Genomic Applications to Salt Marsh Harvest Mouse Conservation: Distribution, Divergence, and Dietary Niche

By

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ABSTRACT

The salt marsh harvest mouse (*Reithrodontomys raviventris*) is an endangered species endemic to coastal wetland habitat in the San Francisco Estuary (SFE). It is >3.5 million years diverged from its extant sister species and is the only mammalian species completely restricted to salt marsh habitat. Over the past 150 years, the SFE has lost over 90% of its historical tidal marsh habitat. Habitat loss and fragmentation has been more pronounced in the central and southern parts of salt marsh harvest mouse range, where the southern subspecies (*R. r. raviventris*) occurs, whereas habitat in the range of the northern subspecies (*R. r halicoetes*) appears to be more stable.

The evolutionary history of the salt marsh harvest mouse is poorly understood, as is its contemporary distribution in the highly fragmented central and southern SFE. In the north, large populations persist on brackish, diked wetlands, where the effects of non-native and upland vegetation are potentially concerns for salt marsh harvest mice. To this end, I applied genomic techniques to aid our understanding of salt marsh harvest mouse historical and modern distribution, evolutionary and demographic history, and use of dietary resources with respect to upland competitors.

I found that modern salt marsh harvest mouse distribution was significantly reduced in the central portions of the bay, where some historically occupied regions appear to be extirpated. Salt marsh harvest mouse occupancy conformed to the area-isolation paradigm of metapopulation theory, such that the probability that a marsh was occupied was positively related to its size and connectivity to nearby marsh habitat. The evolutionary history of the salt marsh harvest mouse has been closely associated with sea level. Population size increased and lineages diverged as sea level rose following the Last Glacial Maximum. Several lineages diverged as the

modern SFE formed, and again as rising sea levels caused vicariance of previously continuous patches of marsh habitat. Finally, the dietary resource use by salt marsh harvest mice was driven largely by affinities for pickleweed (*Salicornia*) and fat-hen (*Atriplex*). Their diet varied seasonally in association with the phenology of their preferred plants, and their dietary niche breadth was narrower than that of three upland-associated competitors, highlighting their specialization on marsh habitat. Overall, the results of my research provided a molecular basis for subspecies delineation, highlighted the significant impact of habitat loss on this endangered species, and identified important dietary resources for salt marsh harvest mice.

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INTRODUCTION

Human activities are impacting global ecosystems with profound magnitude. We are now in an era deemed the "Anthropocene defaunation" (Dirzo et al. 2014) or the "sixth mass extinction" (Barnosky et al. 2011). Extinction risk for species is non-randomly distributed (Pimm et al. 2014). For wild mammals, human population density is one of the strongest correlates of extinction risk (Cardillo et al. 2008). Furthermore, anthropogenic climate change is predicted to become the leading cause of biodiversity loss within the next century (Young et al. 2016).

Anthropogenic influences on the environment that do not directly cause extinction may nonetheless act as catalysts for a series of events resulting in extinction. Environmental changes may impose an "extinction debt" upon wildlife populations – a time-lagged deterministic decline in abundance or species richness until the equilibrium biodiversity for new environmental conditions is reached (Tilman et al. 1994). Population declines can have demographic and genetic consequences that lead to serial increases in extinction risk – a process called the "extinction vortex" (Wright 1931; Gilpin and Soule 1986). Population declines may trigger a demographic process known as the Allee effect, in which small population size inhibits population recovery (Courchamp et al. 1999). For example, population densities may decline such that potential mate encounters are too infrequent to facilitate population growth or maintain population stability (Dennis 1989). Genetic bottlenecks may also contribute to the extinction vortex (Blomqvist et al. 2010). Genetic drift in small populations can lead to the accumulation of maladaptive alleles (Gilpin and Soule 1986). Inbreeding depression may inflate homozygosity, exposing the phenotypic deficiencies associated with maladaptive alleles (Charlesworth and

Willis 2009). Loss of genetic diversity may limit a population's ability to adapt to changing future landscape conditions (Sgro et al. 2011).

Anthropogenic activity may threaten wildlife species not only by reducing the amount of habitat, but also by causing changes to habitat quality and configuration. Introductions of non-native competitors may increase the extirpation risk of native species (Elton 1958; Dueñas et al. 2021). Additionally, native specialists may not take advantage of introduced non-native resources; thus, invasions may facilitate subsequent invasions by non-native generalists across multiple trophic levels (Marvier et al. 2004; Abernethy et al. 2016; Lepczyk and Rubinoff 2017). Due to climate change, the entire globe will likely experience significant changes in landscape configuration and community composition in the near future (Williams et al. 2007; Beaumont et al. 2011; Martinuzzi et al. 2015). It is therefore imperative to understand the efficacy of conservation strategies in the context of such "novel ecosystems" (Hobbs et al. 2006; Hulvey et al. 2013). Whereas generalist species and strong dispersers may be well-equipped to cope with novel ecosystem conditions (Hobbs et al. 2009; Clavel et al. 2011), the most pressing conservation challenge in the context of novel ecosystems may be ensuring the persistence of endemic specialists with small population sizes and limited dispersal ability (Lurgi et al. 2012). To this end, understanding such species' responses to climate change, habitat loss and fragmentation, and non-native community resources, can critically inform conservation of the broad array of species and communities that face inevitable and imminent novel ecosystem developments.

The salt marsh harvest mouse (*Reithrodontomys raviventris*) is a small rodent endemic to the San Francisco Estuary (SFE). They are the only mammalian species in the world that is endemic to coastal marshes (Greenberg et al. 2006), and they possess several morphological and

physiological adaptations to their unique, saline environment, including the ability to tolerate drinking only salt water (Fisler 1963, 1965). Although they are part of a relatively speciose genus (Mammal Diversity Database 2022), they represent >3.5 million years of evolutionary divergence from their closest extant relative, the plains harvest mouse (*R. montanus*), which occurs in parapatry in the central Unites States (Bell et al. 2001; Statham et al. 2016). There are two subspecies of salt marsh harvest mice (Statham et al. 2016). The northern subspecies (*R. r. halicoetes*) occurs in the Suisun and San Pablo Bays, and the southern subspecies (*R. r. raviventris*) occurs in the South San Francisco Bay. Populations in the central part of their range ("Mid-Bay") have been assigned morphologically to the southern subspecies, but no genetic data exist to support the location of the subspecies boundary (Statham et al. 2016).

Salt marsh harvest mice are listed as an endangered species at the State (CDFW 1971), federal (USFWS 1970), and international levels (Whitaker and NatureServe 2018). Their environment, salt marshes in the SFE, has been highly modified by anthropogenic activity (Hobbs et al. 2006) and continues to be imperiled by land development and climate change, particularly sea level rise (Thorne et al. 2018). Their stenotopic nature, their limited range and dispersal abilities, and their highly modified ecosystem makes salt marsh harvest mice an outstanding model for habitat specialists threatened by a synergy of anthropogenically driven conservation challenges. Strengthening our ecological understanding of a model habitat specialist in a novel ecosystem can broadly inform conservation efforts of other species and communities in dynamic human-influenced environments.

Approximately 90% of historical tidal wetlands in the SFE have been lost in the past century (Williams and Faber 2001). The total amount of remaining wetland habitat in the SFE is estimated to be ~125 km^2, but the distribution of salt marsh harvest mice within these remains

unknown despite an abundance of research effort focused on habitat associations (Smith et al. 2018). Salt marsh harvest mouse ecology has historically been closely associated with pickleweed (*Salicornia* spp.; Smith et al. 2018). Dense stands of native tidal vegetation, such as pickleweed, are believed to be important for cover from aerial predation (Shellhammer 1989). However, recent evidence suggests that diked wetlands with abundant non-native vegetation support healthy populations (Sustaita et al. 2011; Smith et al. 2020), and that salt marsh harvest mice readily consume non-native foods (Smith and Kelt 2019).

One of the major challenges to filling knowledge gaps regarding salt marsh harvest mouse ecology is the effort required to obtain reliable field data. Typically, surveys involve five-day live-trapping sessions with strictly regulated protocols requiring multiple visits to the field site each day. Furthermore, it can be challenging to distinguish salt marsh harvest mice from their co-occurring congener, the western harvest mouse (WHM; *R. megalotis*) in the field. Indeed, genetic species identification revealed that morphological keys resulted in false-positive identifications (i.e., a true western harvest mouse called a salt marsh harvest mouse in the field) up to 50% of the time in the range of the southern subspecies (Statham et al. 2016). Morphological methods are improving (Statham et al. 2021), but genetic species verification is considered an essential step for reliable species identification. Considering that much of our knowledge of salt marsh harvest mouse ecology predates genetic species verification, many aspects of their ecology may need to be revisited.

In the first chapter of this dissertation, I developed a non-invasive genetic survey technique to rapidly assess the occupancy status of marshes. I deployed bait stations, which had permanent openings allowing mice to enter and exit freely, and provided these with seed mix and cotton batting. I placed bait stations along two transects at each of two well-monitored sites. Goodyear

Slough represented a robust salt marsh harvest mouse population whereas Benicia Industrial Unit supported a small salt marsh harvest mouse population. After seven days, I collected feces from the bait stations and subjected the feces to a genetic laboratory assay I developed to detect the DNA of salt marsh harvest mice and four sympatric rodents. I tested the laboratory assay rigorously with positive controls from known rodent genetic material and negative controls with no DNA. I tested whether (1) the bait stations effectively collected fecal material, (2) the genetic assay was sensitive to the presence of the target DNA and specific to avoid false-positive detection of non-target DNA, and (3) the frequency of salt marsh harvest mouse detections at the two field sites reflected expectations based on known relative densities as determined by live-trapping efforts.

In Chapter 2, I applied non-invasive genetic surveys to 47 marshes throughout the range of salt marsh harvest mice. The objectives of this study were to (1) assess the occupancy status of numerous marshes that have not been surveyed in decades (if ever), (2) assess the detection probability of the non-invasive genetic survey technique developed in Chapter 1 using a more robust data set, (3) identify whether salt marsh harvest mouse occupancy conforms to the area-isolation paradigm of metapopulation theory, (4) identify vegetation characteristics associated with fine-scale salt marsh harvest mouse occupancy, and (5) identify drivers of regional extirpation by comparing historical and modern occupancy patterns to changes in landscape composition over the past century.

In Chapter 3, I took a step back from the modern distribution of salt marsh harvest mice and investigated the evolutionary and demographic history of the species. I sequenced whole mitogenomes of 101 salt marsh harvest mice, including 17 museum specimens (ca. 1930-1959), which helped to fill sampling gaps where modern populations have been extirpated. The

objectives of this study were to (1) determine the mitogenomic subspecies assignment of three Mid-Bay populations, which are morphologically assigned to the southern subspecies but may be split among the two subspecies based on geography, (2) estimate the divergence times of geographically distinct populations, including that of the two subspecies, (3) estimate the demographic history of salt marsh harvest mice, and (4) relate the evolutionary and demographic history of salt marsh harvest mice to major geological events.

Finally, in Chapter 4, I used DNA metabarcoding to assess the diets of salt marsh harvest mice and three sympatric rodents. The objectives of this study were to (1) determine whether salt marsh harvest mice exhibit preference for certain plants, with particular interest in whether they prefer/avoid non-native plants, (2) examine causes of variation in salt marsh harvest mouse diet over space and time, (3) determine whether salt marsh harvest mice exhibit a more specialized diet than their three potential competitors, and (4) assess the potential for competitive interactions between salt marsh harvest mice and sympatric rodents based on dietary niche overlap.

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CHAPTER 1 – A Novel Non-Invasive Genetic Survey Technique for Small Mammals

This chapter has been accepted for publication in *Journal of Mammalogy*:

Aylward CM, Grahn RA, Barthman-Thompson L, Kelt DA, Sacks BN, Statham MJ (2022) A novel non-invasive genetic survey technique for small mammals. Journal of Mammalogy.

*Non-invasive genetic surveys, often conducted by collecting fecal samples, have become a popular tool for surveying wildlife, but have primarily been applied to species with large and conspicuous scat. Although many small mammals are threatened, endangered, or data deficient, non-invasive genetic surveys have rarely been applied due to the challenges of detecting their inconspicuous fecal pellets. As part of a broader study of the endangered salt marsh harvest mouse (*Reithrodontomys raviventris*), we developed a non-invasive genetic survey technique for the community of small mammals in their putative range. We designed bait stations to passively collect fecal samples from rodents, and developed a multiplex primer set that amplified unique fragment sizes for salt marsh harvest mice and four other sympatric species. We tested the primer set on positive controls and on fecal pellets collected from bait stations at two regularly monitored field sites known to have very different densities of salt marsh harvest mice. The multiplex amplified DNA from all five species, even when all five species were present in a single sample. A positive species identification was made for all field-collected samples, and 43% of these field-collected samples had multi-species detections. The combination of bait stations and genetic species identification proved to be an effective means of non-invasively surveying small mammals in potential salt marsh harvest mouse habitat. The sampling technique should be applicable to a wide variety of small mammals in other systems.*

INTRODUCTION

Traditional methods for surveying small mammals are both labor- and time-intensive, typically requiring a large number of live traps established in some form of an array and sampled over multiple nights. Additionally, human traffic can impact habitat, capturing and handling may stress target species (Pauli et al. 2010), and some species are known to be "trap shy" and less likely to be documented with such efforts. Recent revolutions in the ease and cost-effectiveness of genetic sampling have spurred the use of non-invasive genetic surveys for monitoring species of management or other concern (e.g., Carroll et al. 2018; Statham et al. 2020). Non-invasive genetic surveys have been widely applied to species with large conspicuous scat, such as carnivores (e.g., O'Reilly et al. 2008; Moriarty et al. 2009; Quinn et al. 2019). However, non-invasive genetic surveys have not yet proliferated in smaller study organisms, whose fecal pellets may be more difficult to detect (but see Gillet et al. 2016; Ferreira et al. 2018).

Although non-invasive genetic work on rodents remains uncommon, a wealth of information can be extracted using such sampling. One well-studied example is the Cabrera vole (*Microtus cabrerae*— Barbosa et al. 2012; Ferreira et al. 2018; Sabino-Marques et al. 2018; Proença-Ferreira et al. 2019). Cabrera voles use latrines, which helps facilitate non-invasive collection of feces in the field (Ferreira et al. 2018). However, most small mammals do not regularly defecate in latrines, such that even the collection of fecal samples may be impractical. Accordingly, non-invasive genetic studies generally are uncommon among small mammals, even though many such groups have significantly more species threatened than expected (e.g., Soricomorpha and Rodentia; Entwistle and Stephenson 2000; Schipper et al. 2008).

The salt marsh harvest mouse (*Reithrodontomys raviventris*) is an endangered species endemic to wetlands in the San Francisco Estuary (USFWS 1970; Whitaker and NatureServe

2018). Like many endangered species, large portions of the putative historical range for this species have not been surveyed for decades. Moreover, wetland habitats are intrinsically patchy, heterogeneous, and often fragmented, such that comprehensive sampling is particularly challenging. The resulting gaps in data can hinder conservation efforts and lead to suboptimal allocation of conservation funding and effort (Bland et al. 2017). In addition, genetic species identification is increasingly recognized as essential for salt marsh harvest mouse surveys due to the inadequacy of morphological methods in distinguishing this species from sympatric western harvest mice (*Reithrodontomys megalotis*) (Statham et al. 2016). To facilitate the generation of reliable cost-effective survey data, we developed a non-invasive genetic survey technique for salt marsh harvest mice and four rodents with the potential to co-occur in San Francisco Estuary wetlands (western harvest mice, deer mice [*Peromyscus maniculatus*], California voles [*Microtus californicus*], and house mice [*Mus musculus*]). This allows not only confirmation of the presence of salt marsh harvest mice with minimal disturbance, but for rapid and effective assessment of community composition as well.

Our objectives were to 1) develop a field protocol to non-invasively collect genetic material from small mammals in potential salt marsh harvest mouse habitat, 2) develop a laboratory protocol for the genetic identification of salt marsh harvest mice and potentially co-occurring small mammals, and 3) evaluate the efficacy of these field and laboratory techniques using samples from sites with known species composition.

MATERIALS AND METHODS

Primer Design.—We designed a multiplex of primers that produced a unique fragment size for each target species (e.g., Bozarth et al. 2010; Statham et al. 2020) by aligning 720 bp of sequence from the mitochondrial cytochrome b gene. To determine optimal regions for

conserved primers, nine species of rodents were aligned using Sequencher v5.4.6 (Gene Codes Corporation, Ann Arbor, USA). We included five species with the potential to occur in our study area – California vole (MH729867, AF163891, EF506032, EF506033, EF506106), house mouse (AB819915, AB819919, AF520625), western harvest mouse (KR611927, KR611935, KR611937), salt marsh harvest mouse (AF176254, AY859470, AF176255), and deer mouse (DQ385674, FJ800584, KF949251) – and four related species known to not occur in the study area – Puebla deer mouse (*Peromyscus mekisturus*) (MT078818), pinyon mouse (*Peromyscus truei*) (MN022914), white-footed mouse (*Peromyscus leucopus*) (MG674647), and Mount Pirri isthmus rat (*Isthmomys pirrensis*) (KY754007). Primer sites were chosen by eye based upon regions of high homology for universal primers and shared derived single nucleotide polymorphisms for species-specific primers. Next, we designed a multiplex to detect the five target species likely to occur in our study area. We included one "universal" forward primer that annealed to all target species of the rodent family Cricetidae ("UCricF"; annealed to salt marsh harvest mouse, western harvest mouse, deer mouse, and California vole), and one forward primer that annealed to house mice of the rodent family Muridae ("UMuriF") (Table 1-1). Each forward primer was tagged with the fluorescent dye 6-FAM. In addition, we developed reverse primers for each target species (RravR, RmegR, PmanR, McalR, and MmusR), such that each produced a unique, species-specific fragment size during polymerase chain reaction (PCR) amplification with the forward primers.

Laboratory Methods.—We extracted DNA from fecal samples using EurX Stool DNA Purification Kits (EurX, Gdansk, Poland) according to the manufacturer's protocol. To test the efficacy of the primer multiplex, we conducted PCR on positive control samples from each of the five target species (Table 1-2; n = 10 salt marsh harvest mice, 11 western harvest mice, eight

deer mice, nine California voles, and eight house mice), collected from throughout the range of salt marsh harvest mice. We tested samples with template DNA of each species at three different concentrations (1 ng/µl, 0.1 ng/µl, and 0.01 ng/µl), mixtures of template DNA of all combinations of 2 – 3 species (0.1 ng/µl each), mixes of template DNA of all five target species (0.1 ng/µl each), and negative controls composed solely of deionized water.

We conducted PCRs in a mixture of 1× Qiagen Multiplex PCR Master Mix (Qiagen Inc., Valencia, California, USA), 0.5× Qiagen Q-Solution, 0.6 µM UCricF, 0.3 µM RravR, 0.3 µM RmegR, 0.6 µM PmanR, 0.3 µM McalR, 0.3 µM UMuriF, and 0.3 µM MmusR, with 1 µL of template DNA. All PCRs were conducted in duplicate. We used the following thermal cycle for PCR: 95 °C for 15 minutes; 33 cycles of 94 °C for 30 seconds, 58 °C for 90 seconds, and 72 °C for 60 seconds; and a final extension step at 72 °C for 10 minutes. We mixed PCR products with formamide and LIZ 500 size standard (Applied Biosystems Inc., Foster City, California, USA), and conducted fragment size analysis on a 3730 ABI capillary sequencer (Applied Biosystems Inc.). We assessed fragment sizes relative to LIZ 500 using STRand version 2.4.150 (Toonen and Hughes 2001).

Field Surveys.—We conducted non-invasive surveys at two study areas in the Grizzly Island Wildlife Area (Solano County, California) where salt marsh harvest mouse populations are regularly monitored with live-trapping by the California Department of Fish and Wildlife (CDFW). Goodyear Slough Unit (GYS) harbors a robust population of salt marsh harvest mice (L. Barthman-Thompson, CDFW unpublished data). In contrast, the Benicia Industrial Unit (BIU), a nearby parcel surrounded by a more urban landscape, harbors a much smaller salt marsh harvest mouse population (L. Barthman-Thompson, CDFW unpublished data).

During each survey, we deployed Aegis Mouse Bait Stations (Lipha Tech, Milwaukee, Wisconsin, USA) at 20-m intervals along two transects (Figure 1-1). Our goal was to place seven stations per transect, although unexpected inundation at BIU forced us to modify the path of one transect and to reduce the other to four stations. We placed approximately one tablespoon of seed mix and a handful of cotton bedding into each station (Figure 1-2A). We placed each bait station within the vegetation, approximately 0.5-1 meters above the ground, and tied them to vegetation with colored flagging to help relocate bait stations and secure them in case of strong winds or exceptionally high tides (Figure 1-2B).

We deployed bait stations for seven days, then collected fecal samples from inside, and sometimes on top of, the bait stations. At each bait station, at least 10 fecal pellets were pooled and placed into a 1.5-ml vial filled with 1 ml of >95% ethanol. We sought to include pellets of varying sizes to maximize the chances of detecting multiple species. We used a separate vial for each bait station, and sterilized forceps with bleach and water in between sampling from each bait station. Field work followed guidelines of the American Society of Mammalogists (Sikes et al. 2016).

RESULTS

Positive Controls.—All single-species tests resulted in distinct fragments for each target species and no false-positive amplifications (fragment sizes shown in Table 1-1). Moreover, in controls that contained DNA from all five target species, five distinct peaks corresponded to the fragment sizes of the target species (Figure 1-3).

Species Identification of Field Samples.—Fecal pellets were present in 12 of 13 stations at GYS and at nine of 11 stations at BIU. All fecal samples collected from bait stations (n = 21) successfully amplified PCR products. At GYS, salt marsh harvest mice were detected at eight

stations, western harvest mice at one station, house mice at 11 stations, and California voles at two stations (Table 1-3). Nine stations at GYS had multi-species detections, including one station with three species (salt marsh harvest mouse, western harvest mouse, and house mouse). At BIU, salt marsh harvest mice were detected at one station, western harvest mice at three stations, house mice at seven stations, and California voles at one station. Three stations at BIU had multi-species detections, including the station where salt marsh harvest mice were detected (co-detected with house mice). Deer mice were not detected in any field samples.

DISCUSSION

Bait stations proved an efficient method of sampling marsh sites for small mammals. Each site took approximately four person-hours to conduct a seven-day survey (i.e., two hours for a single technician to deploy stations, and two hours to collect fecal samples a week later). Comparatively, live-trapping surveys of similar sites typically require approximately 22 person-hours. Furthermore, because no animals were handled, permitting and regulation of field work was less extensive than that required for live-trapping efforts. This combination of conveniences could significantly increase survey efficiency. This method of field sampling is also vastly more cost-effective than traditional live-trapping methods. Bait stations can be purchased for approximately $1/20^{th}$ the cost of live traps, and bait station surveys involve fewer field visits, less time per field visit, and fewer personnel, all of which reduces the cost of field work and associated impacts to habitat. Because of the morphological similarity between salt marsh harvest mouse and western harvest mouse, genetic identification following live-trapping is often the only way to identify these congeners with certainty (Statham et al. 2016, 2021). For study systems that do not typically require genetic species identification, the additional cost of genetic species identification would have to be weighed against the cost savings from the bait station

field sampling technique. However, for threatened, rare, or "trap shy" species, and for sensitive habitats, bait stations provide a rigorous and quantifiable means of surveying for species presence as well as community composition.

Given that multiple sites can be surveyed simultaneously by a single researcher, this technique can facilitate a substantial increase in the breadth of sampling for salt marsh harvest mice, significantly improving our understanding of their range. Survey data from bait stations also may be used to answer targeted ecological questions, such as habitat preference and patterns of co-occurrence (MacKenzie et al. 2004), which are priorities for salt marsh harvest mouse conservation (Smith et al. 2018). Our primer set may also be sufficient for use in an aquatic environmental DNA (eDNA) framework (e.g., Ficetola et al. 2008), although we did not test its ability to detect rodent DNA from a non-fecal environmental sample. While this technique has significant potential, the detection probability of our sampling protocol was not formally examined. Further work will need to be performed to refine a survey protocol using bait stations that adequately ensures false-negative survey results are avoided. Furthermore, while our survey technique meets the criteria for non-invasive genetic surveys (see Pauli et al. 2010; Zemanova 2021), surveyors are still required to enter sensitive habitat and the use of bait may alter animal behavior (although not likely to the extent that survival and reproduction would be reduced). Hence, in ecological terms this approach may be better viewed as minimally-invasive (or minimally-disruptive as used by Lefort et al. 2019), but we contend that it is substantially less invasive than traditional live-trapping efforts.

Relative to traditional live-trapping methods, bait station surveys may increase the probability of detecting multiple sympatric species. We found that 43% of occupied stations had multi-species detections, which may increase the chances of detecting a low-density salt marsh

harvest mouse population co-occurring with high densities of other species. To underscore this advantage, the lone station where salt marsh harvest mice were detected at BIU also detected house mice; had this been a live-trapping effort and a house mouse entered the trap first, the salt marsh harvest mouse may have been excluded from the trap and potentially evaded detection. Resolution of this potentiality with live traps requires return visits over multiple nights; bait stations address this concern with a single sampling interval.

Pooling samples from bait stations precludes identification of individuals from samples. Further work should evaluate whether individual animals can be identified from fecal samples, but we have not tested the efficacy of this approach. Furthermore, while we detected salt marsh harvest mice in proportion to *a priori* expectations (i.e., many detections at GYS and few at BIU), further work is needed to quantitatively assess detection probabilities with this technique using repeated survey intervals (e.g., MacKenzie et al. 2002).

To our knowledge, this is the first study to combine bait stations with genetic species identification to survey small mammals with non-invasive techniques. This technique can be extended to other systems where a species' range is understudied or habitat makes them difficult to sample (e.g. arboreal tree voles [*Arborimus* spp.]). Previous studies that have used non-invasive genetic sampling for small mammal surveys relied on sampling from latrines (Ferreira et al. 2018). Other studies have used dog detection teams to find inconspicuous feces of endangered reptiles (Statham et al. 2020) or required extensive visual searches for small mammal feces (Gillet et al. 2016). For study systems where dog detection teams are infeasible or impractical, and feces cannot reliably be identified visually, bait stations can be an effective means to collect multi-species survey data with minimal time commitment and significantly reduced financial investment.

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FIGURES

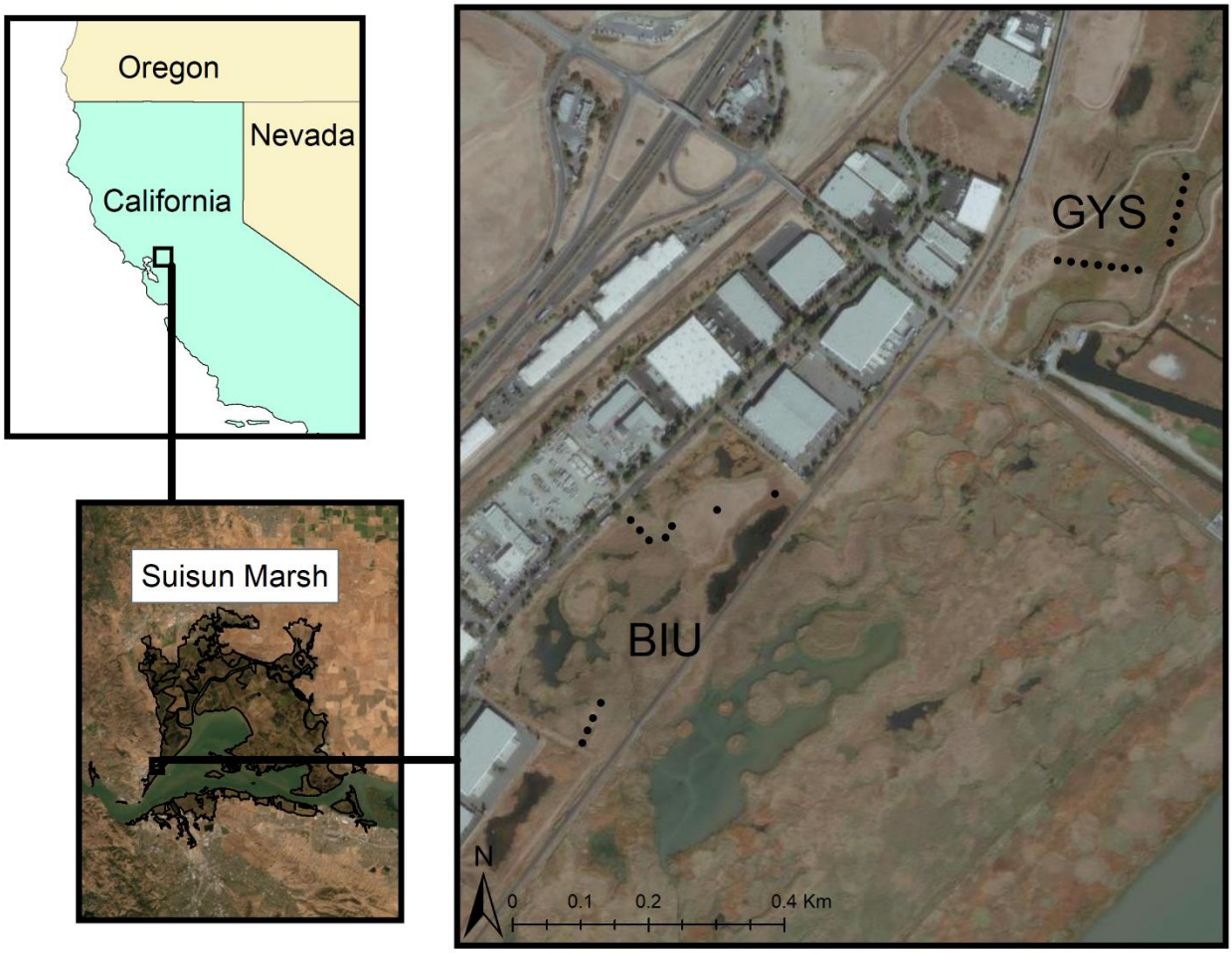

Figure 1-1. Map of field sites at the Grizzly Island Wildlife Area (Solano Co., California): Benicia Industrial Unit (BIU) and Goodyear Slough (GYS). Black dots in the right panel represent bait station locations.

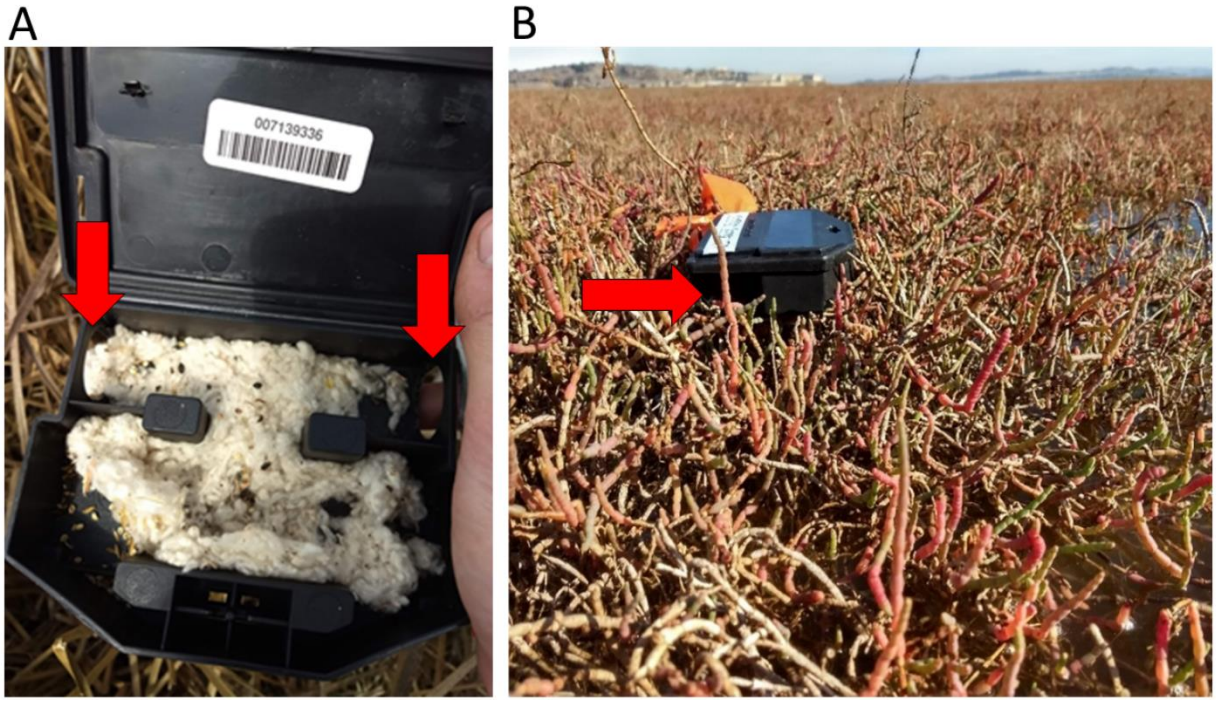

Figure 1-2. Bait stations used in non-invasive surveys for endangered salt marsh harvest mice and sympatric rodents at the Grizzly Island Wildlife Area (Solano County, California). Panel A shows the inside of a station that has been visited by a mouse. Fecal pellets and shells from bait are visible in the cotton bedding. Permanent openings on the left and right sides of the station are noted by red arrows. Panel B shows a bait station in the field. The permanent opening in the side of the station is marked by a red arrow.

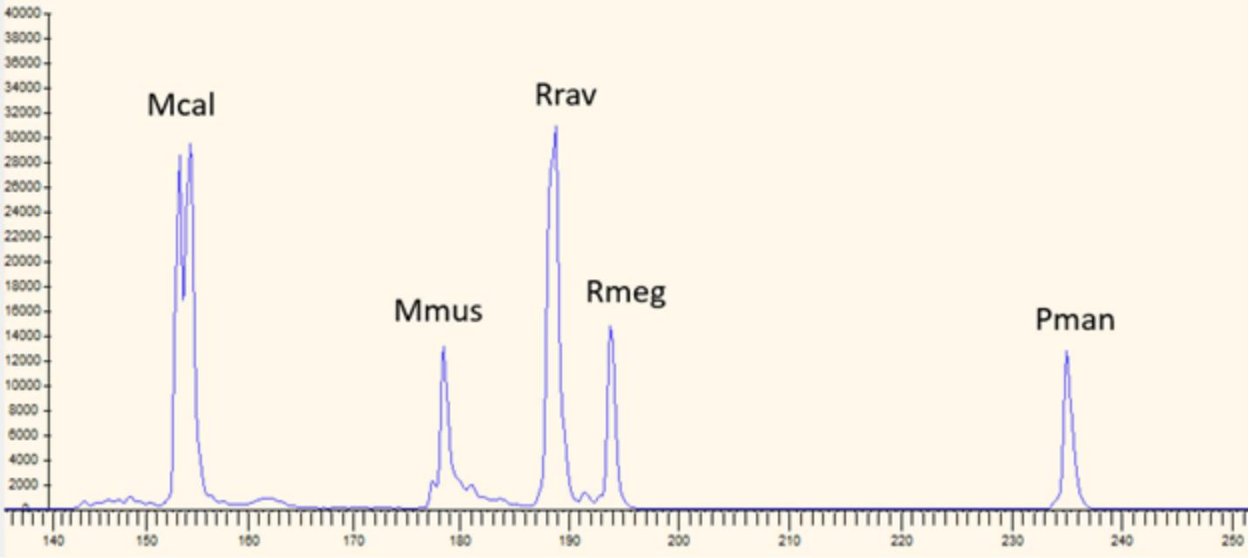

Figure 1-3. All five species detected in a control sample that contained DNA of California vole (Mcal; 154 bp split peak), house mouse (Mmus; 179 bp), salt marsh harvest mouse (Rrav; 189 bp), western harvest mouse (Rmeg; 194 bp), and deer mouse (Pman; 235 bp). Figure shows fragment size in base pairs on the x-axis and relative fluorescence units on the y-axis.

TABLES

Table 1-1. Primers developed in this study to identify two rodent families and five species using unique cytochrome b fragment sizes.

Primer	Sequence	Fragment Size	Target Species
UCricF	GCH ATR CAY TAY ACA TCA GAY ACA	--	--
RravR	GTT TCA CGT TTC TGT GAA TAG GA	189	Salt marsh harvest mouse
RmegR	GGT TAC ATT CAT GTT TCT GTA AAG GTA TAT GAA	194	Western harvest mouse
PmanR	CCT ATG AAT GCT GTT GCT ATT AAA	235	Deer mouse
McalR	TAG ATT CCT CGT CCT ACA TGT AT	154	California vole
UMuriF	GCC ATA CAC TAT ACA TCA GAT ACA	--	--
MmusR	CTA TAA ATG TAT ATG ATC CAT AAT ATA AG	179	House mouse

Table 1-2. DNA samples used as positive controls. We used samples collected from as broadly as possible throughout each of the three bays that comprise salt marsh harvest mouse range (Suisun, San Pablo, South San Francisco). We had few deer mouse (*Peromyscus maniculatus*) localities and no representation from the range of the southern subspecies of salt marsh harvest mouse (South Bay), so we included a sample collected from Monterey County, ~70 km south of the South Bay (*). All other target species had at least two representatives from each of the northern (Suisun and San Pablo Bays) and southern (South San Francisco Bay) salt marsh harvest mouse subspecies' ranges. Representation was greatest for salt marsh harvest mice (*Reithrodontomys raviventris*) and western harvest mice (*R. megalotis*), and included eight paired sites where the congeners were detected during the same trapping session.

Sample ID	Species	Type	Site
M-0142	House mouse	Hair	Alviso, South Bay
M-0396	House mouse	Hair	Newark, South Bay
M-1144	House mouse	Tissue	Palo Alto, South Bay
M-1960	House mouse	Hair	Milpitas, South Bay
M-2277	House mouse	Tissue	Joice Island, Suisun Bay
M-2278	House mouse	Tissue	Goodyear Slough, Suisun Bay
M-2279	House mouse	Tissue	Goodyear Slough, Suisun Bay
M-2325	House mouse	Hair	Montezuma Slough, Suisun Bay
M-0768	Deer mouse	Tissue	Moss Landing, Monterey County*
M-0856	Deer mouse	Hair	Denverton, Suisun Bay
M-1493	Deer mouse	Tissue	Arnold Slough, Suisun Bay
M-1494	Deer mouse	Tissue	Arnold Slough, Suisun Bay
M-1495	Deer mouse	Tissue	Arnold Slough, Suisun Bay
M-1496	Deer mouse	Tissue	Arnold Slough, Suisun Bay
M-1497	Deer mouse	Tissue	Arnold Slough, Suisun Bay
M-1498	Deer mouse	Tissue	Arnold Slough, Suisun Bay
M-1279	Western harvest mouse	Tissue	Joice island, Suisun Bay
M-1351	Western harvest mouse	Tissue	Goodyear Slough, Suisun Bay
M-1693	Western harvest mouse	Hair	Mare Island, San Pablo Bay
M-1910	Western harvest mouse	Tissue	Petaluma Marsh, San Pablo Bay
M-1948	Western harvest mouse	Hair	Dumbarton Marsh, South Bay
M-2006	Western harvest mouse	Tissue	Coyote Creek, South Bay
M-2210	Western harvest mouse	Tissue	Richmond, San Pablo Bay
M-2260	Western harvest mouse	Tissue	Palo Alto, South Bay
M-2263	Western harvest mouse	Tissue	Twitchell Island, Suisun Bay
M-2272	Western harvest mouse	Tissue	Bruener Marsh, San Pablo Bay
M-2319	Western harvest mouse	Tissue	Martinez, Suisun Bay
M-0730	Salt marsh harvest mouse	Tissue	Newark, South Bay
M-1278	Salt marsh harvest mouse	Tissue	Joice Island, Suisun Bay
M-1350	Salt marsh harvest mouse	Tissue	Goodyear Slough, Suisun Bay
M-1692	Salt marsh harvest mouse	Tissue	Mare Island, San Pablo Bay

M-1911	Salt marsh harvest mouse	Tissue	Petaluma Marsh, San Pablo Bay
M-1990	Salt marsh harvest mouse	Tissue	Santa Venitia Marsh, San Pablo Bay
M-2022	Salt marsh harvest mouse	Tissue	Coyote Creek, South Bay
M-2209	Salt marsh harvest mouse	Tissue	Richmond, San Pablo Bay
M-2261	Salt marsh harvest mouse	Tissue	Palo Alto, South Bay
M-2318	Salt marsh harvest mouse	Tissue	Martinez, Suisun Bay
M-0098	California vole	Hair	Napa/Sonoma Marshes, San Pablo Bay
M-1263	California vole	Tissue	McAvoy Harbor, Suisun Bay
M-1458	California vole	Tissue	Hill Slough, Suisun Bay
M-1522	California vole	Tissue	Joice Island, Suisun Bay
M-1701	California vole	Tissue	Mare Island, San Pablo Bay
M-1806	California vole	Tissue	Goodyear Slough, Suisun Bay
M-1876	California vole	Tissue	Newark, South Bay
M-1877	California vole	Tissue	Newark, South Bay
M-2198	California vole	Tissue	Richmond, San Pablo Bay

Table 1-3. Rodent species detected during bait station surveys conducted at Goodyear Slough and Benicia Industrial Unit on the Grizzly Island Wildlife Area, Solano County, California. Rrav = salt marsh harvest mouse, Rmeg = western harvest mouse, Mcal = California vole, Mmus = house mouse.

Detection Type	Number of Stations	
	Goodyear Slough	Benicia Industrial Unit
Rrav only	--	--
Rmeg only	--	1
Mcal only	1	--
Mmus only	2	5
Rrav + Rmeg	--	--
Rrav + Mcal	--	--
Rrav + Mmus	7	1
Rmeg + Mcal	--	1
Rmeg + Mmus	--	1
Mcal + Mmus	1	--
Rrav + Rmeg + Mmus	1	--

CHAPTER 2 – Patch Size and Connectivity Predict Remnant Habitat Occupancy by a Wetland Specialist, the Salt Marsh Harvest Mouse

The area-isolation paradigm of metapopulation theory predicts that larger and more connected patches have a higher probability of occupancy. I tested predictions of the area-isolation paradigm for the endangered salt marsh harvest mouse (Reithrodontomys raviventris), a habitat specialist living in highly fragmented salt marsh habitat in the San Francisco Estuary (California, USA). I surveyed for salt marsh harvest mice at 47 marsh patches throughout their range using a non-invasive genetic survey technique. I used occupancy modeling to estimate the effects of patch size, patch connectivity, matrix urbanization, and several habitat characteristics on occupancy probabilities. I evaluated occupancy at both coarse (e.g., among patches) and fine (e.g., within patches) spatial scales. Patch size, connectivity, and matrix urbanization had significant effects on patch-occupancy; connectivity was the strongest predictor based on AICc. Within patches, occupancy was positively related to the presence of high-tide escape vegetation. My data also revealed the extirpation of several geographically distinct populations, consistent with expectations due to reduced patch sizes and connectivity over the past century. I found that patterns of salt marsh harvest mouse patch-occupancy were consistent with the area-isolation paradigm. In addition, my models provide important guidelines of patch size and connectivity that can inform habitat conservation and restoration for this endangered species. Specifically, my data suggests that selecting restoration sites that are well-connected may be more beneficial than selecting larger, isolated sites.

INTRODUCTION

Habitat fragmentation is one of the leading threats to wildlife worldwide (Haddad et al. 2015). Many endangered species are listed as such due to past and ongoing habitat fragmentation (Kerr and Cihlar 2004). When endangered species live in patchy, fragmented distributions, identifying patch size and connectivity thresholds that facilitate population persistence can inform conservation actions. The "area-isolation paradigm" draws on insights from island biogeography (MacArthur and Wilson 1967; Diamond 1975) and metapopulation theory (Hanski 1991, 1998), and predicts that the probability that a species will occupy a given patch is related proportionally to patch size and inversely to patch isolation. However, it is less clear how species that evolved in more continuous patches of habitat will cope with an increased need for dispersal through a recently fragmented landscape.

Applying the area-isolation paradigm to nature can be challenging because it requires a binary delineation of the landscape into patches of suitable habitat and a matrix of non-habitat, which may be an oversimplification for most species (Hanski 1998; Prugh et al. 2008). However, when species are highly specialized, discrete habitat patches and non-habitat matrix may be a reasonable representation of the landscape (Hanski et al. 1995). Additionally, habitat covariates can be incorporated into models, representing habitat patches more realistically in terms of variable habitat patch quality. Thus, patch-occupancy models that integrate components of habitat and matrix quality with the area-isolation paradigm can be useful for assessing the value of specific habitat patches to some focal species (Wahlberg et al. 1996; Schultz and Crone 2005).

The salt marsh harvest mouse (*Reithrodontomys raviventris*) is an endangered species (USFWS 1970; CDFW 1971) endemic to the coastal wetlands of the San Francisco Bay and connecting tidal bodies, collectively, the San Francisco Estuary (SFE; USFWS 2013). Tidal

wetlands in the SFE have been fragmented over the past century to the point where <10% of historic tidal marsh habitat remains intact (Williams and Faber 2001). Given such levels of habitat loss, understanding minimum marsh patch size and connectivity needed to support salt marsh harvest mouse populations can be useful for predicting occupancy in remaining habitat and in guiding habitat restoration projects (Shellhammer and Duke 2010).

Although much attention has focused on vegetation characteristics of salt marsh harvest mouse habitat (Johnson and Shellhammer 1988; Bias and Morrison 2006; Sustaita et al. 2011; Smith et al. 2018A), as well as some abiotic factors (e.g., distance from roads—Marcot et al. 2020), spatial drivers of occupancy have been largely unexplored (Smith et al. 2018A). Despite a lack of research into patch dynamics and spatial habitat associations, their importance is highlighted by calls for maintenance or restoration of functionally connected habitat patches in the Recovery Plan for Tidal Marsh Ecosystems of Northern and Central California (USFWS 2013). Given the extensive history of habitat loss and threat of future habitat fragmentation from development and sea level rise (Thorne et al. 2018), identifying spatial thresholds that facilitate occupancy is an essential foundation for salt marsh harvest mouse conservation and management (Smith et al. 2018B).

Historically, the SFE was composed of large, continuous marsh patches, contrasting with the modern landscape composed of a mosaic of small marsh patches surrounded most often by water, terrestrial grassland, or urban land cover (Williams and Faber 2001). Salt marsh harvest mice are primarily restricted to marsh habitat; upland and urban habitat edges are thought to be favorable to potential competitors, such as western harvest mice (*R. megalotis*), house mice (*Mus musculus*), and California voles (*Microtus californicus*) (Fisler 1965; Bias and Morrison 2006). Therefore, spatial attributes such as large patch size may buffer salt marsh harvest mice from

negative edge effects. Because salt marsh harvest mouse habitat is inherently dynamic due to tidal action, they must regularly negotiate tide cycles that can force them to take temporary refuge either in emergent vegetation (Johnston 1957; Smith et al. 2014) or by movement to non-inundated habitat (Hadaway and Newman 1971). If no refuge habitat is available, extreme high tide events have the potential to cause local extirpations, necessitating recolonization from nearby patches to maintain long-term population persistence. Therefore, a landscape of many well-connected patches may be more resilient to extreme tidal events than a landscape characterized by one or a few large patches.

Microhabitat characteristics may also drive occupancy at finer (within-patch) scales. Pickleweed (*Salicornia* spp.) cover is hypothesized to be strongly associated with salt marsh harvest mouse presence (USFWS 2013), but diverse vegetation composition may be more beneficial than pickleweed monocultures (Smith et al. 2018A). Gumplant (*Grindelia* spp.) and other tall emergent vegetation may represent important high tide refuge for salt marsh harvest mice during extreme inundation events (Johnston 1957; Smith et al. 2014). Marsh patches with greater biomass of upland vegetation may be more likely to support competitors, such as western harvest mice (Fisler 1965).

Given that salt marsh harvest mice are restricted to marsh habitat (Statham et al. 2022), which is readily delineated from upland and urban habitats, assumptions of the area-isolation paradigm may be strongly applicable to this system. My primary objective was to apply occupancy modeling (MacKenzie et al. 2002) to test the relationship between salt marsh harvest mouse occupancy and patch size, patch connectivity, patch vegetation characteristics, and matrix urbanization (i.e., the proportional composition of urban land cover within matrix habitat). Additionally, I assessed spatial and vegetation characteristics that influenced salt marsh harvest

mouse occupancy at finer scales (i.e., within patches), and compared historical and modern habitat and occupancy data in a dynamic occupancy model (MacKenzie et al. 2003; i.e., multi-season occupancy model with historical and modern representing different "seasons") to estimate relationships between local extinction probabilities and characteristics of habitat change over decades. Finally, occupancy modeling allowed me to quantify the detection probability of a recently developed non-invasive genetic survey technique (Aylward et al. in review).

METHODS

Non-Invasive Genetic Surveys

I conducted non-invasive genetic surveys at 47 marsh patches from September 2020 to December 2021 (Figure 2-1; Supplemental Table S2-1). I identified patches based on the San Francisco Estuary Institute Bay Area EcoAtlas Dataset (SFEI 1998) and manually adjusted any necessary patches based on satellite imagery. Most surveys occurred during late fall and early winter (Oct-Feb) to limit potential seasonal variation in occupancy and/or detection. I placed bait stations within vegetation ~0.5-1 m above ground level along two transects in each marsh patch. I placed stations 20 m apart along transects with a maximum of 10 stations per transect and curtailed the number of stations for transects in smaller marshes (range = 4–10). I fitted bait stations with cotton batting and ~1 tbsp of seed mix (primarily oats, millet, and ground walnut). I checked and re-baited stations after seven days and checked stations again after 14 days, providing two consecutive seven-day survey intervals. During each check, fecal pellets were collected in 2-ml ethanol (>95%) vials; I pooled as many pellets as possible (without over-topping the ethanol) into a single vial for each station. At each station, I recorded several vegetation parameters within a five meter radius: vegetation richness, presence/absence of pickleweed, presence/absence of high tide escape vegetation (HTEV; determined based on expert

opinion according to the height and complexity of vegetation), and presence/absence of terrestrial (upland) grasses. I conducted genetic species identification on fecal pellets following Aylward et al. (in review). Non-invasive genetic surveys were approved by UC Davis Institutional Animal Care and Use Committee and authorized by the California Department of Fish and Wildlife and United States Fish and Wildlife Service Cooperative Agreement.

Patch Occupancy Modeling

At the patch-scale, I considered a patch occupied if salt marsh harvest mice were detected at ≥1 station(s) within the patch. Next, I applied occupancy modeling (MacKenzie et al. 2002) to estimate the probabilities of detection (i.e., the probability of detecting salt marsh harvest mice given they are present) and occupancy (i.e., the probability of salt marsh harvest mouse occurrence) using the R package 'unmarked' (Fiske and Chandler 2011). Occupancy modeling accounts for imperfect detection of survey methods by estimating the probability of detection based on the number of survey sites in which a focal species was detected in ≥1, but not all, survey intervals (MacKenzie et al. 2002). I tested for effects of survey interval (i.e., first seven days vs. second seven days), survey effort (i.e., number of stations in the patch), and maximum high tide height during the survey interval on detection probabilities. I then estimated occupancy probability at the patch level as a function of five parameters: patch size, patch connectivity, matrix urbanization, patch vegetation characteristics, and the capture frequencies of three putative competitors (Supplemental Table S2-2). I defined patch size with and without edge effects of 50 and 200 m (i.e., subtracted 50 and 200 m buffers from the patch perimeter); patch connectivity as the proportion of marsh habitat within 50-m, 200-m, or 1-km buffers from the edge of the target patch; and matrix urbanization as the proportion of the matrix (i.e., non-marsh habitat within 50-m, 200-m, or 1-km of the patch) that was composed of urban land cover.

Vegetation parameters included vegetation richness, pickleweed habitat, HTEV habitat, and terrestrial grass habitat. Vegetation richness was calculated at the patch level as the average richness at all stations within the patch. Pickleweed, HTEV, and terrestrial grass habitats were quantified as the proportion of stations where these habitat characteristics were recorded. Finally, the role of putative competitor species was characterized by the detection frequencies of western harvest mice, house mice, and California voles. I standardized all variables prior to occupancy modeling.

I assessed each of these predictors using univariable models and assessed model performance using AICc. I considered predictors to be biologically informative if their fit to the data was better (i.e., at least 2 AICc units lower) than that of a model with no predictor covariates (i.e., the "null model"). These predictors were selected as candidates for multivariable modeling. For predictors that were calculated at multiple scales (i.e., different buffer sizes for patch size, connectivity, and matrix urbanization), I included only the scale-variant with the lowest AICc. I first constructed a model that included all candidate variables and then applied a backward-stepwise approach (e.g., Hosmer and Lemeshow 2000) to determine the top multivariable model. Each step involved removal of each individual predictor from the model and accepting the model with *n*-1 predictors with the greatest improvement in AICc compared to the model with *n* predictors. When none of the models with *n*-1 predictors represented an improvement in AICc over the model with *n* predictors, I accepted the latter as the top multivariable model. I also constructed a model using patch size and connectivity as the only two predictors, which provided a tool to help evaluate the suitability of any given patch of habitat based on the assumptions of the area-isolation paradigm.

To determine predicted threshold values of spatial variables with respect to salt marsh harvest mouse occupancy, I estimated occupancy probabilities based on univariable models across a range of predictor values for patch size, patch connectivity, and matrix urbanization, then identified the predictor values that corresponded to occupancy probabilities of 0.50 and 0.95 (e.g., Schultz and Crone 2005; Shake et al. 2012). I also calculated the conditional probabilities of patch size and patch connectivity using the multivariable patch size + patch connectivity model; the former was assessed holding patch connectivity equal to zero (i.e., to estimate the relationship between patch size and occupancy of a completely isolated marsh), while the latter was assessed holding patch size equal to 1 ha (i.e., to estimate the relationship between patch connectivity and occupancy of a very small marsh).

Fine-Scale Occupancy Modeling

To better understand microhabitat use by salt marsh harvest mice I estimated the effects of covariates on fine-scale (e.g., within-patch) occupancy and detection. To estimate fine-scale occupancy patterns, I used station-level data from patches where salt marsh harvest mice were detected. I tested the effects of tide height and survey interval on detection probability. I also tested the effects of vegetation richness, dominant vegetation species (a dummy variable, with terrestrial grasses as the reference), presence/absence of pickleweed, presence/absence of HTEV, presence/absence of terrestrial grasses, and distance from the patch perimeter on occupancy (Supplemental Table S2-2). I used mixed-effects modeling in the R package ‘ubms’ (Kellner et al. 2021) with site as a random intercept and fixed effects for all detection and occupancy predictors.

Patch Extinction Modeling

To evaluate factors associated with local extirpation of salt marsh harvest mice, I surveyed museum collections to document marshes where this species was known to occur, and I treated these earlier documentations as representing a first "season" of data in a dynamic occupancy model (MacKenzie et al. 2003). I documented 14 geographically distinct capture locations between 1938 and 1959 (Supplemental Table S2-3), for which their species identity was verified genetically (by sequencing a small fragment of cytochrome *b*; sequencing methods in Statham et al. 2016). I used the SFEI EcoAtlas Historical Baylands dataset to determine the boundaries and calculate patch size of historical marsh patches (SFEI 1998). I used my non-invasive survey data from modern patches that fell within the boundary of a historical marsh patch to represent a second "season". If salt marsh harvest mice were detected at ≥1 modern patch(es) within the boundary of a historical marsh patch, the historical marsh patch was considered occupied in the modern season.

To facilitate compatibility with my modern data set using two survey intervals, I assumed perfect detection and full occupancy of two survey intervals for historical marshes (i.e., first-season detection history of "11"). I applied dynamic occupancy modeling (MacKenzie et al. 2003) to estimate the effects of four predictors on extinction probability: 1) percentage of remaining marsh habitat within the extent of a historical marsh patch, 2) area of the largest remaining marsh patch within the extent of a historical marsh patch, 3) proportion of a historical marsh patch converted to modern urban land cover, and 4) area of a historical marsh patch (Supplemental Table S2-2). Next, I constructed multivariable models based on hypotheses that salt marsh harvest mouse extinction was associated with combined effects of 1) historical patch size and the size of the largest modern patch, 2) historical patch size and the percentage of remaining marsh, 3) historical patch size and percentage of modern urban land cover, 4)

percentage of remaining marsh and percentage of modern urban land cover, and 5) percentage of remaining marsh and the size of the largest modern patch. All dynamic occupancy models were constructed using R package 'unmarked'.

RESULTS

Patch Occupancy Modeling

Non-invasive sampling detected salt marsh harvest mice at 24 of 47 patches (Figure 2-1; Supplemental Table S2-1). The single session detection probability at the patch level (p) was 0.949. Neither tide height, survey effort, nor survey interval substantially improved detection probabilities over the null detection model (ΔAICc < 2; Supplemental Table S2-4), so all patch-occupancy models used the null detection probability. Patch size was best modeled with no buffer, although incorporation of a 50-m buffer was similarly supported (ΔAICc < 2; Supplemental Table S2-5). Patch connectivity and matrix urbanization were most predictive at the 1-km buffer size.

The best-ranked univariable occupancy model was connectivity (i.e., p[.]Ψ[Connectivity]), followed by patch size (Table 2-1A). Additionally, four other univariable models outperformed the null occupancy model (ΔAICc > 2). Among univariable models, connectivity accounted for ~97.0% of AICc weight, followed by patch size, accounting for 2.4% of AICc weight. Occupancy probability was positively related to patch connectivity and patch size, and negatively to matrix urbanization, terrestrial grass habitat, detection frequency of western harvest mice, and vegetation richness. When these variables were incorporated into backward-stepwise multivariable models the top-scoring model related the probability of occupancy positively to patch size and connectivity, and negatively to terrestrial grass habitat

(i.e., p[.]Ψ[Patch Size + Connectivity + Terrestrial Grasses]); β coefficients of all three predictors did not overlap zero (Table 2-2A).

Based on univariable occupancy models, predicted patch occupancy exceeded 0.50 under the following conditions: (a) patch size exceeded 25 ha (Figure 2-2A; Supplemental Table S2-6), (b) patches were surrounded by >16% marsh (i.e., within a 1 km buffer; Figure 2-2B), and (c) the matrix was composed of <48% urban land cover (Figure 2-2C). Predicted occupancy exceeded 0.95 (a) when patch size was >72 ha, or (b) when patches were surrounded by >37% marsh, but even as little as 1% matrix urbanization precluded an occupancy estimate ≥0.95 (Figure 2-2).

When I assessed the conditional probability of patch size on occupancy of a completely isolated marsh (e.g., using the p[.]Ψ[Patch Size + Connectivity] model with connectivity held at zero), patches of 72 and 128 ha corresponded to 0.50 and 0.95 occupancy probability, respectively (Figure 2-2D; Supplemental Table S2-6). However, confidence intervals were wide, with the lower 95% limit <0.06 probability of occupancy even for the largest patches (Figure 2-2D). Assessing conditional probability of patch connectivity on occupancy of a very small marsh patch (e.g., using the p[.]Ψ[Patch Size + Connectivity] model with patch size held at 1 ha), connectivities of 26% and 46% corresponded to 0.50 and 0.95 occupancy probability, respectively (Figure 2-2E). In this case, confidence intervals were narrower, suggesting that connectivity conditioned on small patch size was a more consistent predictor of occupancy than patch size conditioned on patch isolation.

Fine-Scale Occupancy Modeling

I detected salt marsh harvest mice at 150 of 314 stations occurring within marshes occupied by salt marsh harvest mice. Station-level detection probability with the null detection

model was 0.684. The best detection model according to AICc included tide height as a covariate, which had a slightly positive effect on detection (Supplemental Table S2-4). Therefore, I used tide height as a detection predictor in all fine-scale occupancy models. The best-ranked fine-scale occupancy model associated station-level occupancy positively with high tide escape vegetation (HTEV; i.e., the p[Tide Height]Ψ[HTEV] model) and accounted for >99.9% of the AICc weight among univariable fine-scale models (Table 2-1B). HTEV was the only covariate that had a significant β coefficient in fine-scale occupancy models, although four other univariable models showed improvement over the null model (ΔAICc > 2). Within these models, all predictors had 95% CIs that overlapped zero; the presence of pickleweed had a moderately positive effect, the presence of terrestrial grasses had a moderately negative effect, and vegetation richness had a small positive effect. Additionally, 17 categorical types of dominant vegetation had variable but statistically non-significant effects (Supplemental Table S2-7). The largest positive effect sizes tended to be taller, emergent plants such as gumplant and alkali bulrush (*Bolboschoenus maritima*), whereas the largest negative effect sizes tended to be lower-lying plants, such as alkali heath (*Frankenia salina*) and marsh jaumea (*Jaumea carnosa*). When these variables were incorporated into backward-stepwise multivariable models the top-scoring model related fine-scale occupancy probability positively to the presence of HTEV and pickleweed, and variably among dominant vegetation types (i.e., p[Tide Height]Ψ[HTEV + Pickleweed + Dominant Vegetation]; Table 2-2B; Supplemental Table S2-8). HTEV was the only predictor in this model with a 95% CI that did not overlap zero.

Local Extinction Modeling

I detected salt marsh harvest mice within the boundaries of 10 of 14 historically occupied marshes (Supplemental Table S2-3). All extinction models that included a predictor covariate

improved AICc relative to the null model, although small sample size limited the precision of my models (Table 2-3). All 95% confidence intervals surrounding β estimates were wide and overlapped zero. The top model, ranked according to AICc, associated extinction probability negatively with the size of the largest remaining patch of marsh within the extent of a historical marsh patch. The percentage of the historical marsh converted to urban land cover was positively associated with extinction probability; and the percentage of remaining marsh and the historical patch size were negatively associated with extinction probability. The top univariable model outperformed all multivariable models, although two multivariable models resulted in $\Delta AICc < 2$ (Table 2-3). All three top models included the largest remaining patch size as a predictor; one model included the size of the historical marsh patch and another included the percentage of remaining marsh habitat within the extent of the historical marsh patch.

DISCUSSION

Patterns of occupancy by salt marsh harvest mice in the San Francisco Estuary conformed well to the area-isolation paradigm. Connectivity was the strongest univariable predictor and the only predictor in the best-performing multivariable model with confidence intervals not overlapping zero. Connectivity drives patch occupancy for other mammalian habitat specialists as well (Gardiner et al. 2018; Zimbres et al. 2018). Salt marsh harvest mouse gene flow and movement is constrained by non-wetland habitat, such as open water and upland habitat (>2 m elevation; Statham et al. 2022). Therefore, an interconnected network of marsh habitat may be particularly important for coping with extreme inundation events on fine temporal scales (e.g., to provide temporary refuge) and broad temporal scales (e.g., to facilitate recolonization and metapopulation dynamics). Patch size also influenced occupancy, which agrees with previous work suggesting that salt marsh harvest mice respond negatively to edge habitat (Bias

and Morrison 2006; Marcot et al. 2020). The effect of urban land cover in the matrix had a sufficiently negative impact on salt marsh harvest mouse occupancy that any such habitat resulted in <0.95 predicted occupancy. A meta-analysis of patch-occupancy studies found that characteristics of the matrix, rather than patch size and connectivity per se, were often stronger predictors of occupancy across numerous taxa with different life history traits (Prugh et al. 2008), including some habitat specialist rodents (Pita et al. 2007). Taken together, my results suggest a landscape of well-connected, large patches of marsh, with natural intervening matrix (e.g., grasslands, rather than urban land cover) represents optimal conditions for salt marsh harvest mouse persistence.

The influence of habitat on occupancy was scale-dependent. At the broader (patch-level) scale, terrestrial grass was the only habitat variable that had a statistically significant effect on salt marsh harvest mouse occupancy; in contrast, the only such variable at the finer (station-level) scale was high tide escape vegetation (HTEV). The failure of HTEV to emerge in broad-scale models likely reflected the presence of HTEV at many sites both with and without salt marsh harvest mice, suggesting that HTEV alone does not guarantee occupancy but that it is an important feature when other criteria for occupancy are met. Johnston (1957) observed salt marsh harvest mice hiding in tall gumplant bushes during extreme high tide events, and I observed multiple occasions of mice hiding in bait stations placed in gumplant and other tall emergent vegetation during surveys that aligned with high tides. Together, the importance of emergent high tide escape habitat at fine scales and patch connectivity at broad scales implies that refuge (HTEV) and rescue from dynamic tidal conditions (connectivity) are important drivers of long-term persistence, which may become increasingly important in light of rising sea levels (Thorne et al. 2018). Fisler (1965) hypothesized that intrusion of terrestrial grasses into

marsh habitat favored the occupancy of sympatric generalist rodents, such as the western harvest mouse, over that of salt marsh harvest mice. My findings support this hypothesis at the patch level but did not support fine-scale avoidance of terrestrial grasses.

I evaluated thresholds of spatial parameters that corresponded to 0.50 and 0.95 occupancy probabilities in univariable models to provide some guidance for management. However, these thresholds should be interpreted critically and applied to restoration projects on a case-by-case basis. For example, although the univariable patch size model estimated that 72 ha corresponded with 0.95 occupancy probability, if I assumed no connectivity among patches (i.e., using the conditional multivariable patch size + connectivity model), then 72 ha corresponded to just 0.50 occupancy probability, with a lower 95% CI of just 0.06. Broad uncertainty in the conditional model suggests that large patches do not guarantee salt marsh harvest mouse occupancy; rather, the conditional models suggest that small patch size effectively guarantees the absence of salt marsh harvest mice if not compensated by sufficient connectivity to nearby suitable habitat. Supporting this, a significant finding from the patch size + connectivity model was that even very small patches of marsh (e.g., 1 ha) can have high occupancy probability with relatively high confidence if they are well connected to nearby marsh habitat. Thus, my findings suggest that small scale restoration projects have a high probability of success if they are surrounded by a sufficient proportion of (occupied) marsh habitat, and that even large-scale restoration projects may have uncertain outcomes if they are isolated from other occupied marsh habitat.

Although the small number of sites with genetically verified historical presence of salt marsh harvest mice limited statistical power for the extinction models, I believe it is important that all predictors improved model performance relative to the null model. Moreover, the trends I observed were broadly consistent with those based on my contemporary occupancy models.

Extinction models suggested that loss of large patches of habitat were associated with higher likelihood of extirpation. The top performing predictor was the size of the largest remaining patch of marsh habitat, which may imply that a minimum patch size threshold was breached at the four extirpated sites in this study. Historical patch size and the proportion of historical patch remaining also were negatively associated with extinction probability, which further emphasized that smaller extant patches were more vulnerable to extinction. My results suggest that in the future, smaller patches of existing salt marsh harvest mouse habitat will be more vulnerable to extirpation due to ongoing habitat loss.

Ongoing monitoring of salt marsh harvest mice should integrate regular surveys in small and isolated patches of habitat, which are likely the most vulnerable but have not been regularly surveyed in the past (USFWS 2013). The non-invasive genetic survey approach I used in this study has the potential to significantly improve survey efficiency, but its efficacy had not been quantified prior to this study. Based on data presented in this study, bait station surveys were extremely effective at detecting salt marsh harvest mice at the patch level. Of note, I conducted the majority of my surveys in winter, and I do not yet know whether high summer temperatures would affect detection. Future work is needed to determine seasonal effects on detection probability using this approach, but my results from the cold season are promising.

Conclusions

Salt marsh harvest mouse occupancy conformed to the area-isolation paradigm of metapopulation theory. Specifically, larger patch size and greater patch connectivity were associated with higher occupancy probability. My models predicted that small, well-connected marshes were more likely to be occupied than larger, isolated marshes. The abundance of upland vegetation in marsh patches negatively affected occupancy at broad scales, and the presence of

high tide escape vegetation positively affected occupancy at fine scales. Furthermore, local extirpation at four historically occupied sites was associated with measures of habitat loss, particularly the loss of large patches of habitat. My findings highlight important spatial and habitat considerations for future marsh preservation and restoration projects targeting salt marsh harvest mouse habitat.

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FIGURES

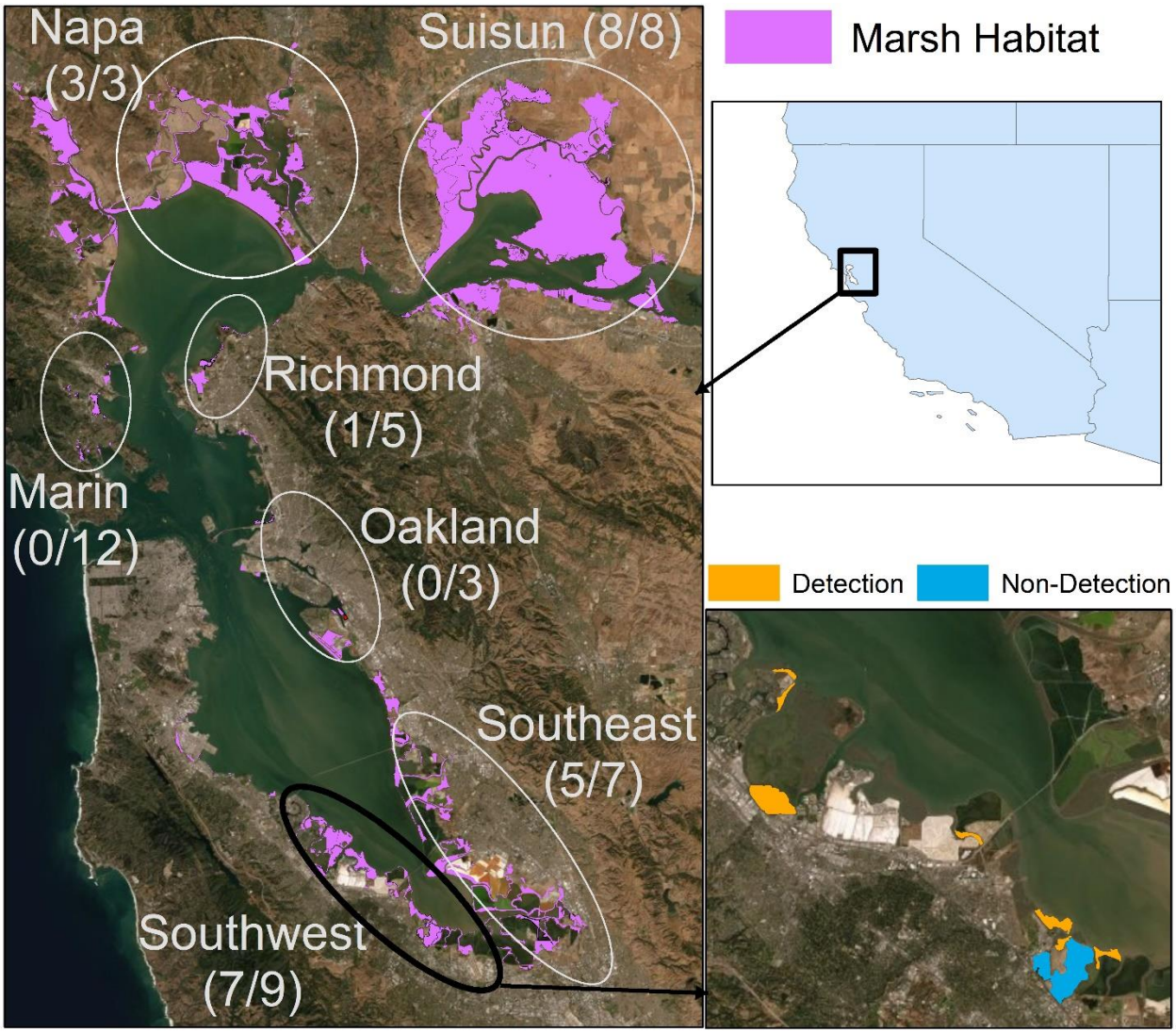

Figure 2-1. Map of the study area and number of occupied/surveyed sites for salt marsh harvest mice (*Reithrodontomys raviventris*). Number of occupied and surveyed sites are listed for each region of the study area. The lower-right inset shows the detection status of specific patches of marsh habitat surveys in the Southwest region. Marsh habitat is colored purple in the large study area pane; detection status is only colored in the Southwest inset pane.

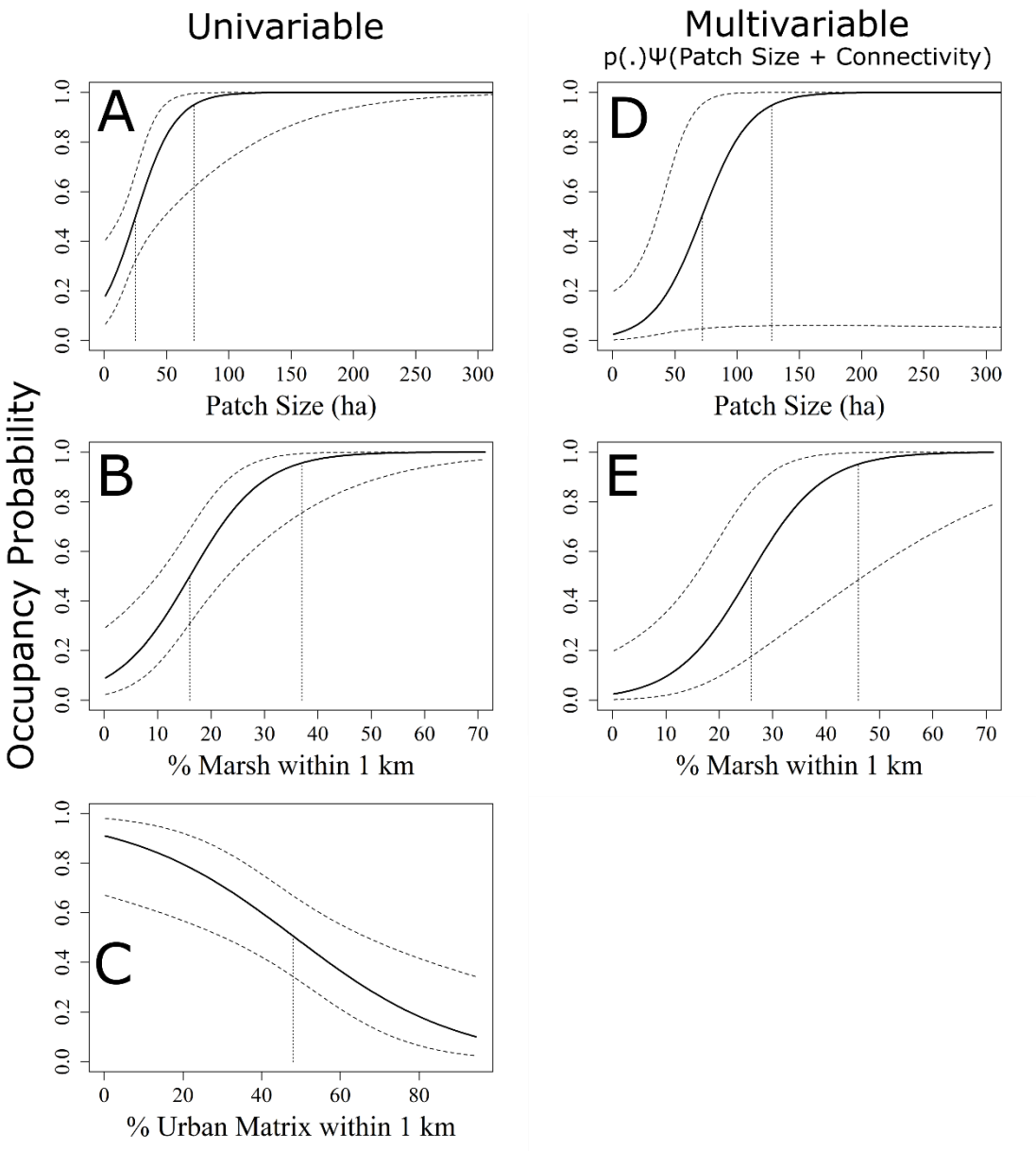

Figure 2-2. Effects of (A) patch size, (B) connectivity, and (C) matrix urbanization on salt marsh harvest mouse (*Reithrodontomys raviventris*) occupancy probability based on univariable models derived from non-invasive genetic survey data. Conditional effects of (D) patch size of a completely isolated marsh patch (0% marsh within 1 km) and (E) connectivity of a small (1 ha) marsh patch on occupancy probability estimated from a bivariable model. Dashed lines represent 95% CIs and vertical dotted lines represent thresholds of 0.50 and 0.95 occupancy probability.

TABLES

Table 2-1. Covariate effects (and 95% confidence intervals) in univariable (A) patch-occupancy models and (B) fine-scale occupancy models for salt marsh harvest mice (Reithrodontomys raviventris). Patch-occupancy models used null detection probability and fine-scale occupancy models used tide height as a predictor of detection probability. Covariate definitions are provided in Table S2-2. Covariate names are bolded for those whose 95% CIs do not overlap zero. Effect sizes and 95% CIs for all 17 categories of Dominant Vegetation (all of which overlapped zero) are provided in Supplemental Table S2-7.

A)

Covariate	Effect (β) and 95% CI	AICc	ΔAICc	AICc weight
Connectivity	2.373 (1.039, 3.707)	62.04	0	97.0%
Patch Size	4.940 (1.266, 8.614)	69.42	7.38	2.4%
Matrix Urbanization	-1.315 (-2.159, -0.471)	73.21	11.17	0.3%
Western Harvest Mouse	-1.553 (-2.159, -0.471)	74.60	12.56	0.2%
Terrestrial Grasses	-0.936 (-1.724, -0.148)	79.57	17.54	<0.1%
Vegetation Richness	-0.697 (-1.361, 0.033)	82.12	20.08	<0.1%
High Tide Escape Vegetation	0.613 (-0.043, 1.269)	83.16	21.13	<0.1%
None (null occupancy)	--	85.02	22.98	<0.1%
House Mouse	-0.393 (-1.015, 0.229)	85.36	23.32	<0.1%
Pickleweed	-0.059 (-0.655, 0.537)	86.98	24.94	<0.1%
California Vole	0.051 (-0.543, 0.645)	86.99	24.95	<0.1%

B)

Covariate	Effect (β) and 95% CI	AICc	ΔAICc	AICc weight
High Tide Escape Vegetation	1.979 (1.140, 2.917)	620.39	0	>99.9%
Dominant Vegetation	--[1]	645.83	25.44	<0.1%
Terrestrial Grasses	-0.365 (-1.425, 0.749)	648.5	28.11	<0.1%
Vegetation Richness	0.048 (-0.237, 0.343)	649.01	28.62	<0.1%
Pickleweed	0.445 (-0.453, 1.325)	649.24	28.85	<0.1%
None (null model)	--	671.75	51.36	<0.1%
Distance from Edge	0.003 (-0.004, 0.009)	677.34	56.95	<0.1%

[1] see Supplemental Table S2-7 for effects of individual dominant vegetation types.

Table 2-2. Covariate effects in the top scoring multivariable (A) patch-occupancy model and (B) fine-scale occupancy model for salt marsh harvest mice (*Reithrodontomys raviventris*). Each model was constructed using a backward stepwise approach with a starting model that included all predictors in univariable models that improved AICc by >2 compared to the null model. Patch-occupancy models were estimated in 'unmarked' (Fiske and Chandler 2011) which provides z- and p-values for individual covariates. Fine-scale occupancy models were estimated in 'ubms' (Kellner et al. 2021) using a mixed modeling approach, which does not provide z-and p-values, thus, 95% CIs were used to infer statistical significance. Covariate names are bolded for those whose 95% CIs do not overlap zero. Covariate definitions are provided in Table S2-2.

A)

Covariate	Effect (β)
Connectivity	2.47 (0.61, 4.33)
Patch Size	9.72 (0.80, 18.64)
Terrestrial Grasses	-2.25 (-4.21, -0.29)

B)

Covariate	Effect (β)
HTEV (presence)	2.32 (1.33, 3.39)
Pickleweed (presence)	0.72 (-0.52, 1.96)
Dominant Vegetation	--[1]

[1]See Supplemental Table S2-8 for effects of individual dominant vegetation types

Table 2-3. Performance of univariable and multivariable models of salt marsh harvest mouse (*Reithrodontomys raviventris*) extinction probability (ε) in historically occupied marsh patches. Extinction covariates were estimated from a dynamic occupancy model with an assumed detection history of "11" in the first season and empirical detection histories from non-invasive genetic surveys in the second season. Covariate effects on extinction probability are given for univariable models.

Covariate	Effect (β) and 95% CI	AICc	ΔAICc	AICc weight
Largest Patch Size	-44.2 (-221.4, 133.0)	19.849	0	49.7%
Largest Patch Size + % Remaining Marsh	--	21.803	1.954	18.7%
Largest Patch Size + Historical Patch Size	--	21.834	1.985	18.4%
% Urban	4.72 (-1.84, 11.28)	25.043	5.194	3.7%
Historical Patch Size	-5.55 (-12.25, 1.15)	26.007	6.158	2.3%
Historical Patch Size + % Remaining Marsh	--	26.144	6.295	2.1%
Historical Patch Size + % Urban	--	26.272	6.423	2.0%
% Remaining Marsh	-6.13 (-13.41, 1.15)	26.700	6.851	1.6%
% Remaining Marsh + % Urban	--	26.999	7.150	1.4%
None (null model)	--	34.487	14.638	<0.1%

SUPPLEMENTAL MATERIAL

Supplemental Table S2-1. Sites surveyed for salt marsh harvest mice (*Reithrodontomys raviventris*; SMHM) using non-invasive genetic surveys. Historical marsh patches marked by an asterisk (*) indicate the nearest historical patch for modern patches that were located outside the boundaries of all historical patches I analyzed.

Site name	SMHM detected?	Survey Month	Lat/Long	Historical Marsh Patch
Hill Slough 8	Y	September 2020	38.223355, -121.983766	Hill Slough
Joice Island	Y	January 2021	38.193134, -121.995888	Grizzly
West Family	Y	October 2020	38.134899, -122.107632	Benicia
Goodyear	Y	October 2020	38.084260, -122.103999	Benicia
Benicia Industrial	Y	September 2020	38.079235, -122.112593	Benicia
Bay Point	Y	November 2021	38.043063, -121.965742	*Martinez
McNabney	Y	November 2021	38.025040, -122.105142	Martinez
Martinez RS	Y	November 2021	38.023487, -122.139784	Martinez
Ringstrom	Y	April 2021	38.222596, -122.408160	*Vallejo
American Canyon	Y	April 2021	38.159459, -122.267419	Vallejo
West Sonoma Creek	Y	April 2021	38.158216, -122.405789	Vallejo
Tiscornia	N	December 2020	37.968433, -122.496085	San Rafael
Creekside	N	December 2020	37.948838, -122.539263	Larkspur / Corte Madera
Piper Park	N	December 2020	37.941359, -122.530573	Larkspur / Corte Madera
Heerdt	N	November 2020	37.940597, -122.511783	Larkspur / Corte Madera
Muzzi North	N	November 2020	37.933903, -122.506422	Larkspur / Corte Madera
Muzzi South	N	November 2020	37.927553, -122.509316	Larkspur / Corte Madera
Marta	N	November 2020	37.925199, -122.502789	Larkspur / Corte Madera
Triangle (Marin)	N	December 2020	37.921935, -122.495986	Larkspur / Corte Madera
Bayfront Park	N	December 2020	37.898121, -122.525796	Bothin / Mill Valley
Alto	N	December 2020	37.893677, -122.526313	Bothin / Mill Valley
Bothin North	N	December 2020	37.887643, -122.523681	Bothin / Mill Valley
Bothin South	N	December 2020	37.883089, -122.523412	Bothin / Mill Valley
Whittell	N	September 2020	38.006959, -122.354394	*Richmond
Giant	N	November 2021	37.989678, -122.358798	*Richmond
Dotson North	N	September 2020	37.982240, -122.364405	*Richmond
Dotson South	N	September 2020	37.979843, -122.366012	*Richmond
San Pablo Creek	Y	November 2020	37.973504, -122.371875	Richmond
McLaughlin	N	November 2021	37.835541, -122.296796	*Oakland
Arrowhead	N	September 2020	37.742231, -122.211754	Oakland
MLK	N	September 2020	37.738201, -122.206583	Oakland
Enterprise	N	September 2020	37.627717, -122.130501	Eden / Hayward
Hayward Pasture	Y	September 2020	37.625533, -122.137148	Eden / Hayward
HARD	Y	September 2020	37.624698, -122.139546	Eden / Hayward
Newark Pasture	N	October 2021	37.463759, -121.933463	Alviso
Triangle (Alviso)	Y	October 2021	37.455639, -121.976588	Alviso

Mallard Slough	Y	October 2021	37.444934, -121.968212	Alviso
New Chicago	Y	October 2021	37.439280, -121.961393	Alviso
Mountain View Slough	Y	October 2021	37.449572, -122.081633	Palo Alto
Renzel	N	December 2020	37.449431, -122.112644	Palo Alto
Mundy	Y	December 2020	37.458886, -122.102832	Palo Alto
Flood Control Basin	N	December 2020	37.441080, -122.100981	Palo Alto
Byxbee (Harbor)	Y	December 2020	37.452365, -122.104944	Palo Alto
Ravenswood Slough	Y	October 2021	37.492135, -122.151426	Redwood City
Inner Bair	Y	October 2021	37.499941, -122.227226	Redwood City
Steinberger Slough	Y	January 2021	37.537791, -122.232153	Redwood City
Radio Point	Y	January 2021	37.547153, -122.226910	Redwood City

Supplemental Table S2-2. Descriptions of covariates used in patch-occupancy models for salt marsh harvest mice (*Reithrodontomys raviventris*).

	Covariate Name	Description
Patch-level	Patch Size	Area of the marsh patch. Area was measured at three scales to account for potential edge effects: 1) no edge effect 2) 50 m edge effect - 50 m buffer subtracted from the boundary of the marsh patch 3) 200 m edge effect - 200 m buffer subtracted from the boundary of the marsh patch
	Patch Connectivity	Percentage of marsh habitat within a buffer distance outside the boundary of the marsh patch, measured at three different scales: 1) 50 m buffer 2) 200 m buffer 3) 1 km buffer
	Matrix Urbanization	Percentage of urban land cover within a buffer distance outside the boundary of the marsh patch, measured at three different scales: 1) 50 m buffer 2) 200 m buffer 3) 1 km buffer
	Vegetation Richness	Mean number of plant species within 5 m of stations
	Pickleweed	Percentage of stations at the site where pickleweed was recorded present within 5 m of the station
	High Tide Escape Vegetation (HTEV)	Percentage of stations at the site where HTEV was recorded present within 5 m of the station (HTEV was determined by expert opinion based on the height and structure of vegetation)
	Terrestrial Grasses	Percentage of stations at the site where terrestrial grass was recorded present within 5 m of the station
	Western Harvest Mouse	Percentage of stations at the site where western harvest mice were detected
	House Mouse	Percentage of stations at the site where house mice were detected
	California Vole	Percentage of stations at the site where California voles were detected
Station-level	Distance to Edge	Distance from the station to the marsh/edge upland
	Dominant Vegetation	Vegetation species that comprised the greatest percent cover within 5 m of the station
	Vegetation Richness	Number of vegetation species within 5 m of the station
	Pickleweed	Presence/absence of pickleweed within 5 m of the station
	High Tide Escape Vegetation	Presence/absence of HTEV within 5 m of the station
	Terrestrial Grasses	Presence/absence of terrestrial grass within 5 m of the station
Extinction modeling	% Marsh	Percentage of modern marsh land cover within the boundary of a historical marsh patch
	Largest Marsh Patch	Area of the largest continuous patch of marsh remaining within the boundary of a historical marsh patch
	% Urban	Percentage of modern urban land cover within the boundary of a historical marsh patch
	Historical Patch Size	Area of a historical marsh patch

Detection Parameters	Tide	Highest high tide height at the nearest monitoring station during the 7-day survey interval
	Interval	Whether the first or second survey interval

Supplemental Table S2-3. List of historical sites, collection year, and number of museum specimens used to verify historical presence in dynamic occupancy modeling for salt marsh harvest mice (*Reithrodontomys raviventris*). Coordinates of museum locations were approximated for locations where exact coordinates were not provided (*). All coordinates are presented with the number of significant digits provided in museum records. Modern occupancy for all sites was determined using non-invasive genetic surveys.

Site	n	Year	Lat/Long	Modern Occupancy
Alviso	10	1959	37.43075, -121.95447	Y
Benicia	2	1959	38.106809, -122.098047*	Y
Bothin / Mill Valley	19	1940	37.8821443, -122.5157516	N
Eden / Hayward	4	1938	37.62233, -122.12892	Y
Hill Slough	1	1959	38.232949, -122.020839*	Y
Joice Island	7	1959	38.191306, -121.944338*	Y
Larkspur / Corte Madera	12	1942	37.9320877, -122.5079271	N
Martinez	12	1945	38.021356, -122.093765*	Y
Oakland	12	1938	37.7439691, -122.2126871	N
Palo Alto	4	1950	37.441434, -122.112559*	Y
Redwood City	8	1950	37.509454, -122.26187	Y
Richmond	15	1959	37.9745281, -122.3765387	Y
San Rafael	2	1942	37.9571842, -122.508492	N
Vallejo	2	1959	38.154270, -122.408255*	Y

Supplemental Table S2-4. Performance of detection probability covariates in patch-occupancy and fine-scale occupancy models for salt marsh harvest mice (*Reithrodontomys raviventris*). Models are ranked by AICc scores.

Model	Detection parameter	Effect	AICc
Patch-Occupancy	Survey Interval	0.078 (-0.646, 0.802)	84.37
	None (null model)	--	85.017
	Survey Effort	-1.081 (-3.443, 1.283)	86.042
	Tide Height	-0.069 (-0.961, 0.823)	86.993
Fine-Scale	Tide Height	0.100 (-0.076, 0.281)	676.185
	Survey Interval	0.429 (-0.034, 0.899)	683.010
	None (null model)	--	683.961

Supplemental Table S2-5. Performance of different buffer sizes in estimating the effects of patch size, connectivity, and urban matrix on salt marsh harvest mouse (*Reithrodontomys raviventris*) occupancy models. Models are ranked by AICc scores.

Covariate	Scale	Effect (β) and 95% CI	AICc	ΔAICc
Patch Size	no edge	4.940 (3.103, 6.777)	69.416	0
	50 m edge	4.910 (2.791, 7.029)	71.437	2.021
	200 m edge	0.582 (0.084, 1.08)	84.754	15.338
Connectivity	1 km buffer	2.373 (1.706, 3.04)	62.035	0
	200 m buffer	1.190 (0.746, 1.634)	76.750	14.715
	50 m buffer	0.904 (0.529, 1.279)	79.747	17.712
Urban Matrix	1 km buffer	-1.315 (-1.757, -0.873)	68.295	0
	200 m buffer	-0.845 (-1.198, -0.492)	76.264	7.969
	50 m buffer	-0.824 (-1.173, -0.475)	77.495	9.200

Supplemental Table S2-6. Covariate values corresponding to 0.50 and 0.95 occupancy probabilities for salt marsh harvest mice (*Reithrodontomys raviventris*) based on univariable patch-occupancy models and a multivariable patch-occupancy model. The multivariable patch-occupancy model included Connectivity and Patch Size as predictors, and occupancy thresholds were estimated conditionally to estimate the effects of connectivity on small (1 ha) patches and of patch size on isolated (0% connectivity) patches. Connectivity was measured as the percentage of marsh habitat with a 1 km buffer of the focal marsh. Matrix Urbanization was measured as the percentage of the matrix (i.e., non-marsh habitat) within a 1 km buffer of the focal marsh that was classified as urban land cover.

Model	Covariate	Occupancy Threshold	
		50%	95%
Univariable			
p(.)Ψ(Connectivity)	Connectivity	16%	37%
p(.)Ψ(Patch Size)	Patch Size	25 ha	72 ha
p(.)Ψ(Matrix Urbanization)	Matrix Urbanization	48%	<1%
Multivariable, conditional			
p(.)Ψ(Connectivity + Patch Size) when Patch Size = 1 ha	Connectivity	26%	46%
p(.)Ψ(Connectivity + Patch Size) when Connectivity = 0%	Patch Size	72 ha	128 ha

Supplemental Table S2-7. Effect sizes and 95% CIs of categories of dominant vegetation in a univariable fine-scale occupancy model for salt marsh harvest mice (*Reithrodontomys raviventris*).

Covariate	Common Name	Effect (β) and 95% CI
Grindelia stricta	Gumplant	2.152 (-0.074, 5.070)
Bolboschoenus maritimus	Alkali bulrush	1.177 (-0.405, 2.834)
Juncus balticus	Baltic rush	1.024 (-0.638, 2.845)
Carpobrotus spp.	Iceplant	1.009 (-1.740, 4.619)
Typha domingensis	Cattail	0.862 (-0.905, 2.954)
Salicornia pacifica	Pickleweed	0.770 (-0.320, 1.824)
Schoenoplectus americana	Tricorner bulrush	0.278 (-1.221, 1.801)
Schoenoplectus acutus	Hardstem bulrush	0.433 (-1.523, 2.447)
Distichlis spicata	Saltgrass	0.195 (-1.204, 1.528)
Spartina spp.	Cordgrass	-0.141 (-2.255, 1.913)
Glaux maritima	Sea milkwort	-0.376 (-3.912, 2.696)
Atriplex prostrata	Fat-hen	-0.681 (-2.901, 1.455)
Euthamia occidentalis	Western goldenrod	-0.834 (-4.474, 2.437)
Phragmites australis	Common reed	-1.070 (-4.353, 1.568)
Frankenia salina	Alkali heath	1.366 (-3.671, 0.596)
Jaumea carnosa	Marsh jaumea	-1.684 (-4.884, 0.745)

Supplemental Table S2-8. Effect sizes and 95% CIs of categories of dominant vegetation in a multivariable fine-scale occupancy model for salt marsh harvest mice (*Reithrodontomys raviventris*). The multivariable model included tide height as a predictor of detection probability and included the presence of high tide escape vegetation, presence of pickleweed, and dominant vegetation type as predictors of occupancy.

Covariate	Common Name	Effect (β) and 95% CI
Grindelia stricta	Gumplant	1.606 (-0.524, 4.360)
Bolboschoenus maritimus	Alkali bulrush	1.038 (-0.681, 2.978)
Typha domingensis	Cattail	0.913 (-0.939, 3.017)
Distichlis spicata	Saltgrass	0.644 (-0.820, 2.145)
Juncus balticus	Baltic Rush	0.601 (-1.136, 2.374)
Carpobrotus spp.	Iceplant	0.586 (-2.247, 4.000)
Salicornia pacifica	Pickleweed	0.360 (-0.856, 1.616)
Schoenoplectus americana	Tricorner bulrush	0.011 (-1.487, 1.647)
Schoenoplectus acutus	Hardstem bulrush	-0.056 (-2.170, 2.137)
Glaux maritima	Sea milkwort	-0.234 (-3.747 2.871)
Spartina spp.	Cordgrass	-0.485 (-2.836, 1.676)
Euthamia occidentalis	Western goldenrod	-0.908 (-4.568, 2.412)
Atriplex prostrata	Fat-hen	-0.977 (-3.407, 1.307)
Phragmites australis	Common reed	-1.243 (-4.718, 1.595)
Frankenia salina	Alkali heath	-1.569 (-3.984, 0.449)
Jaumea carnosa	Marsh jaumea	-1.678 (-5.070, 0.864)

CHAPTER 3 – Effects of Sea Level Rise on the Evolutionary History of an Endangered Habitat Specialist, the Salt Marsh Harvest Mouse

Coastal wetland ecosystems support unique biodiversity, yet their evolutionary histories are poorly understood. The salt marsh harvest mouse (Reithrodontomys raviventris) is an endangered coastal wetland habitat specialist restricted to the San Francisco Estuary (SFE) in California, USA. Salt marsh harvest mice have been categorized into two subspecies, north and south, whose divergence timing and geographic boundaries are poorly understood. I used whole mitochondrial genome sequences of 102 salt marsh harvest mice to characterize phylogeography, demographic history, and to evaluate subspecies delineation of three "Mid-Bay" populations near the putative boundary. A Bayesian Skyline Plot indicated a rapid demographic expansion during the formation of the modern SFE (beginning ~10 kya), and rapid decline consistent with recent anthropogenic activity. Isolation-without-migration models suggested subspecies divergence occurred 8–19 kya, coinciding with the formation of the modern SFE, or earlier. Many populations became isolated within the past ~5 kya, consistent with vicariance resulting from sea level rise. Spatial Analysis of Molecular Variance and Φ_{ST} revealed that two of three Mid-Bay populations were more genetically similar to the northern subspecies, suggesting a mismatch between the morphological and molecular subspecies assignment of these populations. This study aids conservation of this species by clarifying its genetic subspecies boundaries, and by highlighting the unique nature of isolated populations that are increasingly threatened by sea level rise. My data also shows the important role of global glacial cycles and sea level rise in shaping local phylogeographic patterns of this coastal wetland habitat specialist.

INTRODUCTION

Quaternary geological and climatic change have shaped the modern distribution of biodiversity (Hewitt 2000; Svenning et al. 2015). Glacial-interglacial cycles are associated with various processes that affect demography and evolution of vertebrate populations, including changes in sea level (Zhou et al. 2017), exposure and submergence of ephemeral land bridges (Hundertmark et al. 2002), and shifts in the spatial distribution of habitat types (Stone et al. 2002). Understanding how such processes shape modern biodiversity is critical to identifying extant units of evolutionary significance (Funk et al. 2012; Hohenlohe et al. 2021). For a given species, preserving the greatest possible amount of contemporary genetic diversity and evolutionary history maximizes evolutionary potential (Allendorf et al. 2013), which is particularly important for species sensitive to environmental change.

Coastal wetland ecosystems are unique in their geological ephemerality and their relatively narrow global distribution (Duarte et al. 2013). They also are one of the most threatened ecosystems globally (Craft et al. 2009; Barbier et al. 2011), yet support numerous threatened and endangered taxa, which often have developed ecological adaptations to the unique habitat conditions (Greenberg and Maldonado 2006; Walsh et al. 2019). Understanding the interactions between historical climatic conditions and evolutionary history of endemic coastal wetland biodiversity can help predict the effects of future climate change on these ecosystems.

The salt marsh harvest mouse (*Reithrodontomys raviventris*) is a state- and federally listed endangered species that is restricted to coastal wetland habitat in the San Francisco Estuary (SFE) of California, USA (USFWS 2013), and is the only mammalian species entirely restricted to coastal wetland habitat (Greenberg and Maldonado 2006; Statham et al. 2016). It is deeply

divergent (>3.5 mya) from its closest extant relative (Bell et al. 2001; Statham et al. 2016) and has developed numerous adaptations to its saline, semi-aquatic environment. Its range appears limited by salinity levels (Fisler 1965), making it a strong indicator species of salt marsh habitat conditions. Understanding the evolutionary history of salt marsh harvest mice can provide insight regarding broader evolutionary patterns characteristic of coastal wetland habitat in the SFE.

The salt marsh harvest mouse comprises two subspecies, one found in the northern SFE (*R. r. halicoetes*; Dixon 1909) and one in southern SFE (*R. r. raviventris*; Dixon 1908). Original delineation of these subspecies and their geographic boundary was based on morphological data. Molecular data from a limited number of sites has supported the broad subspecies delineation of the three major bays that comprise the SFE (Statham et al. 2016). Additionally, three geographically distinct populations of uncertain provenance occur at intermediate locations (Mid-Bay) near the putative subspecies boundary (Figure 3-1). All three Mid-Bay sites have been assigned to the southern subspecies on morphological grounds, but this classification has not been corroborated with genetic data (Statham et al. 2016; Smith et al. 2018). These populations are small, of significant conservation concern, and in some cases may have been recently extirpated (Aylward et al. in review). Genetically assessing the evolutionary history of these geographically distinct populations and the validity of their current subspecies status is fundamental to potential conservation efforts, particularly those that may involve translocation from other populations.

Geological records of sea level in the modern SFE provide a potential explanation for population divergence during the Holocene, which, if verified, would provide a better scientific basis for subspecific designations. The modern SFE formed during the early Holocene as a result

of rising sea levels following the conclusion of the last glacial maximum (LGM = 29–20 kya; Clark et al. 2009). Sea water entered the SFE through a 2-km wide gap in the Pacific Coast Range (the "Golden Gate") ~10 kya (Atwater 1979). As sea level continued to rise, the SFE expanded to the east and the south, filling Suisun Bay in the northeastern SFE and the South Bay ~6 kya (Goman and Wells 2000; Fard et al. 2021). As sea level rose and marshes expanded, the center of the SFE filled with open water, causing the isolation of marshes that may have promoted subspecies divergence.

Prior to the formation of the modern SFE, during the LGM, sea level was ~100 m lower than today, and the shoreline ~100 km to the west of the modern SFE (Atwater et al. 1977). At that time, habitat in the modern SFE was characterized as mesic coniferous forest, while salt marshes likely were restricted to an area west of the modern SFE, on the present-day continental shelf of the Pacific Ocean. Thus, geological records imply that salt marsh habitat likely has migrated in and out of the modern SFE in association with changing sea levels of glacial and interglacial geological periods, and it is possible that subspecies diverged during one of these more ancient periods of environmental change. Previous estimates of divergence between northern and southern populations included very broad uncertainties (3 to 582 kya; Statham et al. 2016), which preclude determination of whether their divergence occurred within the modern SFE or during the Pleistocene epoch.

I sought to characterize the phylogeography of the salt marsh harvest mouse, estimate timing of significant demographic changes and evolutionary divergence, and evaluate the current subspecies delineation, using whole mitochondrial genomes. I hypothesized that northern and southern populations diverged as a result of rising sea levels after the formation of the modern SFE, within the past 10 kya. Consequently, I also hypothesized that two of the three Mid-Bay

populations would be more genetically similar to northern populations than southern populations, in contrast to current subspecific designations that consider all three to be the southern subspecies. Specifically, I predicted that only the Oakland population would group with the nearby South Bay population, whereas Richmond and Marin, which are geographically most proximate to northern populations and occur north of where the SFE initially filled with sea water, would both group with the nearby San Pablo Bay as part of the northern subspecies (Figure 3-1).

METHODS

I collected 102 genetic samples from throughout the range of the salt marsh harvest mouse, including modern (2010–2021; n = 85) and museum (1930–1959; n = 17) specimens (Supplemental Table S3-1). To facilitate characterization of phylogenetic patterns, I categorized samples into six geographic locations (see Figure 3-1): (1) Suisun Bay (northern subspecies), (2) San Pablo Bay (northern subspecies), (3) South Bay (southern subspecies), (4) Oakland (Mid-Bay; southern subspecies), (5) Richmond (Mid-Bay; southern subspecies), and (6) Marin (Mid-Bay; southern subspecies). For modern samples, I collected ear snips and extracted DNA using Qiagen DNeasy Blood and Tissue Kits (Qiagen, Hilden, Germany) following manufacturer's instructions. For museum samples, I collected whole toe samples from specimens at the Museum of Vertebrate Zoology (University of California, Berkeley) and the Natural History Museum of Los Angeles County (Supplemental Table S3-1). and extracted DNA following the vacuum manifold method from McDonough et al. (2018). Briefly, I digested samples in a solution of water, buffer, SDS, Proteinase K, and DTT for 3–5 days at 55°C; placed the lysate in PB buffer and used a vacuum manifold to pull the lysate/buffer solution through a Qiagen MinElute

column; washed the column by pulling ethanol through the vacuum manifold; and eluted the DNA with EB buffer.

Library preparation and sequencing were conducted by the UC Davis Genome Center using NovaSeq S4 PE150 technology. I assembled mitogenomes for most individuals using NOVOPlasty v3.8.3 (Dierckxsens et al. 2016). If samples did not produce a complete assembly in NOVOPlasty, I used BWA MEM v0.7.17.r1188 (Li and Durbin 2009) for assembly, and I manually edited all assemblies in Sequencher v5.4.6 (Gene Codes Corporation, Ann Arbor, USA).

I aligned sequences in MUSCLE v3.8.31 (Edgar 2004) to an annotated reference sequence of *R. mexicanus* from GenBank (accession KY707307), which I used to determine gene positions. I partitioned the data set into first, second, and third codon positions for 14 loci: 12S, 16S, ATP6, ATP8, COX1, COX2, COX3, CYTB, ND1, ND2, ND3, ND4, ND4L, and ND5. I excluded the reverse-transcribed ND6 from these analyses, as this had a large gap adjacent to ND5 in many samples. Finally, I created a fourth partition of all non-coding tRNA genes. The D-loop was excluded from phylogenetic inference.

I constructed a phylogenetic tree in BEAST v1.10.4 (Drummond and Rambaut 2007) using all partitions to obtain topology and node support. I used the GTR + Γ substitution model for each partition based on results from jModelTest2 (Darriba et al. 2012). Since all of my samples aside from the outgroup were a single species, I used the "coalescent: constant population size" tree prior and a strict molecular clock (e.g., Braulik et al. 2015). I ran 50 million MCMC cycles and sampled every 5,000. I used Tracer v1.7.1 (Rambaut et al. 2018) to observe convergence and ensure effective sample sizes (ESS) >200 for all parameters, Tree Annotator v2.5.2 to construct maximum credibility trees, and visualized trees in FigTree v1.4.4

(http://tree.bio.ed.ac.uk/software/figtree/). To estimate divergence times between clades while minimizing bias due to purifying selection (Subramanian and Lambert 2011), I constructed a tree using only 3rd codon positions following the same process described above. I used a normally distributed tree root prior with a mean of 4.5 mya and standard deviation of 0.5 mya, based on divergence time estimates between salt marsh harvest mice and the outgroup reference sequence (Bell et al. 2001; Arellano et al. 2005; Nava-Garcia et al. 2016; Steppan and Schenk 2017).

I used the R package 'pegas' (Paradis 2010) to calculate nucleotide diversity for each of the six geographic populations. I used two approaches to assign Mid-Bay populations to subspecies. First, I estimated pairwise Φ_{ST} between all population pairs using the Arlequin v3.5.2.2 (Excoffier and Lischer 2010). Second, I used a spatial analysis of molecular variance (SAMOVA) to estimate population structure of the six geographic populations using the program SAMOVA 2.0 (Dupanloup et al. 2002). I ran SAMOVA with the number of populations (K) ranging from 2–5 with 100 simulated annealing processes (as recommended by Dupanloup et al. 2002). I used the first 10,000 bp of mtGenome sequences for SAMOVA due to computational limitations in the program.

I estimated the historical demography of the species and the subspecies using two approaches. First, I used Bayesian Skyline Plots (BSPs) constructed in BEAST to estimate changes in effective population size over time. I constructed BSPs at the species and subspecies levels, with Mid-Bay populations included within subspecies on the basis of Φ_{ST} and SAMOVA results. I used the same partitioning and substitution model scheme as the phylogenetic tree, and I set the root height prior based on TMRCA estimates from the third-codon tree. Second, I used IMa2 (Hey and Nielsen 2007; Hey 2010) to estimate divergence times and pre- and post-divergence population sizes between (a) the two subspecies (with Mid-Bay populations

included), (b) each of the Mid-Bay populations and the known geographic populations representing the southern (South Bay) and northern (combined Suisun Bay and San Pablo Bay) subspecies, and (c) the two major bays of the northern subspecies (San Pablo and Suisun). I ran IMa2 using models of isolation-with-migration and isolation-without-migration and selected the best model using a likelihood-ratio test. I inferred a population mutation rate based on posterior clock rates from BEAST. I ran IMa2 with 50,000 burn-in steps and 100,000 subsequent steps to facilitate estimates of joint likelihood, and ran three replicate runs with different random number seeds to confirm consistency of estimates.

RESULTS

Phylogenetic Divergence

Salt marsh harvest mice were divided broadly into three clades (Figure 3-2). Clade 1 included only individuals from populations in the northern SFE (Suisun, San Pablo); Clade 2 included individuals from the South Bay and Oakland, and Clade 3 included individuals from all six geographic populations. The molecular clock rate estimated from BEAST was 2.4% per million years across the mitogenome and 3.9% per million years for third-codon positions. Based on node ages from the third-codon tree, the time to the most recent common ancestor (TMRCA) for all individuals was approximately 192 (95% CI: 129–259) kya, and TMRCA for Clade 2 and Clade 3 was approximately 90 (62–152) kya. Individuals of the South Bay population primarily occurred in Clade 2, but were also represented in a subclade nested within Clade 3 (Subclade 3b). Of the three Mid-Bay populations, individuals from Oakland were restricted to Clade 2 and Clade 3, specifically within Subclade 3b, which was shared exclusively with South Bay individuals. Individuals from Richmond and Marin were restricted to Clade 3 and were not

represented in Subclade 3b; thus, their phylogenetic relationships were closer to individuals from Suisun Bay and San Pablo Bay than to individuals from South Bay.

Population Diversity and Differentiation

Northern populations (Suisun Bay and San Pablo Bay) had nearly twice the nucleotide diversity of the South Bay (Table 3-1). Richmond exhibited exceptionally low nucleotide diversity. For all levels of K in SAMOVA analyses, Suisun Bay and San Pablo Bay grouped together, and never grouped with the South Bay, in line with my expectations (Table 3-2). At K = 2, Richmond and Marin grouped with Suisun Bay and San Pablo Bay, and Oakland grouped with the South Bay. The number of populations with the greatest proportion of variance among groups (Φ_{CT}) was K = 4, with groups (1) Suisun Bay and San Pablo Bay, (2) Richmond, (3) Marin, and (4) South Bay and Oakland. Pairwise Φ_{ST} reflected the structure supported in SAMOVA. The Φ_{ST} values differed significantly from zero for all pairs except Suisun Bay-San Pablo Bay, Marin-San Pablo Bay and South Bay-Oakland (Table 3-3). Marin and Richmond each had lower Φ_{ST} with Suisun Bay and San Pablo Bay than South Bay, and Oakland had lower Φ_{ST} with South Bay than Suisun Bay and San Pablo Bay. Richmond was the most highly differentiated from all populations.

Historical demography

Bayesian Skyline Plots revealed that salt marsh harvest mice experienced a significant demographic expansion from approximately 25–3 kya, and a significant decline in Ne within the past 3 kya (Figure 3-3). A more ancient demographic expansion occurred approximately 70–50 kya, followed by a slight decline 35–25 kya. The northern populations (San Pablo, Suisun, Marin, and Richmond combined) exhibited a similar pattern of expansion from 25–3 kya,

whereas expansion in the southern populations (Oakland and South Bay combined) expansion began more recently, ~10 kya (Figure 3-3). In addition, the southern populations had lower effective population size than the northern populations throughout their demographic history. Both subspecies and the species as a whole exhibited a recent demographic decline.

Likelihood ratio tests suggested that models of isolation-with-migration did not improve estimates compared to models of isolation-without-migration (Supplemental Table S3-2); therefore, I used models of isolation-without-migration for all population pairs. The northern and southern populations diverged approximately 13.8 (7.8–19.9) kya (Table 3-4). The following four population pairs diverged approximately 5 kya (95% CIs ranged 1.5–11.2 kya): (1) San Pablo Bay and Suisun Bay, (2) Marin and the major northern bays (San Pablo and Suisun combined), (3) Richmond and the major northern bays, and (4) Oakland and South Bay. In agreement with SAMOVA and pairwise Φ_{ST} analyses, IMa2 suggested that divergence times for Oakland were more recent with South Bay than with the combined northern bays (Suisun Bay and San Pablo Bay), and that Marin and Richmond each diverged more recently from the major northern bays than from South Bay (Table 3-4). In general, population sizes were larger for post-divergence populations than ancestral populations, and for the two major northern populations (Suisun and San Pablo bays) than the Mid-Bay and South Bay populations.

DISCUSSION

My objectives were to assess phylogenetic divergence among salt marsh harvest mouse populations, associate the timing of population subdivisions with geographic features and historical changes in the landscape, and reassess the geographic boundary range of the subspecies on the basis of these findings. My findings suggest that salt marsh harvest mice are comprised of three ancient mitochondrial (>50 kya) clades. I found evidence for population

expansion during the formation of the San Francisco Estuary (SFE), approximately 10 kya, and subsequent subdivision of geographically distinct populations as sea level continued to rise and fill the bay. In contrast with current subspecies delimitation, I found that two Mid-Bay populations that occur north of where the bay initially filled with sea water (Marin and Richmond) are more genetically similar to the northern bays (San Pablo and Suisun). My timing estimates were contingent on clock rates inferred from calibration with a single congener, *R. mexicanus*, the only such species with published whole mitogenome reference data. The estimated clock rates (2.4% per million years for whole mitogenomes and 3.9% per million years for 3rd codon positions) fall within the expected range based on similar taxa (Nabholz et al. 2008; Horn et al. 2011; Herman et al. 2014; Platt et al. 2015).

Relationship of Mid-Bay Populations

My findings suggest a mismatch between the currently recognized subspecies delineation and genetic affinities based on mitochondrial DNA. As expected, samples from Oakland, which occur far south of the traditional subspecies boundary, were closely related to individuals from the South Bay population, consistent with their current classification in southern subspecies. However, two of the three Mid-Bay populations that align morphologically with the southern subspecies (Marin and Richmond) were less differentiated and more recently diverged from the two northern populations (San Pablo Bay and Suisun Bay) than from the South Bay. Although any taxonomic revision should be further supported by analysis of nuclear DNA, my findings in this study support assignment of these populations to the northern subspecies, which would alter the ranges of these two taxa (USFWS 2013).

The Mid-Bay populations appear to be either extirpated or at high-risk of extirpation. Recent efforts to sample Oakland and Marin populations have turned up no salt marsh harvest

mice, suggesting they no longer occur there (Aylward et al. in review). All of my samples from Oakland and Marin were obtained from museum collections, highlighting the essential role of natural history collections in aiding our understanding of evolutionary histories (Cook and Light 2019). My estimates of divergence time suggest that these apparent extirpations represent the loss of ~5 thousand years of distinct evolutionary history. The Oakland population was closely related to the extant South Bay population, however, the phylogenetic tree, Φ_{ST}, and SAMOVA, all indicated that the Marin population harbored unique diversity not found elsewhere. While Richmond remains extant, it is likely restricted to a single <4km^2 patch of marsh. Moreover, the nucleotide diversity in samples from Richmond observed in this study were an order of magnitude lower than any other population and the 11 modern mitogenomes were identical outside of low read-depth regions (six ambiguous SNPs and a gap between ND5 and ND6). Nuclear DNA are needed to provide a quantitative estimate of genetic effective population size, but the extent of remaining habitat and mitochondrial data from this study suggest this number is likely dangerously small. Given the extinction risk associated with Mid-Bay populations, my phylogeographic reconstruction provides important context for the conservation of this species, especially if reintroduction is considered as a conservation tool.

Recent (Holocene) Phylogeography

Bayesian Skyline Plots (BSPs) suggested that salt marsh harvest mouse populations expanded gradually following the LGM, then rapidly during the formation of the modern SFE. The latter period, ca. 8–10 kya, corresponded to the expansion of wetlands in the SFE as rapidly as 30 m/yr (Atwater et al. 1977). BSPs also suggested recent sharp population declines in both subspecies. These declines likely reflect the loss of >90% of tidal marsh habitat in the SFE over the past 200 years (Williams and Faber 2001). Although BSPs suggest this decline could have

begun as early at 3–5 kya, which would imply a non-anthropogenic cause of initial declines, I consider the former a more plausible explanation due to uncertainties in mutation rates and high variance associated with the use of a single marker. Nuclear genomic data may be able to reconcile whether demographic declines in salt marsh harvest mice pre-date modern anthropogenic landscape conversion.

As I hypothesized, my data suggest that many geographically distinct populations became isolated and diverged since the initial formation of the modern SFE approximately 10 kya. Oakland diverged from the South Bay, and Marin and Richmond diverged from the two northern bays (Suisun and San Pablo), ~5 kya, when sea level was 4–8 m lower than modern sea level in the SFE (Atwater 1979). Salt marsh harvest mouse gene flow is restricted by open water and upland (>2 m elevation) habitat (Statham et al. 2022). Although the Mid-Bay populations are presently isolated by open water and terrestrial headlands, they may have been connected to nearby populations during this period of lower sea level (see Figure 3-1). For all IMa2 analyses, scenarios of isolation-with-migration did not improve performance compared to models of isolation-without-migration, suggesting that populations never re-established demographic connections following their most recent isolation. This pattern is consistent with the hypothesis that historically continuous populations of salt marsh harvest mice became isolated as sea level rise pushed marsh habitat upward, where steep headlands bifurcated marsh habitat and led to the complete vicariance of the modern geographic populations.

These processes of marsh expansion and subsequent isolation appear to have also contributed to the relationship between the two major northern bays, Suisun and San Pablo. These two populations are isolated by the Carquinez Strait, a narrow (1-km wide), 8-km long passage of water abutting steep headlands that prevent the formation of marsh habitat. I

estimated a divergence time of ~5 kya between these two populations, corresponding to the estimated arrival of sea water and associated marsh plants in Suisun Bay via sea level rise through the Carquinez Strait (Goman and Wells 2000; Fard et al. 2021). Given that salt marsh habitat was not present in Suisun Bay until this time, salt marsh harvest mice likely colonized Suisun Bay from San Pablo Bay as salt marshes expanded eastward, then became isolated as sea level rose and filled the Carquinez Strait. These populations share a significant amount of genetic variation and represent the largest and most genetically diverse modern populations. It appears that a large proportion of genetic variation from the ancestral San Pablo Bay population contributed to the establishment of the Suisun Bay population, avoiding a substantial founder effect. Furthermore, large contemporary population sizes may have helped limit subsequent genetic drift, which likely drove the divergence of the smaller Richmond and Marin populations.

Ancient (pre-Holocene) Phylogeography

My estimates of splitting times did not clarify whether subspecies divergence occurred before or after the formation of the modern SFE. IMa2 estimates ranged from the end of the LGM (20 kya) to the beginning of the formation of the modern SFE (8 kya). The latter part of this interval corresponds to my hypothesis that the subspecies diverged when sea level entered the SFE and open bay water isolated distinct geographic regions of marsh habitat to the north and south of the mouth of the bay. Alternatively, the majority of the confidence region suggests divergence prior to the formation of the modern SFE. During the LGM, sea level was ~100 m lower than today and the shoreline occurred ~100 km west of the modern San Francisco Estuary (Atwater et al. 1977). At this time, habitat in the modern SFE was characterized as a mesic forest, similar to modern coastal forests in northwestern North America (Atwater 1979), and it is likely that salt marsh habitats – and therefore salt marsh harvest mouse populations – were

restricted to areas west of the modern SFE. It is possible that vicariance occurred when the species was located west of the modern SFE (e.g., on the Continental Shelf). Although geological data for this time and geographic area is limited, the flow of the combined ancient Sacramento and San Joaquin rivers, which drained glacial meltwater from approximately half the land area of California through and west of the Golden Gate (Atwater et al. 1977), may have been large enough to isolate multiple ancestral populations. According to BSPs, demographic histories of the two subspecies also diverged during or immediately after the LGM, with only the northern subspecies exhibiting a demographic expansion prior to ~10 kya, supporting the existence of distinct ancestral populations >10 kya, and, therefore, west of the SFE.

Evolutionary and demographic patterns prior to the LGM were challenging to infer for salt marsh harvest mice based on mitogenomic data, likely due to recent (and potentially ancient) losses of genetic variation in this species. The species-level BSP revealed a slight demographic expansion approximately 70–50 kya, which corresponded with the divergence of several phylogenetic clades. Although the demographic signal was lost prior to this expansion, phylogenetic divergence among clades also was indicated approximately 90 kya. These two periods correspond to Marine Isotope Stages (MIS) 3 and 5, respectively, which are the two interglacial periods preceding the LGM. Local geological data suggests the presence of a large estuary and coastal wetland habitat in the SFE during MIS 5, when sea level was comparable to modern sea level, but not during MIS 3, when sea level did not rise to modern levels (Atwater et al. 1977; Atwater 1979). The association of demographic expansion and evolutionary divergence in salt marsh harvest mice with periods of sea level rise broadly agree with geological evidence which suggests that historical periods of rapid sea level rise are correlated with the expansion of

wetland habitat (Cheng et al. 2021). These findings suggest that glaciation events play an important role in shaping the evolutionary history of coastal wetland ecosystems.

Conclusions

My findings suggest that global glaciations and associated changes in sea level can have a strong influence on the evolution of local coastal wetland ecosystems. I found an association between ancient divergence of salt marsh harvest mice and each of the past three significant global periods of sea level rise. I also found that sea level rise over the past 5–10 kya caused divergence among populations in the SFE by submerging ancient, interconnected marsh habitat, highlighting a complex relationship between sea level rise and the expansion and contraction of salt marsh habitat My data largely support my hypotheses of recent population divergence based on modern conditions within the SFE, but also included the possibility of ancient divergence on the modern continental shelf, where a lack of geological data precludes the ability to inform hypotheses of environmental causes of divergence. This study forms the basis for potential revision of subspecies boundaries, although further assessment with nuclear genomic data is needed to support the mitogenome-based delineation.

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FIGURES

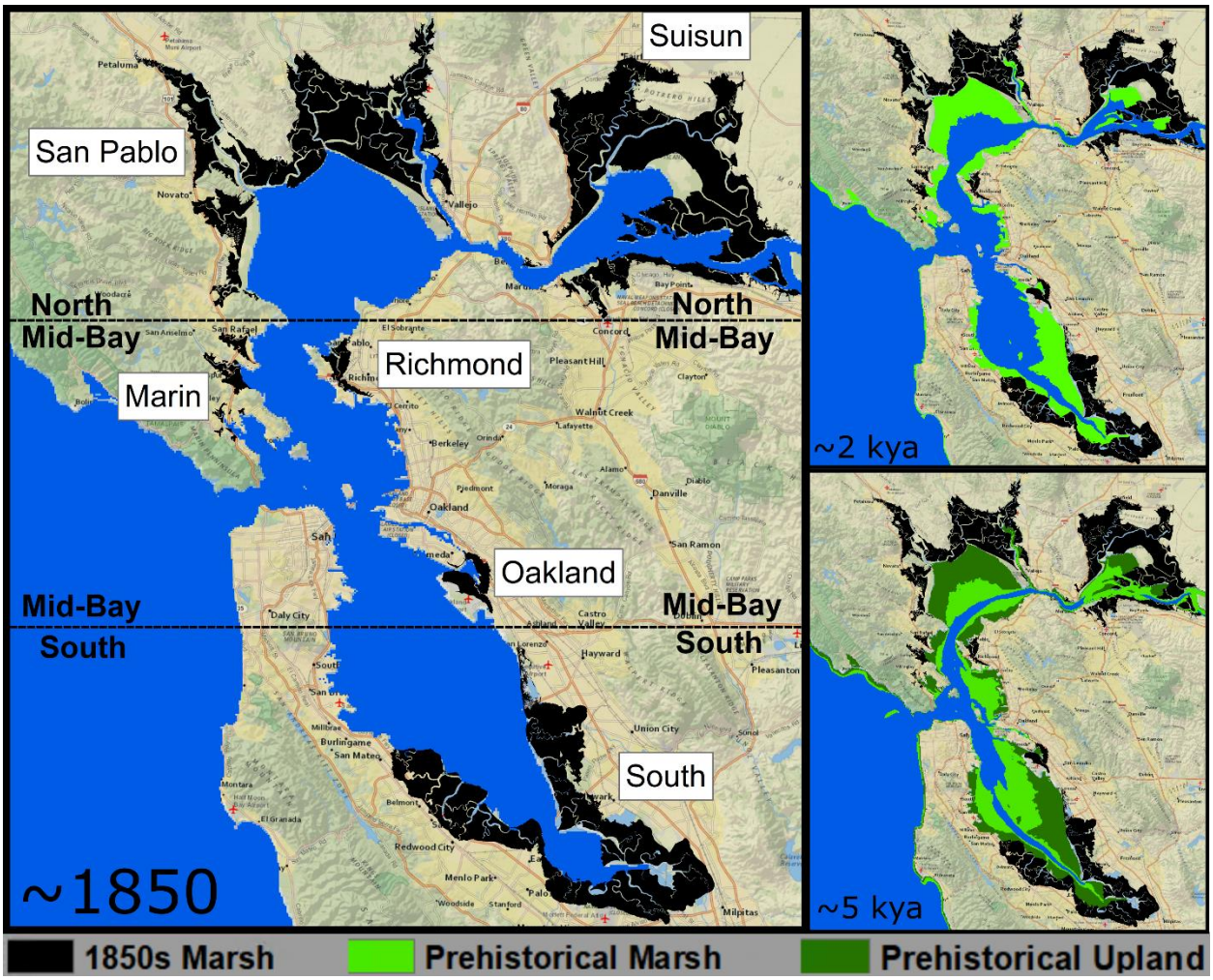

Figure 3-1. Distribution of six geographic populations of salt marsh harvest mice (*Reithrodontomys raviventris*) in this study and estimated prehistorical distribution (ca. 2 and 5 kya) of salt marsh habitat. Recent historical (ca. 1850) distribution of geographic populations (black polygons) are shown for greater visibility due to significant reduction and fragmentation of modern (ca. 2022) habitat. Black dotted lines show separation between the northern populations, Mid-Bay populations, and South Bay population. Historical habitat was estimated based on modern bathymetry of the San Francisco Estuary. Given that sea level 5 kya was 4–8 meters lower than modern sea level (Atwater et al. 1977) and that salt marsh harvest mouse gene flow is restricted by marsh habitat that occurs between 0–2 m elevation (Statham et al. 2022), I categorized 2–8 meters below modern sea level as the potential distribution of marsh habitat 5 kya, and 0–2 meters below modern sea level as the potential distribution of marsh habitat 2 kya.

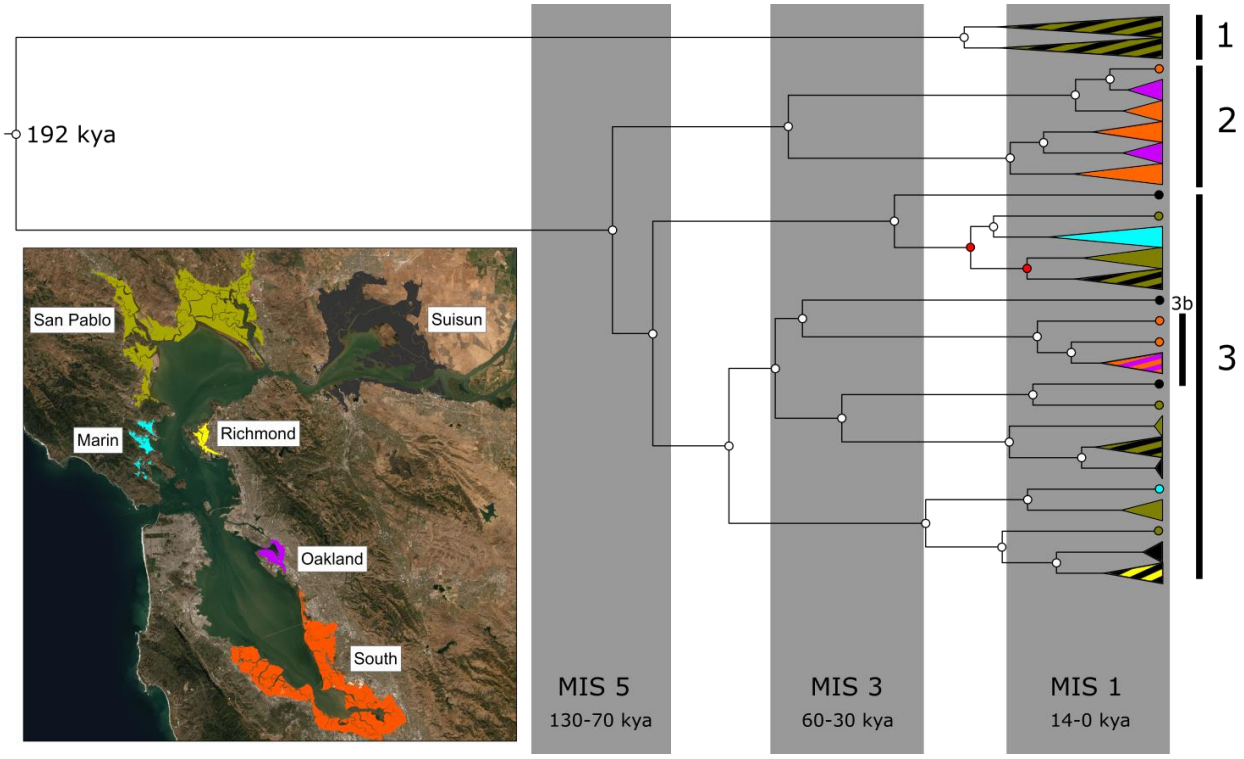

Figure 3-2. Phylogenetic tree of salt marsh harvest mice (*Reithrodontomys raviventris*) estimated from whole genome sequences of 102 samples. The tree was rooted using a reference sequence of *R. mexicanus* (not shown in figure). Clades 1, 2 and 3, and Subclade 3b, are marked with vertical black bars to the right of the tree. Tip colors correspond to each of six geographic populations shown on the map (Black = Suisun Bay, Olive = San Pablo Bay, Light Blue = Marin, Yellow = Richmond, Purple = Oakland, Orange = South Bay). Nodes with a white-filled circle represent >95% posterior support, and those with a red circle represent 90–95% posterior support. Some nodes are collapsed due to either being comprised of individuals from the same location or where there was limited support (<90%) posterior support for internal division. Collapsed nodes comprised of individuals from different geographic populations are filled with diagonal lines representing the colors of each geographic population. The map shows the recent historical (ca. 1850) distribution of geographic populations for greater visibility, as modern wetland habitat has been greatly reduced. Grey bars represent three interglacial periods of high sea level (MIS = Marine Isotope Stage). Node ages in the tree were estimated using a tree constructed from only third-codon positions.

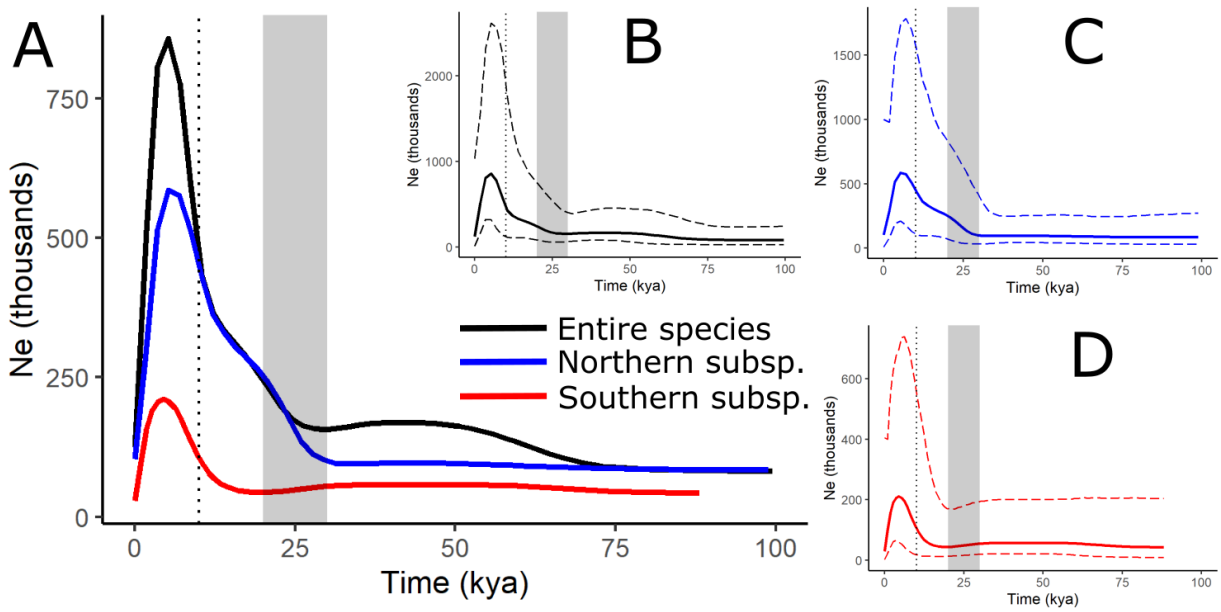

Figure 3-3. Historical effective population size (Ne) of salt marsh harvest mice (*Reithrodontomys raviventris*) estimated from Bayesian Skyline Plots. Effective population size (y-axis) is expressed in thousands of individuals and time (x-axis) is expressed in thousands of years ago (kya). Panel A shows median estimates for the entire species (black) and for the northern (blue) and southern (red) subspecies. The northern subspecies includes Suisun, San Pablo, Marin, and Richmond populations. The southern subspecies includes South Bay and Oakland populations. Panels B, C, and D show median (solid line) and 95% CIs (dashed lines) for the entire species, northern subspecies, and southern subspecies, respectively. Gray shading represents the last glacial maximum (~30–20 kya) and the dashed vertical line represents the initial formation of the modern San Francisco Estuary (~10 kya).

TABLES

Table 3-1. Nucleotide diversity (π) of geographically distinct populations of salt marsh harvest mice (*Reithrodontomys raviventris*) based on whole mitochondrial genomes. Analyses were conducted using the R package 'pegas' (Paradis 2010).

Population	n	π
San Pablo	31	0.00520 (0.00423, 0.00610)
Suisun	25	0.00475 (0.00383, 0.00567)
Marin	4	0.00256 (0.00090, 0.00422)
Richmond	13	0.00016 (0.00011, 0.00021)
Oakland	5	0.00361 (0.00168, 0.00554)
South Bay	29	0.00240 (0.00196, 0.00283)

Table 3-2. Group assignment of six geographic populations of salt marsh harvest mice (*Reithrodotonmys raviventris*) and the proportion of genetic variance among groups (Φ_{CT}) for 2–5 groups estimated using spatial analysis of molecular variance (SAMOVA). Analyses were conducted in the program SAMOVA 2.0 (Dupanloup et al. 2002) using the first 10,000 bp of whole mitochondrial genomes.

Population	K			
	2	3	4	5
San Pablo	A	A	A	A
Suisun	A	A	A	A
Marin	A	B	B	B
Richmond	A	A	C	C
Oakland	B	C	D	D
South	B	C	D	E
Φ_{CT}	0.194	0.320	0.362	0.348

Table 3-3. Genetic differentiation (Φ_{ST}) of six geographic populations of salt marsh harvest mice (*Reithrodontomys raviventris*) using whole mitochondrial genomes. Analyses were conducted in Arlequin v3.5.2.2 (Excoffier and Lischer 2010). Statistically significant Φ_{ST} values ($p < 0.05$) are noted with an asterisk (*).

	San Pablo	Suisun	Marin	Richmond	Oakland	South
San Pablo	--					
Suisun	<0.001	--				
Marin	0.197	0.259*	--			
Richmond	0.451*	0.448*	0.806*	--		
Oakland	0.293*	0.309*	0.319*	0.742*	--	
South	0.360*	0.377*	0.374*	0.579*	<0.001	--

Table 3-4. Estimates of divergence time, modern effective population size of population pairs (Ne_1 and Ne_2), and ancestral effective population size Ne_{Anc} of salt marsh harvest mice (*Reithrodontomys raviventris*). Estimates were generated in IMa2 (Hey and Nielsen 2007; Hey 2010) using a model of isolation-without-migration. For estimating divergence between the two subspecies, the northern subspecies included the Suisun Bay, San Pablo Bay, Richmond, and Marin populations, and the southern subspecies included the South Bay and Oakland populations.

Population Pair	Divergence Time (kya)	Ne_1 (thousands)	Ne_2 (thousands)	Ne_{Anc} (thousands)
(1) Northern subsp. (2) Southern subsp.	13.8 (7.8, 19.9)	65 (44, 95)	28 (15, 51)	18 (4, 86)
(1) San Pablo, (2) Suisun	5.0 (1.7, 7.9)	196 (88, 476)	106 (41.2, 251)	212 (132, 342)
(1) Suisun + San Pablo, (2) Marin	5.3 (1.6, 11.2)	192 (116, 317)	23 (4, 514)	108 (50, 203)
(1) Suisun + San Pablo, (2) Richmond	5.7 (2.9, 10.4)	276 (173, 461)	6 (2, 19)	169 (99, 282)
(1) South Bay, (2) Oakland	5.6 (1.5, 9.3)	72 (28, 168)	88 (30, 587)	40 (16, 103)

SUPPLEMENTAL MATERIAL

Supplemental Table S3-1. Salt marsh harvest mouse (*Reithrodontomys raviventris*) samples used for whole mitochondrial genome sequencing. Modern samples were collected between 2010–2021 and museum specimens I sampled were collected between 1930–1959. Museum samples comprise all of the samples for two geographic populations (Marin and Oakland) where modern sampling has not found evidence of salt marsh harvest mouse presence.

Sample ID	Geographic Population	Museum Collection	Sample Date	Lat/Long
MVZ94834	San Pablo	MVZ	1940	37.9320877, -122.5079271
MVZ130008	San Pablo	MVZ	1959	38.2004954, -122.5748928
MVZ130020	San Pablo	MVZ	1959	38.2004954, -122.5748928
M0805	San Pablo	--	2015	38.11896, -122.4708
M0807	San Pablo	--	2015	38.116189, -122.470833
M1689	San Pablo	--	2018	38.1883, -122.3351
M1690	San Pablo	--	2018	38.1883, -122.3351
M1691	San Pablo	--	2018	38.1883, -122.3351
M1692	San Pablo	--	2018	38.1883, -122.3351
M1694	San Pablo	--	2018	38.0898932, -122.2771702
M1904	San Pablo	--	2019	38.1549650, -122.5437212
M1905	San Pablo	--	2019	38.1549650, -122.5437212
M1906	San Pablo	--	2019	38.1549650, -122.5437212
M1907	San Pablo	--	2019	38.1549650, -122.5437212
M1908	San Pablo	--	2019	38.1549650, -122.5437212
M1909	San Pablo	--	2019	38.1549650, -122.5437212
M1911	San Pablo	--	2019	38.1549650, -122.5437212
M1912	San Pablo	--	2019	38.1549650, -122.5437212
M1913	San Pablo	--	2019	38.1549650, -122.5437212
M2141	San Pablo	--	2020	38.015054, -122.501537
M2142	San Pablo	--	2020	38.015054, -122.501537
M2144	San Pablo	--	2020	38.015054, -122.501537
M2155	San Pablo	--	2020	38.018916, -122.51468
M2157	San Pablo	--	2020	38.018916, -122.51468
M2158	San Pablo	--	2020	38.018916, -122.51468
M2175	San Pablo	--	2020	38.018067, -122.5100232
M2176	San Pablo	--	2020	38.018067, -122.5100232
M2212	San Pablo	--	2020	38.142924, -122.407388
M2213	San Pablo	--	2020	38.150531, -122.406883
M2214	San Pablo	--	2020	38.150531, -122.406883
M2215	San Pablo	--	2020	38.150531, -122.406883
LA27668	Suisun	NHMLAC	1959	38.023019, -122.103166
M1400	Suisun	--	2018	38.1547522, -121.9886375
M1401	Suisun	--	2018	38.1547522, -121.9886375
M1402	Suisun	--	2018	38.1547522, -121.9886375

M1538	Suisun	--	2018	38.2384818, -122.0251044
M1539	Suisun	--	2018	38.2384818, -122.0251044
M1540	Suisun	--	2018	38.2384818, -122.0251044
M1614	Suisun	--	2018	38.231116, -121.994908
M1615	Suisun	--	2018	38.231116, -121.994908
M1616	Suisun	--	2018	38.231116, -121.994908
M1784	Suisun	--	2018	38.0847138, -122.1041361
M1785	Suisun	--	2018	38.0847138, -122.1041361
M1786	Suisun	--	2018	38.0847138, -122.1041361
M1787	Suisun	--	2018	38.0847138, -122.1041361
M1788	Suisun	--	2018	38.0847138, -122.1041361
M1789	Suisun	--	2018	38.0847138, -122.1041361
M1790	Suisun	--	2018	38.0847138, -122.1041361
M1812	Suisun	--	2018	38.1930416, -121.9948305
M1813	Suisun	--	2018	38.1930416, -121.9948305
M1814	Suisun	--	2018	38.1930416, -121.9948305
M2086	Suisun	--	2020	38.15989, -122.095643
M2087	Suisun	--	2020	38.15989, -122.095643
M2089	Suisun	--	2020	38.15989, -122.095643
M2094	Suisun	--	2020	38.15989, -122.095643
M2095	Suisun	--	2020	38.15989, -122.095643
MVZ81302	Marin	MVZ	1938	37.8821443, -122.5157516
MVZ96241	Marin	MVZ	1941	37.9320877, -122.5079271
MVZ102484	Marin	MVZ	1945	37.9571842, -122.508492
MVZ128656	Marin	MVZ	1956	37.9320877, -122.5079271
MVZ126083	Richmond	MVZ	1959	37.9745281, -122.3765387
MVZ126088	Richmond	MVZ	1959	37.9745281, -122.3765387
M2199	Richmond	--	2020	37.97329, -122.37212
M2200	Richmond	--	2020	37.97329, -122.37212
M2201	Richmond	--	2020	37.97329, -122.37212
M2202	Richmond	--	2020	37.97329, -122.37212
M2203	Richmond	--	2020	37.97329, -122.37212
M2204	Richmond	--	2020	37.97329, -122.37212
M2205	Richmond	--	2020	37.97329, -122.37212
M2206	Richmond	--	2020	37.97329, -122.37212
M2207	Richmond	--	2020	37.97329, -122.37212
M2208	Richmond	--	2020	37.97329, -122.37212
M2209	Richmond	--	2020	37.97329, -122.37212
MVZ80671	Oakland	MVZ	1937	37.7439691, -122.2126871
MVZ80673	Oakland	MVZ	1937	37.7439691, -122.2126871
MVZ80674	Oakland	MVZ	1937	37.7439691, -122.2126871
MVZ80837	Oakland	MVZ	1937	37.73659, -122.19581
MVZ80839	Oakland	MVZ	1937	37.73659, -122.19581
MVZ87902	South	MVZ	1939	37.62233, -122.12892

MVZ113406	South	MVZ	1949	37.509454, -122.26187
M0730	South	--	2014	37.48037, -122.00939
M1143	South	--	2016	37.46064, -122.12366
M1673	South	--	2018	37.530065, -122.059591
M1715	South	--	2018	37.5047073, -122.0165336
M1716	South	--	2018	37.5047073, -122.0165336
M1880	South	--	2019	37.6126544, -122.1043138
M1881	South	--	2019	37.6126544, -122.1043138
M1882	South	--	2019	37.6126544, -122.1043138
M1883	South	--	2019	37.6126544, -122.1043138
M1884	South	--	2019	37.6126544, -122.1043138
M1885	South	--	2019	37.6126544, -122.1043138
M2008	South	--	2020	37.4514407, -121.9260363
M2010	South	--	2020	37.452152, -121.9264939
M2012	South	--	2020	37.452152, -121.9264939
M2013	South	--	2020	37.452152, -121.9264939
M2016	South	--	2020	37.4514407, -121.9260363
M2019	South	--	2020	37.4514407, -121.9260363
M2022	South	--	2020	37.4514407, -121.9260363
M2023	South	--	2020	37.4514407, -121.9260363
M2129	South	--	2020	37.626809, -122.138475
M2130	South	--	2020	37.626809, -122.138475
M2131	South	--	2020	37.626809, -122.138475

Supplemental Table S3-2. Results of Log Likelihood Ratio tests to determine whether Isolation-With-Migration outperformed Isolation-Without-Migration in IMa2 analyses.

Population Pair	m0>1 (LR95% CI)	M1>0 (LR95% CI)	LLRtest m0>1	LLRtest m1>0
(1) North ssp., (2) South ssp.	0.000 (0.000, 0.037)	0.033 (0.000, 0.196)	0.000[ns]	0.199[ns]
(1) Suisun + San Pablo, (2) Marin	0.021 (0.000, 2.473)	0.014 (0.000, 5.883)	0.685[ns]	0.263[ns]
(1) South Bay, (2) Marin	0.120 (0.000, 4.233)	0.144 (0.000, 6.642)	0.133[ns]	0.585[ns]
(1) Suisun + San Pablo, (2) Richmond	0.023 (0.000, 0.078)	0.000 (0.000, 0.381)	2.100[ns]	0.000[ns]
(1) South Bay, (2) Richmond	0.023 (0.000, 0.075)	0.000 (0.000, 0.364)	2.425[ns]	0.000[ns]
(1) South Bay, (2) Oakland	0.000 (0.000, 9.411)	0.000 (0.004, 8.481)	0.000[ns]	0.114[ns]
(1) Suisun + San Pablo, (2) Oakland	0.542 (0.000, 12.522)	0.079 (0.000, 12.257)	0.648[ns]	1.340[ns]
(1) San Pablo, (2) Suisun	0.087 (0.000, 0.627)	0.085 (0.000, 0.942)	1.414[ns]	0.456[ns]

[ns] nonsignificant log likelihood ratio test statistic as reported by IMa2.

CHAPTER 4 – Dietary Characterization of the Endangered Salt Marsh Harvest Mouse and Sympatric Rodents using DNA Metabarcoding

*The salt marsh harvest mouse (*Reithrodontomys raviventris*; RERA) is an endangered species endemic to the coastal wetlands of the San Francisco Estuary, California. RERA are specialized to saline coastal wetlands, and their historical range has been severely impacted by landscape conversion and the introduction of non-native plant and rodent species. A better understanding of their diet is needed to assess habitat quality, particularly in relation to potential competitors. I investigated three questions using DNA metabarcoding with ITS2 and trnL markers: (1) Do RERA specialize on the native plant, pickleweed (*Salicornia pacifica*), (2) Do RERA consume non-native plants, and (3) What is the dietary niche breadth and overlap with three sympatric native and non-native rodents? RERA diet was dominated by two plants, native Salicornia and non-native salt bush (*Atriplex *spp.), but included 48 plant genera. RERA diet breadth was narrowest in fall, when they consumed the highest frequencies of Salicornia and Atriplex, and broadest in spring, when the frequencies of these two plants were lowest. Diet breadth was slightly lower for RERA than for co-occurring species in pairwise comparisons. All four species consumed similarly high frequencies of wetland plants, but RERA consumed fewer grasses and upland plants, suggesting that it may be less suited to fragmented habitat than sympatric rodents. Diet overlap was lowest between RERA and the native California vole (*Microtis californicus*). In contrast, RERA diet overlapped substantially with the native western harvest mouse (*R. megalotis*) and non-native house mouse (*Mus musculus*), suggesting potential for competition if these species become sufficiently abundant.*

INTRODUCTION

The salt marsh harvest mouse (*Reithrodontomys raviventris*; RERA; Fig. 4-1) is a habitat specialist occurring solely in the salt marshes of the San Francisco Estuary (SFE), California, USA. They are the only known mammal species restricted to coastal marshes (Greenberg et al. 2006). Despite being listed as an Endangered Species since the inception of the US Endangered Species Act (USFWS 1970), the ecology of RERA remains poorly known. Most prior research effort has emphasized habitat associations (Smith et al. 2018a). Other aspects of RERA ecology, including diet, predation, disease ecology, and interspecific interactions, remain poorly understood (Smith et al. 2018b). Historically, RERA has been considered a specialist of *Salicornia* marsh habitat (Fisler 1965; Shellhammer et al. 1982, USFWS 1984). Recent evidence, however, suggests that RERA may be less specialized to *Salicornia* habitat than believed, particularly in brackish marshes with lower salinity and greater plant diversity (Sustaita et al. 2011; Smith and Kelt 2019; Smith et al. 2020). The SFE has been altered by over a century of anthropogenic impacts, including the loss of >90% of historical tidal marsh habitat (Williams and Faber 2001; Hobbs et al. 2006). Reflecting these threats, RERA is listed as endangered by the state of California (CDFW 1971), the federal government (USFWS 1970), and the International Union for Conservation of Nature (IUCN 2021).

Three other rodent species are commonly detected in SFE marshes, the western harvest mouse (*R. megalotis*; REME), California vole (*Microtus californicus*; MICA), and house mouse (*Mus musculus*; MUMU). REME is native to this region, ranges throughout the western United States, and is considered a habitat generalist (Webster and Jones 1982). REME are less salt tolerant than RERA and are thought to occur primarily in uplands and marsh-upland edge in the SFE (Fisler 1965). MICA also is native and is considered a grassland specialist (Batzli and

Pitelka 1971). MICA are notably larger than both harvest mouse species, and likely are behaviorally dominant to them, although RERA may be better adapted to high salinity conditions (Blaustein 1980; Geissel et al. 1988). Previous work has indicated both negative (Geissel et al. 1988) and positive (Sustaita et al. 2011) associations between RERA and MICA habitat use, but their diet interactions remain unknown. Finally, non-native MUMU commonly co-occur with RERA throughout the SFE (e.g., Bias and Morrison 2006; Marcot et al. 2020). MUMU are highly fecund (Bronson 1979, Pye 1993), opportunistic, and tolerant of a wide range of ecological conditions (Berry 1981). MUMU and RERA are compatible in captivity (Catlett and Shellhammer 1962), and may (Bias and Morrison 2006) or may not (Sustaita et al. 2011) partition habitat, but recapture probabilities for MUMU were positively influenced by RERA densities at Suisun Marsh (Smith et al. 2020).

Non-native plants and animals are abundant in remnant SFE marshes (USFWS 2010; Grewell et al 2014; Smith and Kelt 2019). Non-native species can affect multiple trophic levels of a community, as they may represent novel predators, novel competitors, or novel food resources to different community members (Lepczyk and Rubinoff 2017). Ecological specialists may be particularly sensitive to non-native species, as they may be less likely than generalists to utilize novel resources (Marvier et al. 2004; Abernethy et al. 2016). Indeed, the ecological impacts of non-native species are considered the leading cause of extinction for endemic mammals (Pimm et al. 1995). Given the challenges that ecological specialists must overcome to persist in a highly altered ecosystem, understanding the effects of non-native food resources and non-native intraguild species are critical ecological underpinnings for RERA conservation.

Despite the importance of strong ecological baselines to conservation and management of endangered species, the dietary habits of RERA remain poorly understood. RERA diet has been

inferred from habitat use (USFWS 2010), characterized coarsely by stomach content analysis (Fisler 1965), and measured with cafeteria trials (Smith and Kelt 2019). Rather than providing consensus, these studies have led to divergent views of RERA diet. Habitat associations and stomach content analyses suggest that RERA consume primarily *Salicornia* (Fisler 1965). Conversely, cafeteria trials suggest that RERA are generalist foragers that may prefer some non-native plant species over *Salicornia*. Additionally, comparative dietary interactions of RERA and sympatric rodents have never been investigated.

To address these critical knowledge gaps, I applied DNA metabarcoding to fecal samples collected from rodents in the SFE. Metabarcoding often identifies significantly more dietary taxa at finer taxonomic levels than other methods (Soininen et al. 2009; Valentini et al. 2009; Kartzinel et al. 2015). Additionally, the non-invasive nature of dietary metabarcoding makes it particularly appealing for research on threatened and endangered species (e.g., Iwanowicz et al. 2016; Castle et al. 2020). My objectives were to describe diets of RERA and sympatric rodents and characterize both spatial and temporal dietary variation. I evaluated the hypothesis that RERA has a more specialized diet than sympatric species, and assessed the potential for competition over food resources.

METHODS

Sample Collection

I collected feces from animals captured during regular live-trapping surveys (see Smith et al. 2020 for details of survey design and associated protocols). Preliminary trials showed that feces collected directly from live-trapped animals were more likely to be composed entirely of diet items from the trapping bait, so I endeavored to collect feces from the bedding in traps to characterize diet before consumption of bait. Samples were collected at five sites in coordination

with the California Department of Fish and Wildlife during regular RERA monitoring (Fig. 4-2). One of these sites — the Goodyear Slough Unit (GYS) of the Grizzly Island Wildlife Area — was trapped quarterly over two years (Summer 2018 – Spring 2020, inclusive), allowing me to partition diet into four seasonal data sets; all other sites were trapped once either in summer or late spring, resulting in a total of eight sampling units (Table 4-1). I also surveyed vegetation plots to characterize availability of potential diet items at sampling sites/seasons. Within two weeks of each live-trapping effort, I recorded the presence of all plant genera in 3-m x 3-m quadrats centered at the location of each trap. All methods involving live animals followed guidelines of the American Society of Mammalogists (Sikes et al. 2016), were approved by the UC Davis Institutional Animal Care and Use Committee, and conducted under authority of the Cooperative Agreement between California Department of Fish and Wildlife (CDFW) and the United States Fish and Wildlife Service.

Laboratory Procedures

I extracted DNA from fecal samples using Qiagen Plant Mini Kits (Qiagen, CA, USA). For each captured individual, I extracted DNA from pooled fecal pellets; I targeted >5 pellets from each individual, and final pellet numbers in extractions ranged from 1-13 (mean = 5.7). Library preparation followed the general template of the Illumina 16S metagenomic protocol (Illumina 2015). Since single markers may only amplify a subset of plant taxa in herbivore diets (Goldberg et al. 2020), I applied two commonly used plant metabarcoding markers. I amplified the second internal transcribed spacer (ITS2), which is a longer fragment (~290-340 bp) of nuclear ribosomal DNA with high taxonomic resolution (China Plant Barcode of Life Group et al. 2011), and the P6 loop of the trnL intron, a shorter fragment (~25-90 bp) of chloroplast DNA, which is less likely to be affected by degradation but has coarser taxonomic resolution (Fahner et

al. 2016). I used the R package 'PrimerMiner' (Elbrecht and Leese 2016) to evaluate the compatibility of potential primer pairs with sequences of suspected RERA dietary taxa (based on vegetation surveys and the Suisun Marsh Plant List; CDFW 2017) downloaded from Genbank. I used the primers UniPlantF (Moorhouse-Gann et al. 2018) and ITS-P4 (Cheng et al. 2016) for ITS2, and the primers trnl_g and trnl_h (Taberlet et al. 2007) for trnL. I added sequence overhangs to the 5' ends of amplicon primers to facilitate annealing to Illumina sequencing adapters (compete primer sequences in Appendix A4-1, PrimerMiner scores in Appendix A4-2). I amplified ITS2 using the thermal protocol described in Moorhouse-Gann et al. (2018) and amplified trnL using the thermal protocol described in Taberlet et al. (2007). Given that biological replication (i.e., samples from unique individuals) yields significantly more variation in diet than technical replication (i.e., multiple PCR replicates per individual), I chose to prioritize resources for biological replication and therefore conducted a single PCR replicate for each individual (Mata et al. 2019).

I included 20 positive controls and 12 negative controls per sequencing lane (Appendix A4-3). Positive controls were composed of DNA extracted from plants collected from my field sites. Each set of 20 positive controls included 10 single-species controls to assess sensitivity and to help estimate misassignment error based on the proportion of non-target reads within single-species controls. I also included 10 two-species controls, which had equal concentrations of DNA from two plant taxa and helped to understand potential amplification biases. I used deionized water for negative controls. I sequenced libraries using MiSeq 300 PE for ITS2 and 75 PE for trnL. Sequencing and sample demultiplexing were conducted by the UC Davis Genome Center.

Bioinformatic Processing

I trimmed and quality-filtered sequences using cutadapt (Martin 2011). I identified Amplicon Sequence Variants (ASVs) using DADA2 (Callahan et al. 2016). To identify the taxonomy of ASVs, I created a custom database of ITS2 and trnL sequences of all plant genera known to occur in the SFE using the *batch_download* feature of PrimerMiner, which obtains sequences from both NCBI (Benson et al. 2013) and BOLD (Ratnasingham and Hebert 2007) databases, and I manually re-formatted the reference sequences for use in DADA2. I used the *assignTaxonomy* feature of DADA2 to assign ASVs against the custom database, and used BLAST (Zhang et al. 2000) to corroborate assignments. I assigned ASVs at the genus level, except for some trnL sequences that could not be assigned to a single genus and were therefore assigned to the lowest possible suprageneric level (e.g., family or multiple genera).

I conducted sequence processing and assignment independently for each MiSeq lane. After taxonomic assignment, I retained only ASVs that comprised >0.01% of the total sequence reads in a lane. I then used positive and negative controls to inform filtering parameters to account for misassigned ASVs (O'Rourke et al. 2020). Based on the negative and positive controls, I discarded any sample with <5,000 (trnL) or <3,000 (ITS2) sequencing reads, or with <20% of reads successfully matching plant taxa; and within samples, I discarded any taxa comprising <0.5% (trnL) or <1.0% (ITS2) of reads. After applying those filters, I removed any taxa that likely originated from a source other than wild RERA diet (*Avena*, *Helianthus*, *Juglans*, *Panicum*, and *Phalaris* from trapping bait, and *Gossypium* from trap bedding).

Salt Marsh Harvest Mouse Diet

I recorded presence/absence of diet taxa within individual diets. I calculated the frequency of occurrence (FO = the proportion of individuals that consumed a given diet item) of diet items within RERA samples pooled across all sampling locations and seasons. I then

categorized diet items as native versus non-native, by life form (grass, shrub, forb, vine), and by habitat (e.g., whether they were typical of wetlands or of uplands; determined from Jepson eFlora [Jepson Flora Project 2021]), and I estimated FO for each category. I chose to use presence/absence-based data (i.e., FO) due to the complexity of estimating biomass from relative read abundance (e.g., Deagle et al. 2019) with two different markers that detected different suites of species (see Appendix B). My sample sizes were sufficient to produce a strong correlation between FO and RRA (Appendix A4-4), so use of FO was unlikely to affect downstream analyses. I estimated plant availability at the site level by calculating FO of plant genera among all quadrats at a site.

I evaluated seasonal variation in RERA diet at Goodyear Slough. For this analysis I pooled data by season across the two years, and estimated FO of diet items within each season. I tested for significant seasonal differences using the *anosim* function in the 'vegan' R package (Oksanen et al. 2020). I compared diet to plant availability with Manly's Selection Index (W_i; Manly et al. 2002) using the R package 'adehabitatHS' (Calange 2011). I considered diet items to be "selected" when $W_i \pm 95\%$ confidence intervals > 1, and "avoided" when $W_i \pm 95\%$ CI < 1. Since vegetation availability data were collected at the genus level, I excluded any diet items identified at a coarser taxonomic level from selection analyses. I quantified dietary niche breadth as the effective number of species (^{1}D; Hill 1973; Chao et al. 2014) derived from Shannon's Diversity Index (Shannon and Weaver 1949) for unequal sample sizes and presence/absence data (Chao et al. 2014). I used the R package 'iNEXT' (Chao et al. 2014) to estimate ^{1}D and 95% CIs using 500 bootstrap replicates. I considered seasonal differences in dietary niche breadth significant if 95% CIs were non-overlapping. Additionally, I estimated dietary niche overlap between pairs of seasons using Jaccard's Similarity Index (J_s), calculated in 'vegan'. To visualize

dietary niches in ordination space, I conducted non-metric multi-dimensional scaling (nMDS) based on Jaccard Distances (J_D) using the metaMDS function in 'vegan', and calculated 95% confidence ellipses for each season. I conducted nMDS over a range of dimensions (k) and selected the minimum number of dimensions (k = 3) in which stress of the ordination was <0.10.

I evaluated spatial variation in RERA diet from five sites sampled in late spring and summer (henceforth, "summer" sampling units): Goodyear Slough (GYS; summer) and Crescent Unit (CRES; late spring) of the Grizzly Island Wildlife Area, Ponds 1&2 (HS12; summer) and Area 9 (HS9; summer) of the Hill Slough Wildlife Area, and Eden Landing Ecological Reserve (EDEN; late spring). I estimated the mean and variance of FO for each diet item, and the mean and variance of plant availability, across the five sampling units. I calculated W_i of diet items within each sampling unit and combined across all sampling units.

Diet of Salt Marsh Harvest Mice and Sympatric Rodents

I calculated FO for all diet items and for all rodent species pooled across all sampling units. Further comparative analyses (dietary niche breadth and overlap) were limited to pairwise comparisons with RERA and used only those sampling units where I had dietary information for >4 individuals of both species. To ensure that large sample sizes at one site (i.e., Goodyear Slough) did not bias interpretations of dietary niche breadth and overlap, I calculated FO of diet items at individual sites and then averaged these site-level FOs, thereby giving equal weight to the population-level diet information at each site. I compared RERA diets to REME (three sampling units), MICA (three), and MUMU (four). I calculated ^{1}D and J_s for comparison across each species pair and considered dietary niche breadth significantly difference if 95% CIs of ^{1}D were non-overlapping.. Additionally, I visualized dietary niches of species in ordination space with nMDS based on J_D, and I used 95% confidence ellipses to qualitatively assess diet overlap.

RESULTS

Field Sampling

I collected fecal samples from 327 unique individuals from the eight sampling units. Six samples were discarded during bioinformatic filtering (see Appendix B), leaving 321 samples for subsequent analyses. Sample sizes were significantly larger for RERA (n = 245) than for REME (n = 30), MUMU (n = 26), or MICA (n = 20). Sample sizes also were heavily weighted towards Goodyear Slough (n = 246) due to quarterly sampling over two years. All plants used in controls were reliably detected with the exception of *Schoenoplectus*, which is considered a likely food item for RERA but was absent from my results.

Salt Marsh Harvest Mouse Diet

I documented 53 taxa, including 48 genera and 5 higher-order identifications, in the diet of RERA (Appendix A4-5). When data were pooled across all eight sampling units, seven plant genera presented a FO > 10% (Fig. 4-3A). *Salicornia* and *Atriplex* stood out from the rest of the dataset (FO > 0.50), and *Distichlis*, *Grindelia*, *Rumex, Lepidium*, and *Phragmites* had moderate FOs (> 0.10). RERA diet was dominated by wetland forbs/subshrubs (Appendix A4-6), and both native and non-native items were prevalent.

Diets at Goodyear Slough varied seasonally (ANOSIM; R = 0.173, p = 0.001). *Salicornia* and *Atriplex* were consumed at high frequencies year-round, whereas several taxa were consumed either at moderate frequency year-round (*Lepidium*) or at high frequency but seasonally (*Distichlis*, *Grindelia*, *Rumex*, *Phragmites*, and *Cuscuta*) (Fig. 4-3B; Appendix A4-7). Wetland forbs/subshrubs were eaten frequently in all seasons, whereas upland plants (grasses and forb/subshrubs) were consumed primarily in spring (Appendix A4-6). RERA selected five diet items in at least one season (Table 4-2A; Fig. 4-3C). *Salicornia* and *Atriplex* were each

selected in three seasons, and were never avoided. Three genera were selected in summer (*Phragmites*, *Rumex*, and *Salicornia*), fall (*Atriplex*, *Grindelia*, and *Salicornia*), or winter (*Atriplex*, *Rumex*, and *Salicornia*), and only one in spring (*Atriplex*). RERA avoided *Juncus* in all seasons, and *Distichlis* (summer and fall) and *Phragmites* (fall and spring) in two seasons. Combining data across all seasons, RERA selected *Atriplex*, *Grindelia*, *Rumex*, and *Salicornia*, and avoided *Distichlis* and *Juncus*. Dietary niche breadth (^{1}D) was significantly lower in fall than all other seasons (Fig. 4-4A). Fall and spring exhibited the lowest similarity (J_s) with respect to other seasons (Table 4-3). Seasonal diets overlapped in ordination (nMDS) space, although confidence ellipses varied in breadth in accordance with estimates of seasonal dietary niche breadth (Fig. 4-4B).

At all five summer sampling units, RERA frequently consumed *Salicornia* (FO ≥ 0.50; mean FO = 0.76; Fig. 4-3C; Appendix A4-8). *Atriplex* (mean FO = 0.40) had a FO ≥ 0.50 in two of five sampling units. *Frankenia*, *Lepidium*, and *Phragmites* were the only other taxa with FO ≥ 0.50 at any given sampling unit. Pooling samples across all summer sites, RERA selected *Atriplex*, *Frankenia*, *Phragmites*, *Rumex*, and *Salicornia* (Table 4-2B).

Comparison of Diet to Co-Occurring Rodents

The FOs of REME and MUMU were qualitatively similar to those of RERA (Fig. 4-5; Appendix A4-9). The dominant items in RERA diet (*Salicornia* and *Atriplex*) were also the two most frequently consumed foods by REME (FO = 0.57 and 0.73, respectively) and MUMU (FO = 0.69 and 0.50, respectively). *Salicornia* was the most frequently eaten food by MICA (FO = 0.85), but *Atriplex* was relatively sparse in their diets (FO = 0.15). Notably, grasses (e.g., *Distichlis*, *Phragmites*, *Hordeum*, and *Festuca*) and upland plants (e.g., *Sonchus*, Cynareae) were

more prominent in the diets of sympatric rodents than that of RERA. MICA diet was the most distinct, driven by a low frequency of *Atriplex* and high frequency of rushes (*Juncus*).

RERA had significantly lower dietary niche breadth than all three sympatric rodents (Fig. 4-6). Finally, dietary niche overlap in nMDS space was very high with both REME and MUMU, which effectively subsumed RERA dietary niche space (Fig. 4-7A, 4-7B). In contrast, ordination highlighted that the diets of RERA and MICA were effectively distinct (Fig. 4-7C).

DISCUSSION

Salt marsh harvest mouse diet was dominated by *Salicornia* and *Atriplex* year-round, but also included a wide variety of other native and non-native plants. Seasonal niche breadth was narrowest in fall when they consumed primarily *Salicornia*, *Atriplex*, and *Grindelia*. RERA diet was less diverse than the diets of sympatric rodents due to less frequent consumption of grasses and upland plants.

Salt Marsh Harvest Mouse Diet

Salt marsh harvest mice consumed at least 48 genera of plants in this study. Despite high taxonomic richness in RERA diet, the overwhelming majority was composed of the native *Salicornia* and the non-native *Atriplex*. *Salicornia* was present in the majority of RERA fecal samples in every sampling unit in the study area, was the most frequently consumed item in six of eight sampling units, and ranked second to *Atriplex* at the other two. *Salicornia* was selected in three of four seasons at Goodyear Slough and across five summer sampling units with varying plant composition. These data support the traditional view that *Salicornia* is a staple in the diet of RERA.

Equally important, however, is that *Atriplex* was nearly as prominent in RERA diet as *Salicornia*. A primary difference between the two plants was the lower availability of *Atriplex*, which led to relatively less consumption overall but high selection coefficients. These data were consistent with cafeteria trials that suggested a strong affinity for *Atriplex* (Smith and Kelt 2019). In addition, RERA selected several other non-native plants. The Tidal Marsh Recovery Plan (USFWS 2010) emphasized conservation concerns associated with the invasion of marshes by non-native *Lepidium latifolium*. However, RERA consumed *Lepidium* year-round in proportion to its availability, indicating that low-to-moderate availability of this plant did not adversely affect RERA. I did not sample sites where *Lepidium* dominated the vegetative cover, so the impacts of more intense invasions of *Lepidium* remain uninvestigated. Future work to quantify the nutritional value of native and non-native diet items and their effects on individual survival would provide further clarity on the implications of non-native plants for RERA population health.

Overall, these data support a hypothesis that *Salicornia* stands including mixtures of plants such as *Atriplex, Frankenia,* and *Grindelia* may provide more value to RERA than those with *Salicornia* alone (Fisler 1965; Shellhammer et al. 1982). In particular, a growing body of work from Suisun Marsh, where brackish water promotes more diverse plant communities, has emphasized the importance of mixed vegetative communities over *Salicornia*-dominated sites (Botti et al. 1986; Sustaita et al. 2011; Smith and Kelt 2019). My data clarified that *Salicornia* is an important element in RERA diet, but that their diets were not strictly specialized.

Seasonal Changes in Salt Marsh Harvest Mouse Diet

Optimal foraging theory suggests that animals will specialize on preferred foods when they are available, and that they will broaden their diets when preferred foods are unavailable

(MacArthur and Pianka 1966; Stephens and Krebs 1986). In fall, RERA diet narrowed sharply and was overwhelmingly composed of three species (*Salicornia*, *Atriplex*, and *Grindelia*). In spring, however, consumption of these three plants declined and their dietary breadth expanded accordingly. I suspect that RERA foraging patterns may largely be driven by affinities for these three plants. Dietary seasonality, in turn, likely is driven by plant phenology. Fall, when RERA diet narrowed to focus almost exclusively on *Salicornia*, *Atriplex*, and *Grindelia*, is the peak seeding period for these three plants (Hutchings and Russell 1989; Jepson eFlora Project 2021), and is followed by dormancy or dieback in late winter and early spring, which coincided with reduced consumption by RERA. Whereas annual dieback of *Atriplex* has led some to suggest that this plant has limited value to RERA in winter and spring (Botti et al. 1986; USFWS 2010), my data suggest substantial consumption of *Atriplex* year-round despite seasonal dieback. In contrast, some non-native plants, such as *Phragmites*, were consumed primarily during one season, and were avoided most of the year. It is possible that *Phragmites* seeds do not persist in the environment as long as *Atriplex*, thus limiting their seasonal availability as forage.

Seasonal space use may play an important role in seasonal dietary patterns of RERA. *Grindelia* provides refuge for RERA during high tides (USFWS 2010). In particular, RERA often seek refuge in emergent *Grindelia* during extreme diurnal high tide events in late fall and early winter, whereas other rodents are more likely to retreat to uplands (Johnston 1957). I observed higher frequencies of *Grindelia* in RERA diets during fall and winter, which may reflect an increase in habitat use associated with seasonally high tides. Taken together, these observations suggest that *Grindelia* may provide an important combination of high tide refuge, cover from predators, and forage to RERA during extreme diurnal high tides of late fall and early winter.

The diet of RERA broadened in spring, with increased consumption of upland plants that were negligible in the diets in other seasons. This was particularly notable for upland grasses, which is consistent with previous RERA stomach content analyses (Fisler 1965). RERA remain largely restricted to marsh habitat with the exception of spring forays into terrestrial grasslands (Zetterquist 1977; Shellhammer et al. 1982; USFWS 2010). Fisler (1965) speculated that vegetative cover in grasslands was insufficient for RERA outside the spring growing season. Geissel et al. (1988) suggested that RERA retreated to uplands in response to springtime population irruptions of larger-bodied voles. Although I cannot discern whether competition or seasonal resource exploitation drove this pattern, my data support the hypothesis that utilization of terrestrial grasslands by RERA is largely limited to spring.

Several seasonal patterns in my data mirrored observations from cafeteria trials (Smith and Kelt 2019). Seasonal selection indices of *Salicornia* and *Atriplex* in this study were high in fall and low in spring, corresponding with seasonal preference rankings in cafeteria trials. In contrast, my data showed high FO of these plants in summer as well, whereas feeding trials did not. My data also aligned with feeding trials that suggested increased preference for annual grasses in spring. On the other hand, feeding trials suggested high or moderate preference for *Juncus* in multiple seasons; I found low consumption of *Juncus* both overall and in proportion to availability in the present study. Despite high availability and high FO in MICA diet, I detected *Juncus* in just one of 189 RERA samples at Goodyear Slough. Another major conclusion from feeding trials was a strong preference for *Polypogon*. My ability to corroborate this result may have been limited by low availability or absence of this plant from most of the study sites. *Polypogon* was rare at Goodyear Slough and relatively common at Crescent Unit, and consumption by RERA occurred in proportion to its availability.

Dietary Comparisons to Co-Occurring Rodents

Diet of the endangered RERA overlapped substantially with that of the widespread REME, driven primarily by high frequencies of *Salicornia* and *Atriplex*. Although the kidney physiology of REME suggests capability to consume *Salicornia*, they were unable to survive in feeding trials after consuming even small amounts of this plant (Coulombe 1970). Similarly, captive REME starved when presented with only *Salicornia* and *Distichlis* as food sources (Fisler 1965). Nonetheless, my data revealed that wild REME regularly consumed both of these genera (Fig. 4-5, Appendix A4-9). REME were the only species to consume *Atriplex* (which grows primarily in diked wetlands) more frequently than *Salicornia* (which occurs frequently in both diked and tidal wetlands). This pattern most likely reflects differential space use, as REME are more abundant on diked wetlands than tidal wetlands, and RERA and MUMU abundances do not differ among wetland types (Smith et al. 2020). REME also consumed grasses (*Distichlis*, *Festuca*, *Hordeum*, and *Phragmites*) and upland plants such as thistles (*Sonchus* and Cynareae) with greater frequency than did RERA.

I also documented considerable dietary overlap between MUMU and RERA, driven by high frequencies of *Salicornia* and *Atriplex*. Similar to REME, MUMU consumed more grasses (*Distichlis*, *Phragmites*, *Hordeum*, and *Festuca*) and upland plants (*Sonchus*, Cynareae) than did RERA. In studies of habitat use in the SFE, MUMU were more closely associated with terrestrial grasses and fragmented habitat assemblages than were RERA (Bias 1994; Bias and Morrison 2006). Interestingly, despite a relatively generalist diet, only a single house mouse (of 26) consumed *Grindelia*, which was one of the most frequently consumed plants for the three native rodents (Fig. 4-5, Appendix A4-9).

Relative to RERA, the most distinct diet was that of MICA, primarily due to reduced use of *Atriplex* and a high frequency of *Juncus*. Although few plant species were consumed by a single rodent species in this study, MICA was the only species to utilize *Juncus* to a great extent. Despite being characterized as grassland specialists, MICA in this study consumed lower frequencies of terrestrial grasses than either REME or MUMU. Instead, MICA diet was dominated by *Salicornia*, *Juncus*, and *Distichlis*, differing from more upland locations in the SFE, where they primarily consume terrestrial grasses (Batzli and Pitelka 1971). In fact, the diet of MICA in this study more closely resembled that of Amargosa voles (*M. c. scirpensis*), a subspecies endemic to wetlands in the Mohave Desert (Castle et al. 2020), than MICA occupying the uplands adjacent to SFE marshes (Batzli and Pitelka 1971).

The diet of RERA was more restricted (Fig. 4-5) and significantly less diverse (Fig. 4-6) than that of sympatric species. Preference for *Salicornia* and *Atriplex* was notably greater for RERA, while sympatric species consumed higher proportions of several other species (Fig. 4-5). Notably, many of these latter plants were restricted to uplands, indicating that sympatric rodents are better equipped than RERA to utilize resources in edge habitats. Indeed, I note that REME, MUMU, and MICA generally are considered upland species, thus, my characterizations of their diets are specific to the individuals occurring on the upland/marshland edges and likely not reflective of these species as a whole. Habitat fragmentation and small patch size reduce the probability of RERA occurrence (Bias and Morrison 2006; Marcot et al. 2020), and occupancy of marsh habitat by REME and MUMU may be dependent upon the degree of habitat fragmentation and penetration of terrestrial grass microhabitats into the marsh (Fisler 1965; Bias and Morrison 2006). My results support these important management issues, adding to a growing

literature suggesting that fragmentation of marsh habitat and the associated increase in edge habitat are potential threats to RERA with respect to competition from upland-adapted rodents.

Conclusions

I characterized the diet of RERA and three sympatric rodents in remnant coastal marsh habitat of the SFE. *Salicornia* and *Atriplex* were prominent in RERA diet across sites and seasons. RERA diet narrowed sharply in fall during peak seed production of *Salicornia*, *Atriplex*, and *Grindelia*, which appeared to be favored food items. RERA consumption of terrestrial grass was largely restricted to spring, coinciding with previously documented patterns of seasonal use of upland habitats. RERA diet overlapped substantially with REME and the non-native MUMU, but not with the native MICA. My data provide the first comprehensive characterization of RERA diet in the wild. This information fills critical knowledge gaps in the ecology of RERA and can guide habitat and vegetation management decisions to benefit conservation of the species. Moreover, this study lays the groundwork for future investigation of competition affecting this endangered species.

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Zhang Z, Schwartz S, Wagner L, Miller W (2000) A greedy algorithm for aligning DNA sequences. Journal of Computational Biology 7: 203-214.

FIGURES

Figure 4-1. Salt marsh harvest mouse (*Reithrodontomys raviventris)* in salt marsh habitat in Suisun Marsh, California, USA.

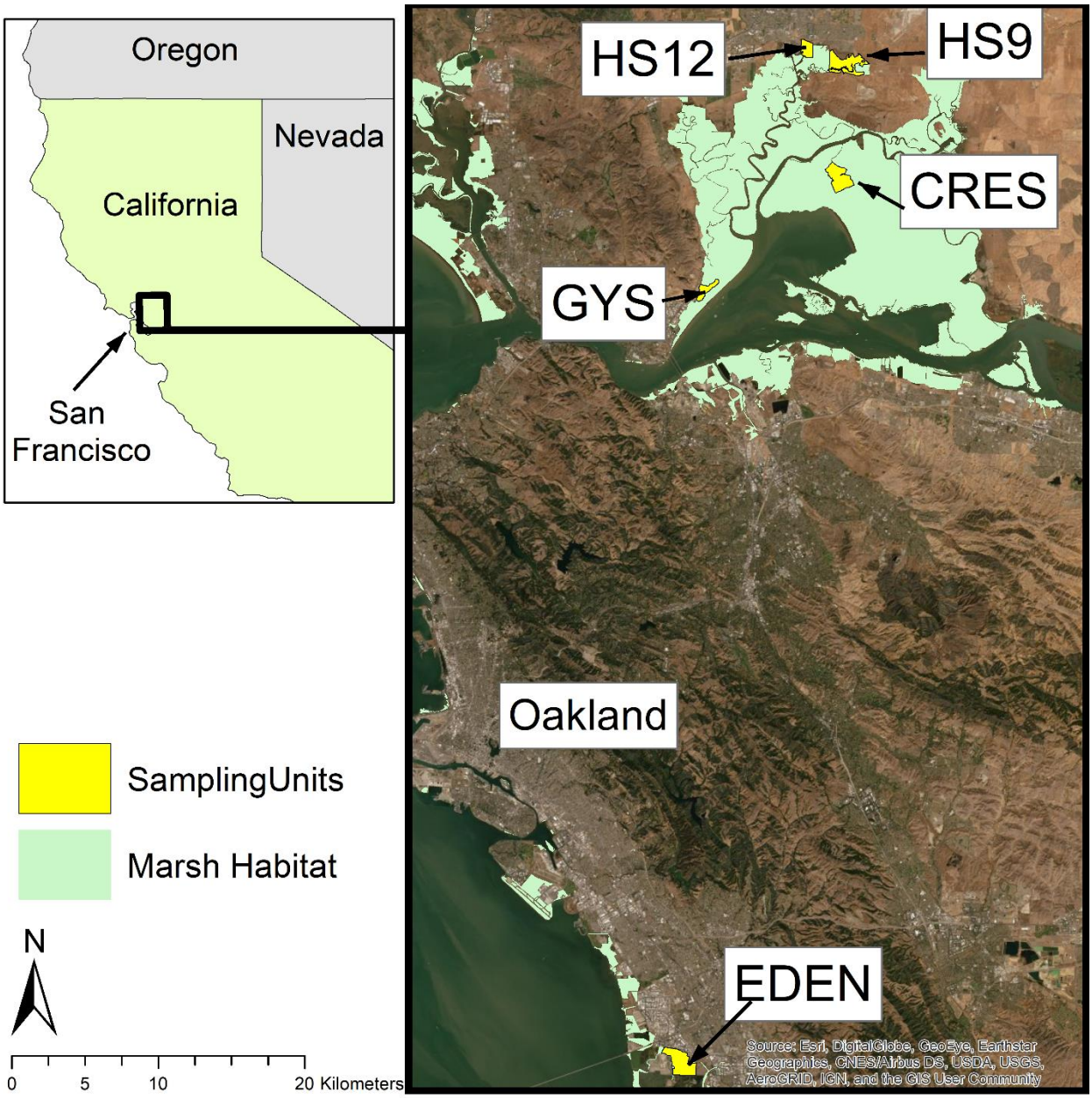

Figure 4-2. Map of sampling locations in this study. Five sites were live-trapped for salt marsh harvest mice (*Reithrodontomys raviventris*) and other small mammals and fecal pellets were collected to characterize diet with DNA metabarcoding. Sites included Goodyear Slough (GYS) and Crescent Unit (CRES) of the Grizzly Island Wildlife Area, Ponds 1&2 (HS12) and Area 9 (HS9) of the Hill Slough Wildlife Area, and Eden Landing Ecological Reserve (EDEN). GYS was sampled quarterly over two years to provide seasonal dietary data. All other sites were trapped opportunistically on one occasion each, either in late spring (CRES and EDEN) or summer (HS12 and HS9).

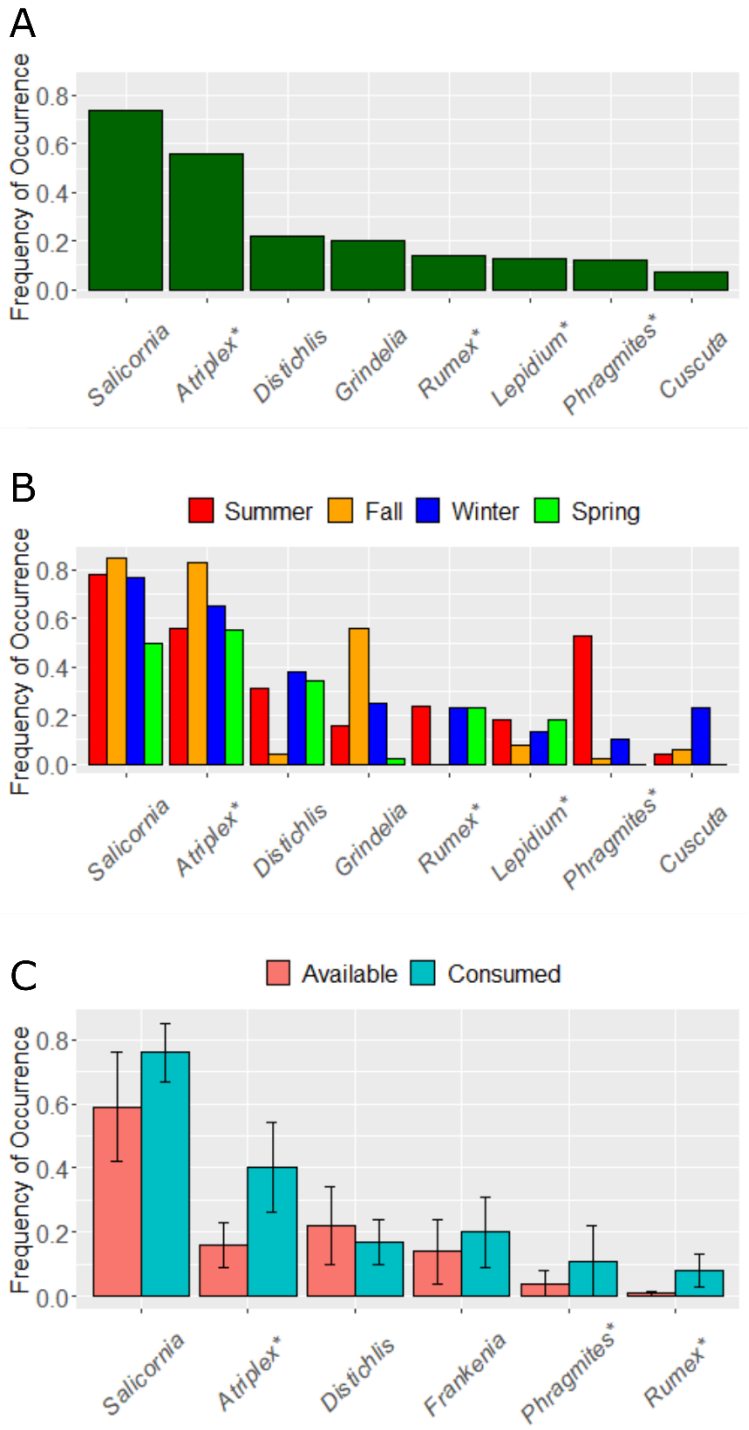

Figure 4-3. Frequency of occurrence (FO) of plant genera consumed by salt marsh harvest mice (*Reithrodontomys raviventris*). (A) FO of the eight most frequently-consumed plants pooled across all sites and seasons (n = 245 individuals). Over 40 additional genera were consumed at lower frequencies. (B) Seasonal FO of the top eight plants consumed at Goodyear Slough. (C) Mean and SE of FO in diets compared to FO in vegetation quadrats sampled at five summer sampling units. In all panels, non-native plants are denoted with an asterisk (*).

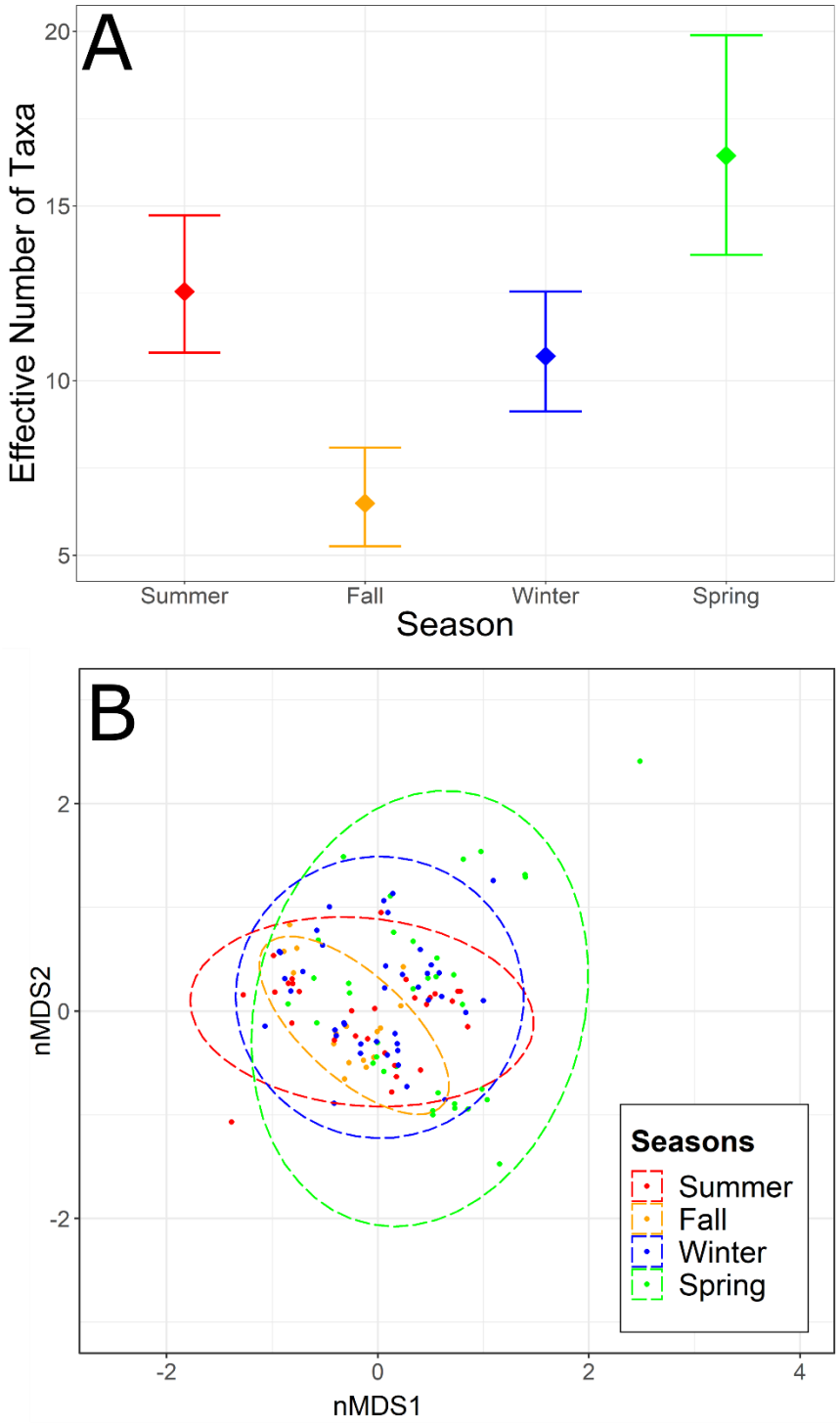

Figure 4-4. (A) Dietary niche breadth (effective number of taxa; ^{1}D; Hill 1973) of salt marsh harvest mouse (*Reithrodontomys raviventris*) diet in four seasons over two years at Goodyear Slough. . (B) Non-metric multidimensional scaling ordination of seasonal salt marsh harvest mouse (*Reithrodontomys raviventris*) diet at Goodyear Slough. Dots represent individual animals, and dashed lines represent 95% confidence ellipses.

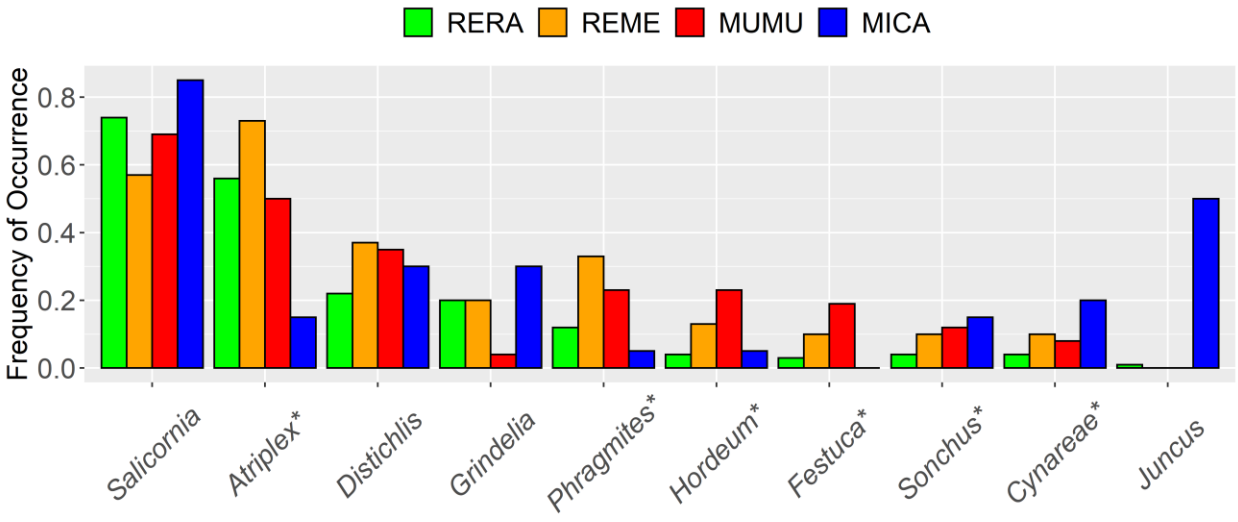

Figure 4-5. Frequency of occurrence of ten important diet items in salt marsh harvest mouse (*Reithrodontomys raviventris*; RERA), western harvest mouse (*R. megalotis*; REME), house mouse (*Mus musculus*; MUMU), and California vole (*Microtus californicus*; MICA) diets. For data across all dietary items, see Appendix A4-9. In all panels, non-native plants are denoted with an asterisk (*).

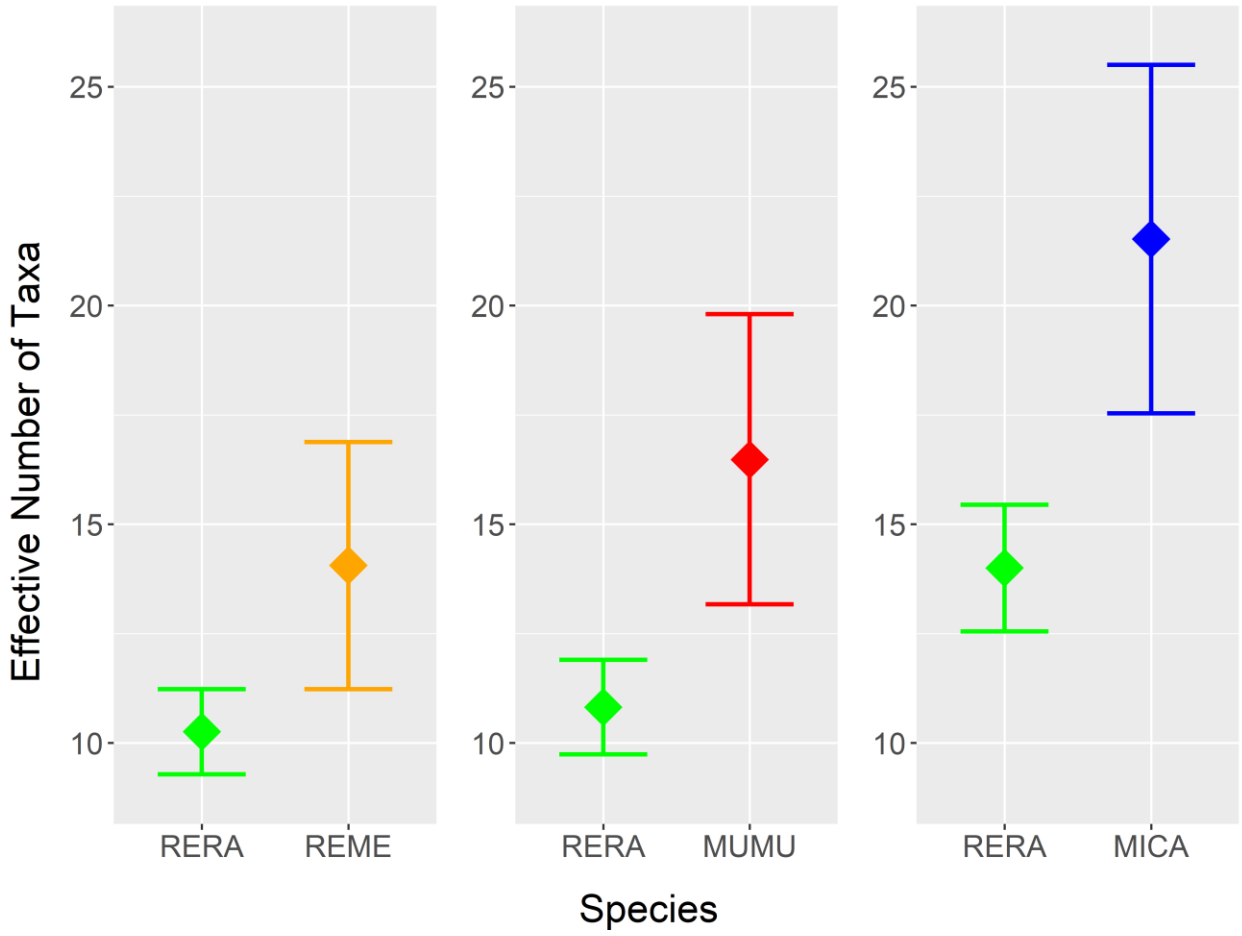

Figure 4-6. Pairwise comparisons of dietary niche breadth (effective number of taxa; 1D; Hill 1973) of salt marsh harvest mouse (*Reithrodontomys raviventris*; RERA), western harvest mouse (*R. megalotis*; REME), house mouse (*Mus musculus*; MUMU), and California vole (*Microtus californicus*; MICA). Comparisons were conducted pairwise because sample sizes of non-RERA were inconsistent throughout space and time, therefore only allowing valid comparisons at a different suite of sites/seasons for each species pair. RERA/REME comparisons were conducted at Goodyear Slough (GYS) in summer, fall, and winter; RERA/MUMU comparisons were conducted at GYS (summer and fall), Hill Slough Wildlife Area Ponds 1&2 (summer) and Eden Landing Ecological Reserve (EDEN; spring); and RERA/MICA comparisons were conducted at GYS (summer and spring) and EDEN (spring).

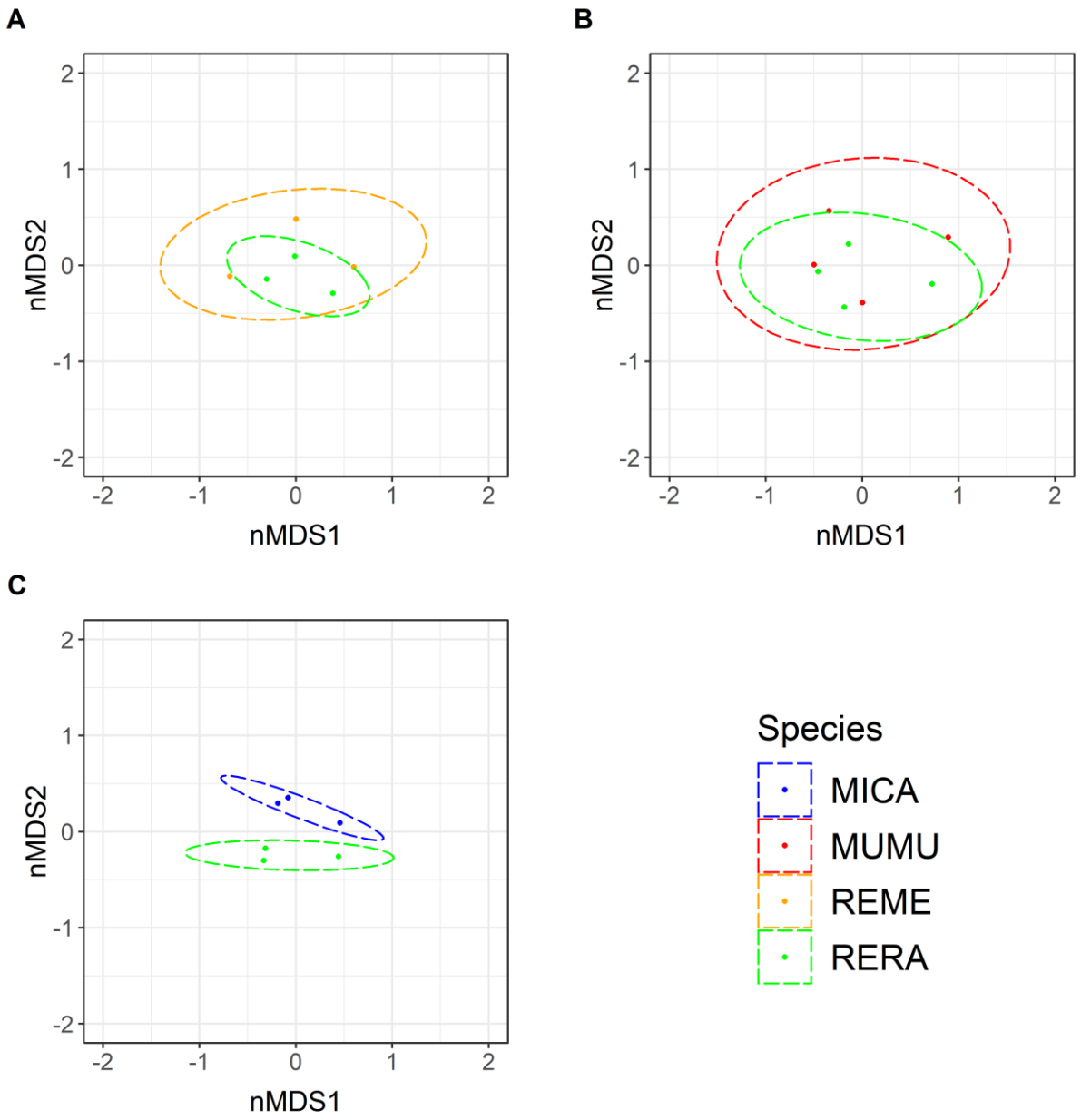

Figure 4-7. Population-level dietary overlap as represented in ordination (nMDS) plots of (A) salt marsh harvest mouse (*Reithrodontomys raviventris*; RERA) compared with western harvest mouse (*R. megalotis*; REME), (B) RERA compared with house mouse (*Mus musculus*; MUMU), and (C) RERA compared with California voles (*Microtus californicus*; MICA). Dots represent population-level diet using frequency of occurrence data. Ellipses show 95% confidence intervals.

TABLES

Table 4-1. Number of individuals of four rodent species captured during each season at each site: Salt marsh harvest mouse (*Reithrodontomys raviventris*; RERA), western harvest mouse (*R. megalotis*; REME), house mouse (*Mus musculus*; MUMU), and California vole (*Microtus californicus*; MICA). Goodyear Slough (GYS) was surveyed during all four seasons (Su = Summer, Fa = Fall, Wi = Winter, and Sp = Spring), whereas Hill Slough 1&2 (HS12), Hill Slough 9 (HS9), Crescent Unit (CRES), and Eden Landing (EDEN) were sampled only in summer or late spring. Due to varying sample sizes by site and season, interspecific comparisons of diet were conducted on a pairwise basis between RERA and one other species independently, and only at sites where diet data were available for ≥4 individuals of both species.

	Sampling Unit								
Species	GYS (Su)	GYS (Fa)	GYS (Wi)	GYS (Sp)	HS12	HS9	CRES	EDEN	Total
RERA	45[abc]	52[ab]	48[a]	44[c]	8[b]	14	13	21[bc]	245
REME	13[a]	7[a]	7[a]	2	0	1	0	0	30
MUMU	8[b]	4[b]	1	1	7[b]	0	1	4[b]	26
MICA	4[c]	0	0	10[c]	0	0	1	5[c]	20

[a] indicates sites with sufficient sample size to be included in comparisons of RERA and REME diet

[b] denotes a site included in RERA / MUMU comparisons

[c] denotes a site included in RERA / MICA comparisons

Table 4-2: Manly's Selection Index (W_i) for plant genera in salt marsh harvest mouse (*Reithrodontomys raviventris*) diets in (A) four seasons at Goodyear Slough, and (B) summer at 5 locations/sites: Goodyear Slough (GYS), Hill Slough 1&2 (HS12), Hill Slough 9 (HS9), Crescent Unit (CRES), and Eden Landing (EDEN). Tables include all diet items with significant selection (*; $W_i \pm 95\%$ CI > 1) or avoidance (†; $W_i \pm 95\%$ CI < 1) in at least one season/site or when all sites/seasons were pooled. Dashes (--) indicate a diet item that was absent from the site and therefore does not have a selection coefficient.

A

Seasonal	Manly's Selection Index (*Wi*)				
Genus	Summer	Fall	Winter	Spring	All Seasons
Atriplex	1.45 (0.91, 1.99)	2.43 (1.90, 2.97)*	2.36 (1.61, 3.10)*	3.36 (1.90, 4.82)*	2.30 (1.90, 2.70)*
Distichlis	0.58 (0.25, 0.90)†	0.06 (-0.07, 0.20)†	0.73 (0.32, 1.12)	0.64 (0.25, 1.03)	0.49 (0.31, 0.67)†
Grindelia	0.88 (-0.10, 1.86)	6.57 (4.34, 8.80)*	1.56 (0.37, 2.75)	0.13 (-0.28, 0.53)†	1.84 (1.06, 2.61)*
Juncus	0.00 (0.00, 0.00)†	0.00 (0.00, 0.00)†	0.07 (-0.15, 0.29)†	0.07 (-0.14, 0.30)†	0.04 (-0.05, 0.12)†
Phragmites	3.40 (1.79, 5.00)*	0.17 (-0.34, 0.68)†	0.68 (-0.21, 1.56)	0.00 (0.00, 0.00)†	1.12 (0.52, 1.72)
Rumex	8.95 (2.52, 15.38)*	0.00 (0.00, 0.00)†	6.85 (1.33, 12.38)*	7.70 (0.80, 14.60)	6.76 (3.44, 10.07)*
Salicornia	1.37 (1.08, 1.66)*	1.32 (1.04, 1.60)*	1.29 (1.04, 1.74)*	0.82 (0.46, 1.17)	1.27 (1.10, 1.45)*

B

Spatial	Manly's Selection Index (*Wi*)					
Genus	GYS	HS12	HS9	CRES	EDEN	All Sites
Atriplex	1.45 (0.91, 1.99)	2.96 (1.62, 4.31)*	4.45 (-8.10, 16.99)	3.53 (-0.06, 7.12)	1.26 (-2.17, 4.69)	2.14 (1.35, 2.93)*
Distichlis	0.58 (0.25, 0.90)†	0.82 (-0.75, 2.38)	0.72 (-0.11, 1.56)	--	--	0.72 (0.35, 1.10)
Frankenia	--	1.83 (0.29, 3.38)	4.45 (-8.10, 16.99)	--	1.13 (0.36, 1.90)	2.79 (1.30, 4.29)*
Phragmites	3.40 (1.79, 5.00)*	--	--	--	--	3.52 (1.81, 5.23)*
Rumex	8.95 (2.52, 15.38)*	0.00 (0.00, 0.00)†	--	12.35 (-9.19, 33.90)	--	8.61 (2.34, 14.87)*
Salicornia	1.37 (1.08, 1.66)*	2.56 (0.15, 4.97)	1.73 (0.35, 3.10)	0.79 (0.40, 1.17)	1.33 (0.74, 1.91)	1.79 (1.38, 2.20)*

Table 4-3. Measures of Jaccard Similarity (J_s; range 0-1) between seasonal diet of salt marsh harvest mice (*Reithrodontomys raviventris*) at Goodyear Slough.

	Jaccard's Similarity (J_s)				
Season	Summer	Fall	Winter	Spring	Mean
Summer	--	0.396	0.569	0.476	0.480
Fall		--	0.515	0.291	0.401
Winter			--	0.448	0.511
Spring				--	0.405

SUPPLEMENTAL MATERIAL

Appendix A

Table A4-1. Primer sequences used in this study. In complete primer sequences, brackets denote the sequence of the 5' overhang used for annealing to indexed adapters for Illumina sequencing. Note that these overhangs were complimentary to indexed adapters developed in-house and do not necessarily anneal to commercial indexed adapters. Nucleotide sequences following bracketed sequences represent the nucleotide sequences from referenced literature.

Amplicon Primer Name	Complete Primer Sequence	Reference
trnl_g	5-[TCTTTCCCTACACGACGCTCTTCCGATC]GGGCAATCCTGAGCCAA-3	Taberlet et al. 2007
trnl_h	5-[GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC]CCATTGAGTCTCTGCACCTATC-3	Taberlet et al. 2007
UniPlantF	5-[TCTTTCCCTACACGACGCTCTTCCGATC]TGTGAATTGCARRATYCMG-3	Moorhouse-Gann et al. 2018
ITS-p4	5-[GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC]CCGCTTAKTGATATGCTTAAA-3	Cheng et al. 2016

Table A4-2. *in silico* PCR penalty scores of primer pairs used in this study against sequences of select taxa downloaded from Genbank. Scores were calculated using the *evaluate_primer* tool in the R package 'PrimerMiner', with default settings. Sequences that produced failing scores (>120) are denoted by an asterisk (*). Failing scores did not appear systemic for any taxon. *Schoenoplectus* was not amplified by either of the two primer pairs despite strong *in silico* scores.

	ITS2				trnL		
Template	UniPlantF	ITS-p4	Score	Template	trnl_g	trnl_h	Score
Atriplex 1	0	0	0	*Atriplex* 1	0	24.8	24.8
Atriplex 2	0	0	0	*Atriplex* 2	0	24.8	24.8
Atriplex 3	0	0	0	*Atriplex* 3	0	24.8	24.8
Atriplex 4	0	0	0	*Bromus* 1	0	0	0
Atriplex 5	0	0	0	*Bromus* 2	0	0	0
Atriplex 6*	0	174.7	174.7	*Bromus* 3	0	0	0
Atriplex 7	0	0	0	*Cotula* 1	0	7.8	7.8
Bolboschoenus 1	0	73.8	73.8	*Cuscuta* 1	0	6.2	6.2
Bromus 1	0	73.8	73.8	*Cuscuta* 2	0	6.2	6.2
Bromus 2	0	73.8	73.8	*Cuscuta* 3*	0	345.8	345.8
Cotula 1	0	0	0	*Cuscuta* 4	0	6.2	6.2
Cotula 1	0	0	0	*Distichlis* 1	0	0	0
Cuscuta 1	0	0	0	*Distichlis* 2	0	0	0
Cuscuta 2	0	0	0	*Distichlis* 3	0	0	0
Cuscuta 3	0	0	0	*Festuca* 1	0	0	0
Distichlis 1	0	0	0	*Festuca* 2	0	0	0
Distichlis 2	0	0	0	*Grindelia* 1	0	7.8	7.8
Distichlis 3	0	0	0	*Juncus* 1	0	6.2	6.2
Distichlis 4	0	0	0	*Juncus* 2	0	6.2	6.2
Festuca 1	0	73.8	73.8	*Juncus* 3	0	6.2	6.2
Festuca 2	0	0	0	*Juncus* 4	0	6.2	6.2
Festuca 3	0	0	0	*Juncus* 5	0	6.2	6.2
Frankenia 1	0	0	0	*Lepidium* 1	0	0	0
Grindelia 1	0	0	0	*Lepidium* 2	0	0	0
Juncus 1	0	94.2	94.2	*Phragmites* 1	0	0	0
Juncus 2	0	94.2	94.2	*Polypogon* 1	0	0	0
Juncus 3	0	94.2	94.2	*Polypogon* 2	0	0	0
Lepidium 1	0	0	0	*Salicornia* 1	0	24.8	24.8
Lepidium 2	0	0	0	*Salicornia* 2	0	24.8	24.8
Lepidium 3	0	0	0	*Salicornia* 3	0	24.8	24.8
Phragmites 1	0	73.8	73.8	*Salicornia* 4	0	24.8	24.8
Polypogon 1	0	73.8	73.8	*Salicornia* 5	0	24.8	24.8

Polypogon 2	0	73.8	73.8	*Schoenoplectus* 1	0	7.8	7.8
Salicornia 1	0	0	0	*Schoenoplectus* 2	73.8	7.8	81.6
Salicornia 2	0	105.2	105.2	*Schoenoplectus* 3	0	7.8	7.8
Salicornia 3	0	0	0	*Triglochin* 1	0	0	0
Salicornia 4	0	0	0	*Triglochin* 2	0	0	0
Salicornia 5	0	105.2	105.2	*Typha* 1	0	7.8	7.8
Salicornia 6	0	0	0	*Typha* 2	0	7.8	7.8
Salsola 1*	0	270.9	270.9				
Salsola 2	0	0	0				
Schoenoplectus 1	0	73.8	73.8				
Schoenoplectus 2*	0	134.8	134.8				
Schoenoplectus 3	0	73.8	73.8				
Schoenoplectus 4	0	0	0				
Triglochin 1	0	0	0				
Triglochin 2	0	0	0				
Typha 1	0	0	0				

Table A4-3. Positive controls used during metabarcoding library preparation and sequencing. Four sequencing lanes were used in this study, one per marker per year. In each lane, I used ten single-species controls, with the expectation of recovering 100% of reads from that species, and ten "50-50" controls with two plant species, with the expectation of recovering 50% reads from each species, assuming no amplification bias. Percentages of target species reads shown are post-bioinformatic filtering. Species included in controls include *Grindelia stricta, Salicornia pacifica, Polypogon monspeliensis, Triglochin maritima, Schoenoplectus americanus, Juncus balticus, Lepidium latifolium, Bolboschoenus maritima, Achillea millefolium, Rosa californica, Distichlis spicata,* and *Atriplex prostrata*. Although *in silico* PCR scores suggested both primer sets would amplify *Schoenoplectus*, neither produced any sequences that could be identified as *Schoenoplectus* from the positive control. One *Polypogon* positive control produced sequences assigned to *Bromus* in the ITS2 marker, but these two species did not co-occur in any diet sample, so I believe this misassignment did not affect dietary inference.

2018-2019 lanes	% target species reads		2019-2020 lanes	% target species reads	
Plant Genus	trnL	ITS2	Plant Genus	trnL	ITS2
Single-species controls					
Grindelia	100	100	*Salicornia*	100	100
Salicornia	100	100	*Atriplex*	100	100
Polypogon	100	100	*Distichlis*	100	100
Triglochin	100	100	*Grindelia*	100	100
Schoenoplectus	0	0	*Schoenoplectus*	0	0
Juncus	100	0	*Lepidium*	100	100
Lepidium	100	100	*Bolboschoenus*	100	0
Bolboschoenus	100	100	*Juncus*	100	0
Achillea	100	100	*Polypogon*	100	64
Rosa	100	100	*Frankenia*	100	100
50-50 controls					
Grindelia/Salicornia	53/47	51/49	*Salicornia/Atriplex*	60/40	50/50
Polypogon/Triglochin	88/12	23/77	*Distichlis/Grindelia*	49/51	30/70
Juncus/Schoenoplectus	100/0	0/0	*Schoenoplectus/Lepidium*	0/100	0/100
Bolboschoenus/Lepidium	2/98	17/83	*Bolboschoenus/Juncus*	2/98	0/0
Achillea/Rosa	17/83	64/36	*Polypogon/Frankenia*	67/33	3/97
Grindelia/Polypogon	60/40	94/6	*Salicornia/Lepidium*	32/68	41/59
Salicornia/Triglochin	93/7	50/50	*Atriplex/Bolboschoenus*	93/7	98/2
Schoenoplectus/Lepidium	0/100	0/100	*Distichlis/Juncus*	35/65	100/0
Juncus/Achillea	72/28	0/100	*Grindelia/Polypogon*	59/41	95/5
Bolboschoenus/Rosa	3/97	7/93	*Schoenoplectus/Frankenia*	0/100	0/100

Table A4-4. Correlation coefficient (*r*) of Frequency of Occurrence (FO) data and Relative Read Abundance (RRA) data within each marker data set for salt marsh harvest mice (*Reithrodontomys raviventris*; RERA), western harvest mice (*R. megalotis*; REME), house mice (*Mus musculus*; MUMU) and California voles (*Microtus californicus*; MICA). Correlation strength scaled with sample size and reached high levels even in the rodent species with the smallest sample size ($n = 20$) in this study.

		r of FO and RRA	
Species	n	trnL	ITS2
RERA	245	0.978	0.991
REME	30	0.944	0.986
MUMU	26	0.934	0.960
MICA	20	0.888	0.937

Table A4-5. Frequency of Occurrence (FO) of diet items in salt marsh harvest mouse (*Reithrodontomys raviventris*) diet (*n* = 245) pooled across all sites and seasons. * = non-native taxa.

Taxon	FO	Taxon	FO
Salicornia	0.743	*Baccharis*	0.012
*Atriplex**	0.563	*Cordylanthus*	0.012
Distichlis	0.224	*Foeniculum**	0.012
Grindelia	0.200	*Lactuca**	0.012
*Rumex**	0.139	*Salsola**	0.012
*Lepidium**	0.127	*Apium**	0.008
*Phragmites**	0.122	*Carduus**	0.008
Polygonaceae	0.073	*Chenopodium**	0.008
Cuscuta	0.065	*Conium**	0.008
*Cotula**	0.057	*Elymus**	0.008
Frankenia	0.057	*Glaux*	0.008
*Hordeum**	0.045	*Juncus*	0.008
Baccharis/Euthamia	0.041	*Potentilla*	0.008
Cynareae*	0.041	*Sambucus*	0.008
*Sonchus**	0.041	*Bolboschoenus*	0.004
*Lotus**	0.033	*Cressa*	0.004
Parapholis	0.033	*Euthamia*	0.004
Convolvulaceae	0.029	*Geranium**	0.004
*Festuca**	0.029	*Hainardia**	0.004
Jaumea	0.029	*Lathyrus*	0.004
Spergularia	0.029	*Matricaria*	0.004
Achillea	0.024	*Mesembryanthemum*	0.004
Typha	0.024	*Polygonum*	0.004
*Echinochloa**	0.020	*Raphanus**	0.004
*Polypogon**	0.020	Rosaceae	0.004
Triglochin	0.020	*Trifolium*	0.004
Solanum	0.016		

Table A4-6. Frequency of Occurrence (FO) of plant forms in salt marsh harvest mouse (*Reithrodontomys raviventris*) diet pooled across all sites and seasons (*n* = 245). Plant forms and habitats were determined from CalFlora.org.

Season	Plant Form					
	Forb/Subshrub (Wetland)	Grass (Wetland)	Forb/Subshrub (Upland)	Grass (Upland)	Vine	Shrub
Summer	0.933	0.689	0.200	0.067	0.089	0.000
Fall	1.000	0.096	0.038	0.000	0.077	0.000
Winter	0.958	0.521	0.063	0.000	0.271	0.000
Spring	0.932	0.341	0.295	0.318	0.045	0.045
Overall	0.958	0.402	0.143	0.090	0.122	0.011

Table A4-7. Seasonal Frequency of Occurrence (FO) of plant taxa in salt marsh harvest mouse (*Reithrodontomys raviventris*) diet in four seasons at Goodyear Slough.

Diet Taxon	Summer	Fall	Winter	Spring	Overall
Salicornia	0.778	0.846	0.771	0.500	0.730
Atriplex	0.556	0.827	0.646	0.545	0.651
Distichlis	0.311	0.038	0.375	0.341	0.259
Grindelia	0.156	0.558	0.250	0.023	0.259
Rumex	0.244	0.000	0.229	0.227	0.169
Phragmites	0.533	0.019	0.104	0.000	0.159
Lepidium	0.178	0.077	0.125	0.182	0.138
Cuscuta	0.044	0.058	0.229	0.000	0.085
Polygonaceae	0.244	0.038	0.063	0.000	0.085
Baccharis/Euthamia	0.044	0.019	0.104	0.045	0.053
Hordeum	0.044	0.000	0.000	0.159	0.048
Cynareae	0.044	0.000	0.000	0.136	0.042
Convolvulaceae	0.044	0.019	0.042	0.045	0.037
Cotula	0.089	0.000	0.000	0.068	0.037
Sonchus	0.089	0.000	0.000	0.068	0.037
Achillea	0.089	0.019	0.021	0.000	0.032
Jaumea	0.000	0.038	0.063	0.023	0.032
Typha	0.022	0.038	0.021	0.045	0.032
Festuca	0.000	0.000	0.000	0.114	0.026
Echinochloa	0.000	0.019	0.063	0.000	0.021
Baccharis	0.022	0.038	0.000	0.000	0.016
Foeniculum	0.067	0.000	0.000	0.000	0.016
Lactuca	0.022	0.000	0.042	0.000	0.016
Parapholis	0.000	0.000	0.000	0.068	0.016
Solanum	0.022	0.019	0.000	0.023	0.016
Carduus	0.000	0.000	0.000	0.045	0.011
Conium	0.000	0.000	0.000	0.045	0.011
Juncus	0.000	0.000	0.021	0.023	0.011
Lotus	0.000	0.000	0.000	0.045	0.011
Potentilla	0.022	0.000	0.000	0.023	0.011
Salsola	0.000	0.000	0.021	0.023	0.011
Sambucus	0.000	0.000	0.000	0.045	0.011
Triglochin	0.000	0.000	0.000	0.045	0.011
Apium	0.000	0.000	0.021	0.000	0.005
Bolboschoenus	0.000	0.019	0.000	0.000	0.005
Chenopodium	0.000	0.000	0.000	0.023	0.005
Elymus	0.000	0.000	0.000	0.023	0.005
Euthamia	0.000	0.019	0.000	0.000	0.005
Hainardia	0.000	0.000	0.000	0.023	0.005
Lathyrus	0.000	0.000	0.000	0.023	0.005

Matricaria	0.000	0.000	0.021	0.000	0.005
Polypogon	0.022	0.000	0.000	0.000	0.005
Raphanus	0.000	0.000	0.000	0.023	0.005
Rosaceae	0.000	0.019	0.000	0.000	0.005
Trifolium	0.000	0.019	0.000	0.000	0.005

Table A4-8. Spatial variation in Frequency of Occurrence (FO) of taxa in salt marsh harvest mouse (*Reithrodontomys raviventris*) diet at five sampling units surveyed in late spring or summer: Crescent Unit (CRES), Eden Landing (EDEN), Goodyear Slough (GYS; summer), Hill Slough 1&2 (HS12), and Hill Slough 9 (HS9).

Diet Taxon	Site					
	CRES	EDEN	GYS (Su)	HS12	HS9	Mean
Salicornia	0.923	0.952	0.778	0.625	0.500	0.756
Atriplex	0.308	0.048	0.556	0.875	0.214	0.400
Frankenia	0.000	0.429	0.000	0.500	0.071	0.200
Distichlis	0.000	0.000	0.311	0.250	0.286	0.169
Lepidium	0.000	0.000	0.178	0.500	0.071	0.150
Cotula	0.462	0.000	0.089	0.125	0.000	0.135
Phragmites	0.000	0.000	0.533	0.000	0.000	0.107
Polygonaceae	0.000	0.000	0.244	0.125	0.071	0.088
Lotus	0.000	0.000	0.000	0.000	0.429	0.086
Rumex	0.154	0.000	0.244	0.000	0.000	0.080
Sonchus	0.077	0.048	0.089	0.125	0.000	0.068
Spergularia	0.000	0.333	0.000	0.000	0.000	0.067
Polypogon	0.308	0.000	0.022	0.000	0.000	0.066
Parapholis	0.000	0.190	0.000	0.000	0.071	0.052
Triglochin	0.000	0.000	0.000	0.000	0.214	0.043
Cordylanthus	0.000	0.000	0.000	0.000	0.214	0.043
Hordeum	0.154	0.000	0.044	0.000	0.000	0.040
Grindelia	0.000	0.000	0.156	0.000	0.000	0.031
Festuca	0.154	0.000	0.000	0.000	0.000	0.031
Solanum	0.000	0.000	0.022	0.125	0.000	0.029
Glaux	0.000	0.000	0.000	0.000	0.143	0.029
Cynareae	0.000	0.095	0.044	0.000	0.000	0.028
Chenopodium	0.000	0.000	0.000	0.125	0.000	0.025
Salsola	0.000	0.000	0.000	0.125	0.000	0.025
Achillea	0.000	0.000	0.089	0.000	0.000	0.018
Elymus	0.077	0.000	0.000	0.000	0.000	0.015
Jaumea	0.000	0.000	0.000	0.000	0.071	0.014
Apium	0.000	0.000	0.000	0.000	0.071	0.014
Polygonum	0.000	0.000	0.000	0.000	0.071	0.014
Foeniculum	0.000	0.000	0.067	0.000	0.000	0.013
Cressa	0.000	0.048	0.000	0.000	0.000	0.010
Echinochloa	0.000	0.048	0.000	0.000	0.000	0.010
Geranium	0.000	0.048	0.000	0.000	0.000	0.010
Mesembryanthemum	0.000	0.048	0.000	0.000	0.000	0.010
Baccharis/Euthamia	0.000	0.000	0.044	0.000	0.000	0.009
Convolvulaceae	0.000	0.000	0.044	0.000	0.000	0.009

Cuscuta	0.000	0.000	0.044	0.000	0.000	0.009
Typha	0.000	0.000	0.022	0.000	0.000	0.004
Potentilla	0.000	0.000	0.022	0.000	0.000	0.004
Baccharis	0.000	0.000	0.022	0.000	0.000	0.004
Lactuca	0.000	0.000	0.022	0.000	0.000	0.004

Table A4-9. Frequency of diet items for salt marsh harvest mice (*Reithrodontomys raviventris*; RERA), western harvest mice (*R. megalotis*; REME), house mice (*Mus musculus*; MUMU), and California voles (*Microtus californicus*; MICA). Data were pooled across all sites and seasons. Sample sizes were significantly weighted toward Goodyear Slough (n = 245 out of 327), thus pooled frequencies are biased against some taxa that were absent from Goodyear Slough but prominent elsewhere (e.g., *Frankenia*).

Diet Item	RERA	REME	MUMU	MICA
Salicornia	0.743	0.567	0.692	0.850
Atriplex	0.563	0.733	0.500	0.150
Distichlis	0.224	0.367	0.346	0.300
Grindelia	0.200	0.200	0.038	0.300
Phragmites	0.122	0.333	0.231	0.050
Juncus	0.008	0	0	0.500
Hordeum	0.045	0.133	0.231	0.050
Baccharis/Euthamia	0.041	0.100	0	0.300
Frankenia	0.057	0	0.115	0.250
Cynareae	0.041	0.100	0.077	0.200
Sonchus	0.041	0.100	0.115	0.150
Rumex	0.139	0.033	0.077	0.100
Convolvulaceae	0.029	0.033	0.077	0.200
Festuca	0.029	0.100	0.192	0
Lepidium	0.127	0.067	0.077	0.050
Polygonaceae	0.073	0.167	0.038	0
Cotula	0.057	0	0.115	0.100
Cuscuta	0.065	0.100	0.038	0.050
Potentilla	0.008	0	0	0.200
Achillea	0.024	0.033	0	0.150
Spergularia	0.029	0	0.077	0.100
Jaumea	0.029	0.067	0	0.100
Euthamia	0.004	0	0	0.150
Asparagus	0	0	0	0.150
Sinapis	0	0	0.038	0.100
Calystegia	0	0.033	0	0.100
Bromus	0	0	0.077	0.050
Raphanus	0.004	0.067	0.038	0
Sambucus	0.008	0	0	0.100
Typha	0.024	0	0.077	0
Baccharis	0.012	0.033	0	0.050
Apium	0.008	0.033	0	0.050
Brassica	0	0	0.038	0.050
Cressa	0.004	0.033	0	0.050
Foeniculum	0.012	0.067	0	0
Parapholis	0.033	0	0.038	0

Polygonum	0.004	0.067	0	0
Lactuca	0.012	0	0	0.050
Echinochloa	0.020	0	0.038	0
Polypogon	0.020	0	0.038	0
Salsola	0.012	0	0.038	0
Solanum	0.016	0.033	0	0
Carduus	0.008	0	0.038	0
Chenopodium	0.008	0	0.038	0
Elymus	0.008	0	0.038	0
Bolboschoenus	0.004	0	0.038	0
Mesembryanthemum	0.004	0	0.038	0
Glaux	0.008	0.033	0	0
Lotus	0.033	0	0	0
Triglochin	0.020	0	0	0
Cordylanthus	0.012	0	0	0
Conium	0.008	0	0	0
Geranium	0.004	0	0	0
Hainardia	0.004	0	0	0
Lathyrus	0.004	0	0	0
Matricaria	0.004	0	0	0
Rosaceae	0.004	0	0	0
Trifolium	0.004	0	0	0

Appendix B: Performance of Metabarcoding Markers

I used two loci to identify plant items in the diet of salt marsh harvest mice (*Reithrodontomys raviventris*; RERA) and co-occurring rodents. Although dietary metabarcoding studies frequently are performed with a single marker (e.g., trnL: Kartzinel et al. 2015; or ITS2: Iwanowicz et al. 2016), there are clear advantages to using multiple markers. As one example, use of multiple markers targeting a single taxonomic group – plants, in this case – greatly improved the ability to detect the complete taxonomic breadth of plants within the diet of Idaho ground squirrel (*Urocitellus brunneus*; Goldberg et al. 2020).

I assessed the performance of multiple markers targeting the same taxonomic group of diet items using a four-step process. First, I tallied the number of unique amplicon sequence variants (ASVs) detected by each marker (after bioinformatic filtering; thus, "detection" required an ASV to comprise > 0.01% of the reads in a sequencing lane). Second, I calculated the number and proportion of ASVs that were successfully assigned to a plant taxon (as opposed to unassigned reads that I suppose were fungal or bacterial). Third, I determined the number of unique taxa identified by each marker. Finally, I counted the number of samples that were filtered out from each marker's data set during bioinformatic processing.

I detected 1,226 ASVs in the ITS2 data set, of which 427 (35%) were assigned to a plant taxon (Table A4-1). I detected 481 ASVs in the trnL data set, of which 211 (44%) were assigned to a plant taxon. Data filtering led to the removal of more ITS2 samples (n = 72) than trnL samples (n = 9). The majority of discarded samples had too few sequence reads assigned to plant taxa (60% of discards in ITS2; 89% in trnL). I examined the trnL data of the samples that were discarded from the ITS2 dataset to determine whether the ITS2 failures may have been due to taxonomy (e.g., they only contained taxa amplified by trnL primers). The trnL data of these

samples regularly contained plant taxa that were commonly detected in successful ITS2 samples, suggesting that taxonomy was not responsible for ITS2 failures. Overall, only six of 327 (1.7%) samples failed to yield dietary information from both markers. Overall, I detected 62 genera; 13 by ITS2 alone, 12 by trnL alone, and 37 by both markers (Table A4-2). FO and RRA were strongly correlated within each single-marker data set but were consistently slightly higher for ITS2 (Table A4-4).

My study echoed the findings of Goldberg et al. (2020) that multiple markers provide complementary (i.e., not redundant) identification of dietary items, thereby improving the taxonomic breadth of detection in dietary metabarcoding. Furthermore, I found that a substantial and methodical system of positive and negative controls helped to understand the limitations of each marker and design marker-specific bioinformatic filters.

In this study, trnL provided true dietary information for nearly all samples while approximately 20% of ITS2 samples did not pass bioinformatic filtering procedures. One key advantage that may have contributed to the relative success of trnL in this study is its smaller fragment size (23-92 bp; versus 298-333 bp ITS2), which increased the chances of amplification in severely degraded DNA, such as DNA that has been digested. Furthermore, trnL had a higher proportion of ASVs assigned to plant taxa. Degenerate ITS2 primers, which were selected to increase sensitivity to a broader range of plants, may have been disadvantageous due to the swamping of diet DNA by that of non-target taxa. Nonetheless, my results aligned with previous studies suggesting ITS2 provides finer taxonomic resolution and fewer ambiguous identifications at the genus level (CPBOL Group 2011). Fine taxonomic resolution was important in this study with respect to grasses, which were common in the trapping bait. Several ASVs in the trnL dataset could only be identified as grasses (Poaceae) and were therefore discarded as potentially

introduced to RERA diet through bait used in the trapping process, whereas ITS2 had no ambiguous ASVs at the genus level and therefore easily distinguished grass taxa present in bait from grass taxa only present in the wild. For dietary studies that require the use of bait, the fine taxonomic resolution of ITS2 may be a significant advantage to distinguish such taxa from closely related natural components of the target species' diet. Additionally, although I did not use RRA data due to my multi-marker approach, ITS2 had slightly higher correspondence between RRA and FO for all four species' diets.

Positive and negative controls provided important guidance in determining appropriate bioinformatic filters in this study. Bioinformatic decisions can alter the outcome of metabarcoding studies and are often determined ambiguously. Recent work has highlighted the importance of positive controls to objectively guide computational decisions in metabarcoding (O'Rourke et al. 2020). I used data from my controls to understand the sensitivity of my markers to particular taxa, estimate error rates, identify potential primer biases, and set a threshold number of reads for a sample to be retained/discarded. One interesting finding from my controls was that several taxa that received passing scores in *in silico* PCR tests were unable to be amplified by one or both of my primers. My results underscore the importance of positive controls to evaluate primer sensitivity and sources of bias in dietary metabarcoding data.

Appendix B References

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Table B4-1. Numbers of ASVs, taxa, and samples filtered out from each marker data set. Numbers of ASVs and numbers of plant ASVs are presented for each sequencing lane separately.

	trnL	ITS2
Number of Reads	11.5M (2018-19) / 10.7M (2019-20)	10.4M (2018-19) / 8.6M (2019-20)
Number of Plant Reads	10.5M (91.1%) / 10.6M (99.1%)	9.2M (89.6%) / 8.2M (95.1%)
Number of ASVs	202 (2018-19) / 179 (2019-20)	832 (2018-19) / 394 (2019-20)
Number of Plant ASVs	94 (47%) / 117 (65%)	237 (28%) / 190 (48%)
Taxa Detected	54	50
Genera Detected	49	50
Samples Filtered Out	9 (3%)	72 (22%)

Table B4-2. Genera detected by trnL and ITS2 in diets of rodents in this study.

Genus	trnL	ITS2
Achillea	X	X
Apium	X	X
Asparagus	X	-
Atriplex	X	X
Baccharis	-	X
Bolboschoenus	X	X
Brassica	X	X
Bromus	X	X
Calystegia	-	X
Carduus	-	X
Chenopodium	X	-
Conium	-	X
Cordylanthus	X	-
Cotula	X	X
Cressa	X	-
Cuscuta	X	X
Distichlis	X	X
Echinochloa	X	X
Elymus	X	X
Epilobium	X	X
Erodium	X	-
Euthamia	-	X
Festuca	X	X
Foeniculum	X	X
Frankenia	X	X
Geranium	X	X
Glaux	X	X
Grindelia	X	X
Hainardia	-	X
Hordeum	X	X
Jaumea	X	X
Juncus	X	-
Lactuca	X	X
Lathyrus	X	X
Lepidium	X	X
Lotus	X	X
Lythrum	X	X
Matricaria	-	X
Melilotus	X	-
Mesembryanthemum	X	-
Parapholis	X	X
Persicaria	-	X

Phragmites	X	X
Polygonum	X	-
Polypogon	X	X
Potentilla	X	X
Raphanus	X	X
Rosa	-	X
Rubus	-	X
Rumex	X	-
Salicornia	X	X
Salsola	X	X
Sambucus	-	X
Sinapis	X	X
Solanum	X	X
Sonchus	X	X
Sorghum	X	-
Spergularia	X	X
Symphyotrichum	-	X
Trifolium	X	X
Triglochin	-	X
Typha	X	-
Family/Multi-Genus		
Baccharis/Euthamia	X	-
Convolvulaceae	X	-
Cynareae	X	-
Polygonaceae	X	-
Rosaceae	X	-