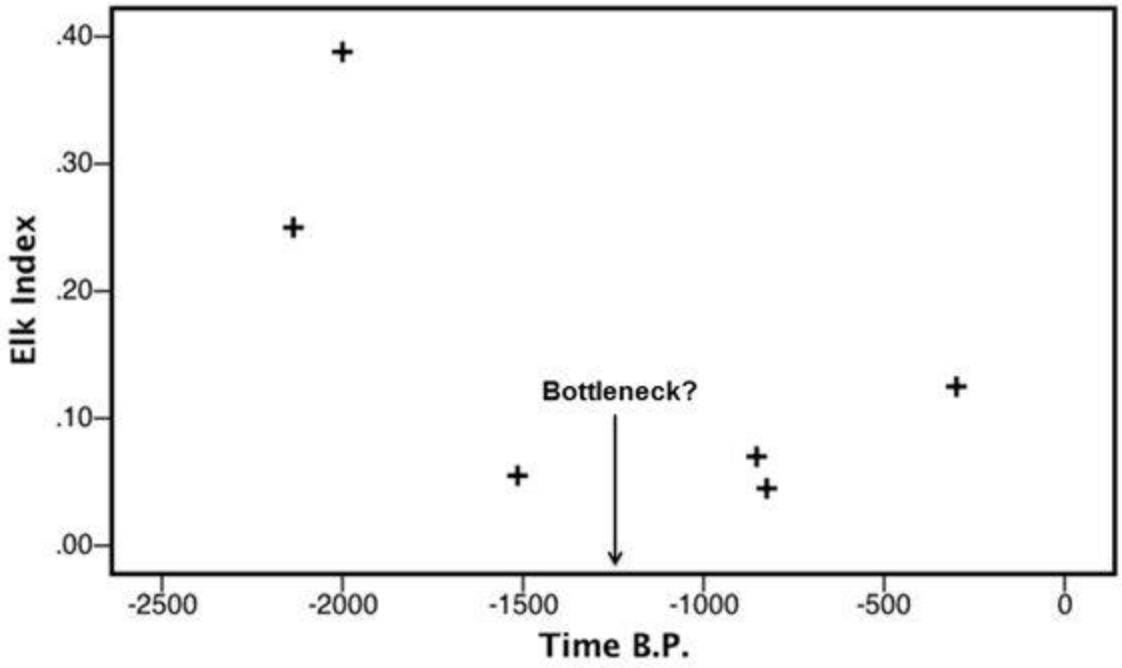

Figure 4.3. Distribution of the Elk Index (Σ [NISP Elk]/ Σ NISP [Terrestrial Mammals]) across a tight cluster of sites located in the Coyote Hills area of the southeast bay (total NISP = 2,168). (Does not include potentially intrusive rodents and lagomorphs).

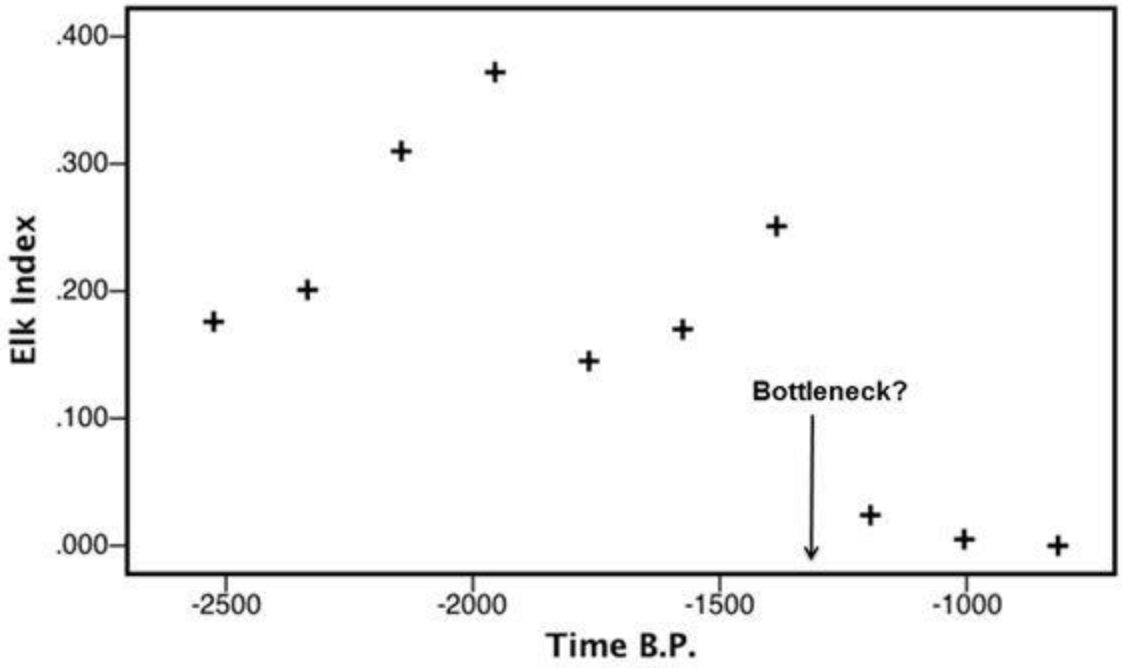

Figure 4.4. Distribution of the Elk Index (Σ [NISP Elk]/ Σ NISP [Terrestrial Mammals]) across the occupational history of the Emeryville Shellmound (total NISP = 6,032). (Does not include potentially intrusive rodents and lagomorphs).