Technical Report in Fulfillment of Agreement No. A07-0008 between AECOM Technical Services Inc. and the Regents of the University of California, and Agreement No. 4600011293 between Department of Water Resources and the Regents of the University of California

Salt Marsh Harvest Mouse Landscape Genetics and Connectivity within the Suisun Bay Area Recovery Unit

A salt marsh harvest mouse from Grizzly Island Wildlife Area, Goodyear Slough, Suisun. Photo: Katie Smith.

Technical Report in Fulfillment of Agreement No. A07-0008 between AECOM Technical Services Inc. and the Regents of the University of California, and Agreement No. 4600011293 between Department of Water Resources and the Regents of the University of California

Salt Marsh Harvest Mouse Landscape Genetics and Connectivity within the Suisun Bay Area Recovery Unit

Prepared by:

Mark J. Statham
University of California, Davis
Veterinary Genetics Lab

Benjamin N. Sacks
University of California, Davis
Veterinary Genetics Lab

Other Project Partners:

Laureen Barthman-Thompson
Suisun Marsh Unit
California Department of Fish and Wildlife

Sarah Estrella
Suisun Marsh Unit
California Department of Fish and Wildlife

Caitlin Roddy
Suisun Marsh Planning Section
California Department of Water Resources

Prepared for:

California Department of Water Resources
Division of Environmental Services, Suisun Marsh Branch
3500 Industrial Boulevard
West Sacramento, CA 95691

Contact:
Cliff Feldheim, Chief
Suisun Marsh Branch
(916) 379-9693
Cliff.Feldheim@water.ca.gov

June 2019

State of California

Gavin Newsom, Governor

California Natural Resources Agency

Wade Crowfoot, Secretary for Natural Resources

Department of Water Resources

Karla Nemeth, Director

Cindy Messer

Chief Deputy Director

Michelle Banonis, J.D.

Assistant Chief Deputy Director

Kasey Schimke
Assistant Director
Legislative Affairs Office

Erin Mellon
Assistant Director
Public Affairs Office

Spencer Kenner, J.D.
Assistant Director
Office of the Chief Counsel

Kristopher Tjernell
Deputy Director
Integrated Watershed Management Program

Michael Sabbaghian
Deputy Director (Acting)
Security and Emergency Management Program

Katherine S. Kishaba
Deputy Director
Business Operations Management Program

Joel Ledesma, P.E.
Deputy Director
State Water Project

Taryn Ravazzini, J.D.
Deputy Director
Statewide Groundwater Management

Eric Koch, P.E.
Deputy Director
Flood Management and Dam Safety

David Whitsell
Chief Auditor
Internal Audit Office

Stephanie Varrelman
Chief
Office of Workforce Equality

Gary Lippner, P.E.
Deputy Director
Delta Conveyance Bay-Delta Office

Anecita Agustinez
Tribal Policy Advisor

Dean Messer
Chief
Division of Environmental Services

Prepared under the supervision of

Cliff Feldheim
Chief
Susuin Marsh Branch

This page intentionally left blank

TABLE OF CONTENTS

Appendix

Figures

Tables

List of Acronyms and Other Abbreviations

µL	microliter
°C	degrees Celsius
A	average number of alleles per locus
AECOM	AECOM Technical Services, Inc.
AICc	Akaike Information Criterion, corrected for small sample size
Ar	allelic richness
CDFW	California Department of Fish and Wildlife
CWA	California Waterfowl Association
D_A	a measure of genetic distance
EBRP	East Bay Regional Park
ER	Ecological Reserve
F_{ST}	fixation index, a measure of genetic distance
GIWA	Grizzly Island Wildlife Area
H_E	expected heterozygosity
H_O	observed heterozygosity
IBD	Isolation by Distance
K	number of genetic clusters
m	meter(s)
MLPE	maximum-likelihood population effects
MVSD	Mountain View Sanitary District
Ne	effective population size
PCR	polymerase chain reaction
Pr	rarefied number of private alleles
R_{max}	maximum resistance
SBARU	Suisun Bay Area Recovery Unit
SRCD	Suisun Resource Conservation District
SMHM	salt marsh harvest mouse
USFWS	U.S. Fish and Wildlife Service
VHA	Viable Habitat Area
WA	Wildlife Area
WHM	western harvest mouse

This page intentionally left blank

Acknowledgements

Laureen Barthman-Thompson and the field crew at the California Department of Fish and Wildlife and California Department of Water Resources were vital partners in this work. Susan Fresquez contributed to all aspects of field sampling and laboratory work. Luis Hernandez helped collect genetic samples at the earlier stages of this work. Lexi Mendoza helped with the screening of novel microsatellite loci and with genotyping. Liz Kierepka helped with geographic information system work and with the program Circuitscape, and Cody Aylward helped with landscape genetics model testing in R.

This page intentionally left blank

EXECUTIVE SUMMARY

The salt marsh harvest mouse (SMHM) is a federally listed and state listed endangered species endemic to the coastal marshes of the San Francisco Estuary of California. The largest tracts of wetland habitat for the species remaining within its historical range are found around Suisun Bay. This study involved trapping SMHM individuals and collecting 538 genetic samples from across the USFWS Suisun Bay Area Recovery Unit. The researchers investigated the genetic diversity and genetic relationship among SMHM from 26 trapping locations, which included multiple sites in each USFWS Recovery Plan marsh complex. A landscape genetic analysis was conducted to assess the role of landscape features (cover type and elevation) in the creation of population substructure for SMHM.

The main findings and achievements of this study are summarized below:

- This study included the first next-generation DNA sequencing of the SMHM. A small fraction of the data generated was used to develop novel microsatellite markers (genetic tools) for the study of the species.
- The Suisun Bay Area Recovery Unit supports multiple populations of SMHM. The most genetically distinct population in the recovery unit is present at Ryer Island. SMHM individuals along the Contra Costa County shoreline (at Point Edith and McNabney Marsh) were also identified as distinct. Mice from the northern marshes of Suisun Bay were distinct from those on Ryer Island and the Contra Costa County shoreline.
- Based on the landscape genetic analysis of SMHM from across Suisun Bay, the best explanation for the subdivision observed was that water and elevation constrain gene flow and mouse movement. This information can be used to help to locate other potentially distinct populations of SMHM. The information also highlights how isolated many other marshlands are, especially around central San Francisco Bay, which has implications for any relict populations of SMHM remaining in the area.
- SMHM located in the northern marshes of Suisun Bay constitute a single population. Geographic distance between locations was the best predictor of genetic distance. From a conservation management perspective, this area covers the majority of four USFWS Recovery Plan marsh complexes.
- On the Contra Costa County shoreline, the mice at McNabney Marsh were differentiated from those at neighboring Point Edith, which is likely due to genetic drift caused by geographic isolation and/or small population size.
- This study has established the existence of the SMHM at McNabney Marsh, Sherman Island, and McAvoy Harbor.
- Researchers also genetically verified the continued occurrence of SMHM at several rarely surveyed locations such as Ryer Island, and Point Edith.
- During trapping at Bay Point along the Contra Costa County shoreline, researchers for this study failed to catch a single SMHM, despite catching 19 western harvest mice. These results indicate that SMHM may not be present at Bay Point.

Products Funded by Agreement No. A07-0008

Included as attachments/appendices/tables

Appendix Data Set: Genetic species identification of 777 harvest mice collected throughout the Suisun Bay Area Recovery Unit.

Note: This data set will also be provided to the California Department of Fish and Wildlife (CDFW). As work has proceeded, much of the data has been provided to CDFW, which has used the information to aid in its conservation and monitoring work.

Genetic Tool: Novel microsatellite markers for genetic analyses of harvest mice (Table 1, shown in Section 6 of this report).

These markers provide tools for genetic analyses of salt marsh harvest mice, western harvest mice, and likely other related harvest mice (genus *Reithrodontomys*).

This report is intended to serve as the foundation for a peer-reviewed journal article on the same topic. When that paper is published, it will be submitted as an addendum to this report.

1 BACKGROUND AND JUSTIFICATION

1.1 INTRODUCTION

The salt marsh harvest mouse (SMHM; *Reithrodontomys raviventris*) is endemic to the coastal marshes of the San Francisco Estuary of California (Shellhammer 1982). The SMHM is separated into two morphologically and genetically distinct subspecies: the southern *Reithrodontomys raviventris raviventris* of the south San Francisco Bay area and the northern *R. r. halicoetes* of San Pablo and Suisun bays (Fisler 1965; Statham et al. 2016). Since the 1800s, >90% of the tidal marshes in the San Francisco Bay have been lost to filling and diking (Shellhammer et al. 1982; Williams and Faber 2001). Continued destruction and fragmentation of the remaining habitat led both the U.S. federal government and the State of California to list the species as endangered (USFWS 1970; 2013).

The U.S. Fish and Wildlife Service (USFWS) *Recovery Plan for Tidal Marsh Ecosystems of Northern and Central California* (USFWS 2013) states that to achieve the long-term recovery of the SMHM, well-distributed populations of the species must be maintained across its range. The range of genetic variation must be maintained to allow the species' future evolution and resilience to environmental change, and to reduce the risk of inbreeding depression. A full inventory is needed that encompasses the genetic variation of SMHM, as well as its population subdivision, levels of gene flow, estimates of inbreeding, and genetic effective population size (Recovery Plan Priority 4.3.1).

The diked and tidal marshes of Suisun Bay represent the largest remaining tracts of habitat for the SMHM within its historical range. These mice often occur at high densities; thus, taken together, the marshes likely hold the largest remaining populations of the species (Smith et al. 2018). From a USFWS recovery plan perspective, the marshes of Suisun Bay constitute the Suisun Bay Area Recovery Unit (SBARU). The SBARU is itself divided into five marsh complexes. Within each marsh complex, Viable Habitat Areas (VHAs) have been designated at specified locations. One of the criteria for down-listing or delisting SMHM is that the VHAs in marsh complexes must be connected to allow the mice to function as one large population over time.

1.2 OBJECTIVES OF THE STUDY

The primary objective of this study was to assess the distribution, population subdivision, genetic diversity, and levels of gene flow among SMHM within the SBARU. An additional objective was to assess the role played by habitat features and topography in creating population substructure within the SBARU. To address these objectives, researchers trapped SMHM widely across the SBARU; collected genetic samples, conducted a population genetic study; and conducted analyses to identify which landscape features were associated with population subdivision.

This study builds on previous research funded under USFWS Section 6 grants no. F12AP00298-0001-007A, 2011.

2 METHODS

2.1 FIELDWORK AND GENETIC SAMPLE COLLECTION

The researchers for this study collaborated closely with California Department of Fish and Wildlife (CDFW) partners to obtain harvest mouse genetic samples. Many of the genetic samples were obtained during ongoing trapping and monitoring efforts conducted by CDFW (Benicia, Blacklock, Bradmoor, Crescent Unit, Denverton, Gold Hills, Goodyear, Grey Goose, Grizzly Island, Hill Slough, Joice Island, Lower Joice Island, and Peytonia). Additional sites that were not part of ongoing monitoring activities were chosen to provide a diverse set of sampling locations spread across the SBARU (Figure 1). These sites included Bay Point, Collinsville, McAvoy Harbor, McNabney Marsh, Point Edith, Ryer Island, and Sherman Island.

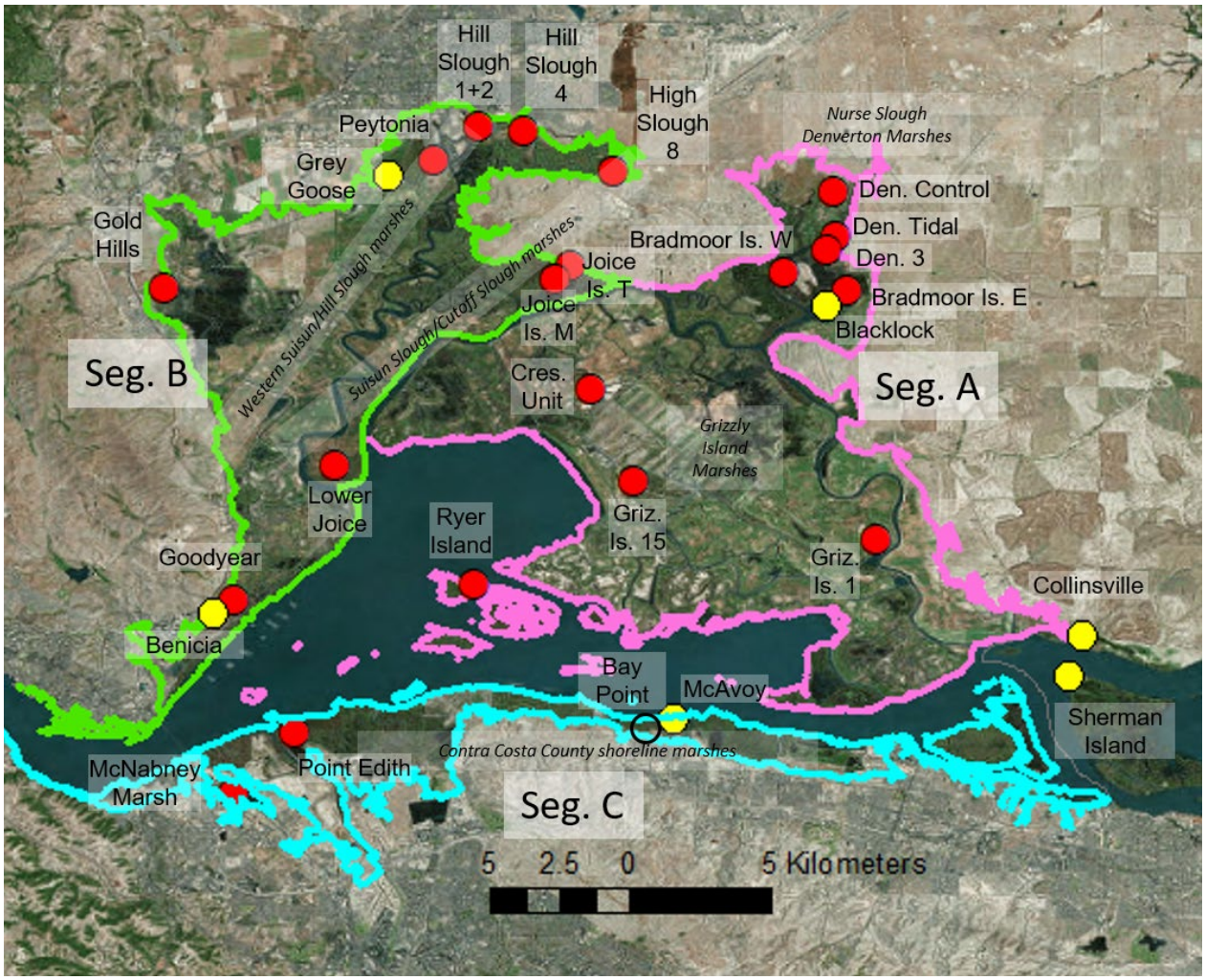
In total, harvest mice were trapped at 27 locations, including multiple sites within each of the five designated marsh complexes. The study used approximately 100 Sherman live traps (H. B. Sherman Traps, Tallahassee, Florida), spaced at intervals of approximately 10 meters (m), with the layout depending on the wetland shape. Traps were baited with a mixture of bird seed and ground walnut, and cotton batting was added for warmth. The traps were set the traps at dusk, and checked at dawn. Hair was plucked from the harvest mice as a source of DNA. Prior to sampling from an individual, the forceps were physically wiped down with a clean tissue, sterilized in a 2% bleach solution, rinsed with water to remove the bleach, and dried with a second tissue (Statham et al. 2016). The hair was stored in 95–100% ethanol until the DNA was extracted. Animal trapping, handling, and genetic sampling procedures were approved by the University of California, Davis, Institutional Animal Care and Use Committee (IACUC No. 19686) and authorized by the appropriate federal and state agencies.

2.2 LABORATORY METHODS

DNA was extracted from 777 samples using one of two methods. The first method involved digesting samples in a hair lysis buffer (Statham et al. 2016) and purifying the DNA with a modified phenol chloroform method (Sambrook and Russell 2001). The second method involved using a DNeasy Blood & Tissue kit (Qiagen Ltd) with modification of the digestion buffer to include 20 microliters (µL) 1M dithiothreitol (DTT), 300 µL buffer ATL, and 20 µL proteinase K. Each extraction set included a negative control to monitor for contamination.

2.3 GENETIC SPECIES IDENTIFICATION

The methods of Statham et al. (2016) were used to genetically identify harvest mice as either SMHM or western harvest mouse (WHM) (*Reithrodontomys megalotis*). A polymerase chain reaction (PCR) was used to amplify and portion of the cytochrome *b* gene. The DNA sequence of the PCR product was determined using an ABI 3730 capillary sequencer. SMHM and WHM form well-defined, reciprocally monophyletic clades with this gene (Statham et al. 2016). The associated sequence divergence between the two species, which exceeded 10%, was used to discriminate between SMHM and WHM with certainty.



Note: Red circles indicate trapping locations where 10 or more salt marsh harvest mice were caught; yellow circles indicate where three or fewer mice were caught. No salt marsh harvest mice were trapped at Bay Point (indicated with an open circle). Pink outlining indicates Suisun Bay Area Recovery Unit Segment A, green indicates Segment B, and blue indicates Segment C.

Source: Data compiled by University of California, Davis in 2019

Figure 1. Salt Marsh Harvest Mouse Trapping Locations within the Suisun Bay Area Recovery Unit

2.4 NEXT-GENERATION DNA SEQUENCING FOR MICROSATELLITE DISCOVERY AND DEVELOPMENT

Next-generation DNA sequencing was used to sequence a massive amount of DNA, which was used to develop new genetic tools for population genetic analyses of harvest mice. A shotgun sequencing approach was used to sequence genomic DNA from two SMHM and one WHM. Sequencing libraries were prepared using the NEBNext Ultra DNA Library Prep Kit for Illumina and NEBNext Multiplex Oligos for Illumina (both New England Biolabs) to individually barcode two SMHM and one WHM. The pooled library was sequenced using a paired end run of 250 base pairs on a MiSeq platform (Illumina) at the DNA Technologies and Expression Analysis Cores of the University of California, Davis, Genome Center.

The researchers used ngsShoRT (Chen et al. 2014) to remove Illumina adapter sequences, bases under a minimum quality score of 25, and low-quality reads where 50% of the bases were under 2, and to trim low-quality 3' prime

end bases. Fastqc (Andrews 2010) was used to assess the quality of sequences after filtering. The resulting paired-read sequences were screened for microsatellite repeats using PAL_FINDER_v0.02.04 (Castoe et al. 2012). A total of 55 potentially amplifiable loci were identified for further investigation including di-, tri-, or tetra-nucleotide repeats. The researchers followed the selection criteria of Hernandez et al. (2014):

(1) Repeat motif occurring at average frequency (to avoid transposable elements).

(2) Both forward and reverse primers occurring only once among reads.

(3) More than six repeats.

(4) Only perfect repeats.

(5) No tri- and tetra-nucleotide repeats composed of two or three tandem bases, respectively (e.g., TCC, TAAA).

These contigs were then used to redesign primers in Primer 3 (Rozen and Skaletsky 2000; Untergasser et al. 2012). Individual primer pairs were screened for amplification success in SMHM following the methods of Reponen et al. (2014). Then, based on the performance in single locus reactions, Multiplex Manager software (Holleley and Geerts 2009) was used to aid in determining the best locus combinations for two multiplexes.

2.5 MICROSATELLITE ANALYSES

Microsatellite analyses were used to analyze the genetic diversity and population substructure within SMHM. All SMHM samples were screened with 20 microsatellite loci. Loci Rrav 1, 6, 8, 10, 13, 18, 21, 29, 36 were from Reponen et al. (2014); R34 was from Vázquez-Domínguez and Espindola (2013) and the loci Rrav 40, 43, 44, 46, 47, 49, 51, 57, 61, 62 were developed in this study (Table 1, shown in Section 6 of this report). The loci were amplified in four multiplexes using the Qiagen Multiplex PCR Kit (Valencia, California) according to manufacturer's guidelines, and with the following thermal profile: 15 minutes at 95 degrees Celsius (°C), 33 cycles of 30 seconds at 94°C, 1.5 minutes at 58°C, and 1 minute at 72°C, and a 10-minute extension at 72°C. Products were electrophoresed on an ABI 3730 capillary sequencer and alleles were scored relative to an internal size standard, Genescan 500 LIZ (Applied Biosystems), using STRand software (Locke et al. 2007). All hair samples were genotyped in duplicate.

Unless otherwise stated, all subsequent statistical analyses were conducted for sampling locations with 10 or more individuals. Tests for deviations from Hardy-Weinberg and linkage equilibrium were conducted using Genepop (http://genepop.curtin.edu.au/) with default Markov chain parameters. Correction for multiple tests was achieved using the sequential Bonferroni method (Rice 1989). The observed heterozygosity (H_O), expected heterozygosity (H_E), and average number of alleles per locus (A) were calculated in the Excel Microsatellite toolkit (Park 2001). The allelic richness (Ar) and the rarefied number of private alleles (Pr) were calculated for 10 diploid individuals in HP-Rare v1.1 (Kalinowski 2005). The pairwise fixation index (F_{ST}) among sampling sites was estimated using Arlequin 3.5 (Excoffier and Lischer 2010). Nei's D_A genetic distance (Takezaki and Nei 1996) was calculated using the program Populations 1.2.32 (Langella 1999). This genetic distance was also sued in the program pPopulations to generate a neighbor joining tree with 200 bootstrap replicates.

Population subdivision across the entire SBARU using all SMHM was investigated using the model-based Bayesian clustering method implemented in the program Structure v 2.3.3 (Pritchard et al. 2000) using the admixture model

with correlated allele frequencies, without prior information (Falush et al. 2003). Iterations were run assuming numbers of genetic clusters (K) ranging 1 from 10, with a burn-in of 50,000 cycles followed by a run of 50,000 cycles. Simulations were run five times at each value of K to assess consistency across runs.

2.6 LANDSCAPE GENETIC ANALYSES

The degree to which landscape variables restrict gene flow among SMHM populations was examined. Spatial data for landscape variables was obtained from the following public sources: U.S. Forest Service (cover types) and Data Basins (Digital Elevation Model). The layer was clipped to the study area in ArcMap 10.4 (Environmental Systems Research Institute (ESRI) 2015). Cover types were grouped into the following broad categories: Barren, Cropland, Grassland, Urban, Waterways, Wetland, and Woodland. SMHM are restricted to salt and brackish marshes that occur at sea level (Shellhammer 1982). Mean higher high water for Suisun Bay is at 2 m and represents the highest elevation of salt marshes. Therefore, the elevation layer was separated into two categories: 2 m or less and more than 2 m.

Rather than assigning *a priori* resistance values to landscape characteristics, the researchers chose to follow a causal modeling approach (Cushman et al. 2006) and to test a range of values, allowing the data to determine the optimal weighting scheme. For each landscape variable, resistance surfaces were generated where the variable of interest was weighted 2, 5, 10, 25, 50, 100, 200, and 500, while other variables were weighted 1 (Roffler et al. 2016). The same resistance weights were used for elevations greater than 2 m. In total, 65 models were generated, including a null model of Euclidian distance (Isolation by Distance [IBD]). Resistance distance between all pairs of populations was calculated using Circuitscape v 4.05 (McRae et al. 2008). Each landscape distance was evaluated against the genetic distance (Nei's D_A). The relationship was tested at two different scales: bay-wide and among the northern marshes. The bay-wide analysis consisted of 20 sites (each representing 10 or more individuals) from across Suisun Bay. The northern marshes consisted of 17 sites, excluding the two Contra Costa County shoreline populations, as well as Ryer Island. This allowed an examination of landscape distances among populations not separated by Suisun Bay.

The analysis involved testing among resistance values for each landscape variable using maximum-likelihood population effects (MLPE) models (Clarke et al. 2002). The optimal maximum resistance (R_{max}) for each landscape variable was determined as the resistance value with the lowest Akaike Information Criterion, corrected for small sample size (AICc) in MLPE models (Shirk et al. 2018). Where AICc indicated similar support for two or more resistance values, the resistance with the highest correlation (R^2) was used.

Multivariate models were constructed for all possible combinations of landscape variables supported by the univariate analysis. MLPE was used for multivariate modeling using lme4 (Bates et al. 2015). Landscape variables estimated from Circuitscape resistance values incorporate geographic Euclidean distance. Therefore, geographic Euclidean distance was removed from each landscape variable to isolate the impact of the landscape variable on landscape resistance. In addition, Euclidean geographic distance was included in each model as an independent covariate (Tucker et al. 2017; Aylward et al. in prep.). Finally, the analysis excluded all models with significant multicollinearity (one or more variables with a Variance Inflation Factor greater than 10) and tested for uninformative landscape variables (coefficient 95% confidence intervals included 0). The final model selection, including a null model of Euclidean distance only, was conducted by AICc. Based on the best supported model, a combined resistance surface was made in ArcMap. This combined resistance surface was then used to generate a current map of SMHM connectivity in the program Circuitscape.

2.7 GENETIC EFFECTIVE POPULATION SIZE

The genetic effective population size (Ne) of different SMHM populations was estimated using the linkage disequilibrium method (Waples and Do 2008) in the program NeEstimator V2.1 (Do et al. 2014). Genetic effective population size was estimated for the genetic populations identified in the study as well as for marsh complexes identified in the *Recovery Plan for Tidal Marsh Ecosystems of Northern and Central California* (USFWS 2013).

3 RESULTS

3.1 GENETIC SPECIES IDENTIFICATION

A total of 777 harvest mouse genetic samples were collected for this study from across the SBARU. Using mitochondrial DNA sequence analysis, 566 SMHM and 211 WHM were identified (Appendix 1). This analysis established the existence of the SMHM at Sherman Island, McAvoy Harbor, and possibly Gold Hills, and verified the continued occurrence of SMHM at several rarely surveyed locations such as Ryer Island, McNabney Marsh, and Point Edith. The only location where no SMHM were caught was EBRP Bay Point along the Contra Costa County shoreline. From this point forward, the analysis was restricted to examination of the SMHM.

3.2 NEXT-GENERATION SEQUENCING AND MICROSATELLITE LOCUS DEVELOPMENT

A total of 18 million sequences (paired reads) were obtained from the MiSeq shotgun sequencing. After filtering to remove low-quality reads, 297,598 sequences were identified as containing putative microsatellite repeat units across the three harvest mice examined. Based on stringent criteria, 40 loci were selected for further screening and testing. After these loci were tested in single locus reactions, 10 were selected for combination into two new multiplexes for analyses of SMHM.

3.3 MICROSATELLITE ANALYSES

All SMHM samples were examined with 20 microsatellite loci. Loci Rrav 13, 18, and 40 were excluded because of difficulties in calling alleles. Locus Rrav 8 was previously shown to be fixed for different alleles in *Reithrodontomys raviventris* and *R. megalotis* (Reponen et al. 2014; Statham et al. 2016). This locus was used to confirm the species identifications from the mitochondrial analysis. Locus Rrav 8 was monomorphic within SMHM, and therefore, was excluded from subsequent analyses. Among the remaining 16 loci, a single occurrence was noted in a single population of significant deviation from linkage equilibrium after sequential Bonfferoni correction for multiple tests. There were five occurrences where loci were significantly out of Hardy-Weinberg equilibrium after sequential Bonfferoni correction for multiple tests; these consisted of two occurrences of Rrav 10 and three of Rrav 36. Missing genotypes were also noted at locus Rrav 36, which taken together may indicate the presence of null alleles. The presence of null alleles has only a small effect on assignment tests and genetic distance estimates (Carlsson 2008). Therefore, both of these loci were retained, and all subsequent analyses were conducted using 16 loci.

3.4 GENETIC DIVERSITY

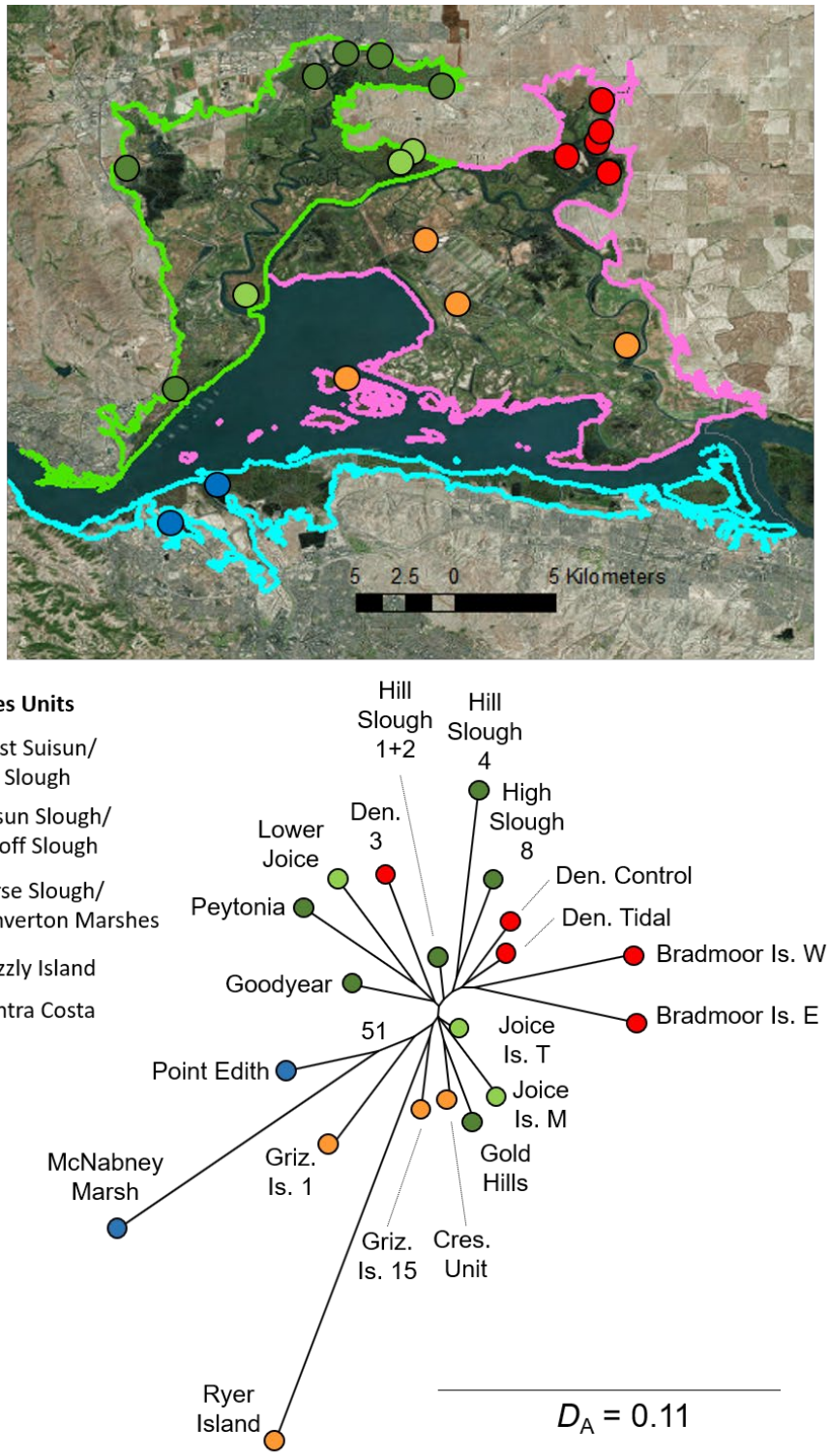
Genotype data were obtained for 558 of 566 *Reithrodontomys raviventris* samples. The genotypes of 20 replicated individuals were identified and removed, resulting in a data set of 538 genotyped *R. raviventris* individuals. Across this bay-wide data set, the number of alleles per locus ranged from three to 18. Summary statistics were calculated for locations with more than 10 individuals (Table 2). Across locations the mean number of alleles per locus was 4.8; the mean H_E was 0.63 and the mean H_O was 0.59. The lowest H_O (0.51) was observed in McNabney Marsh and Bradmoor Island East. The mean allelic richness (Ar) rarified to control for sample size was 4.3. The lowest Ar (3.8) was identified at Ryer Island and McNabney Marsh, indicating reduced genetic diversity at both locations. The rarified average number of private alleles per locus per population was generally low, with seven of 20 locations not possessing any private alleles, thus indicating shared ancestry and/or gene flow with other populations. The

largest numbers of private alleles were identified at Ryer Island (0.13), Goodyear (0.08), Crescent Unit (0.07), and Lower Joice Island (0.06). These populations tended to be geographically distant or more isolated from other sampling locations.

3.5 POPULATION SUBDIVISION

Pairwise F_{ST} was calculated for all sampling locations with 10 or more individuals. The majority of pairwise comparisons between locations indicated significant differentiation (Table 3). The highest F_{ST} (0.138) was identified between Ryer Island and McNabney Marsh. The majority of pairwise comparisons between each of these populations (Ryer Island and McNabney Marsh) and others were also relatively high, indicating that they were generally isolated (Table 3).

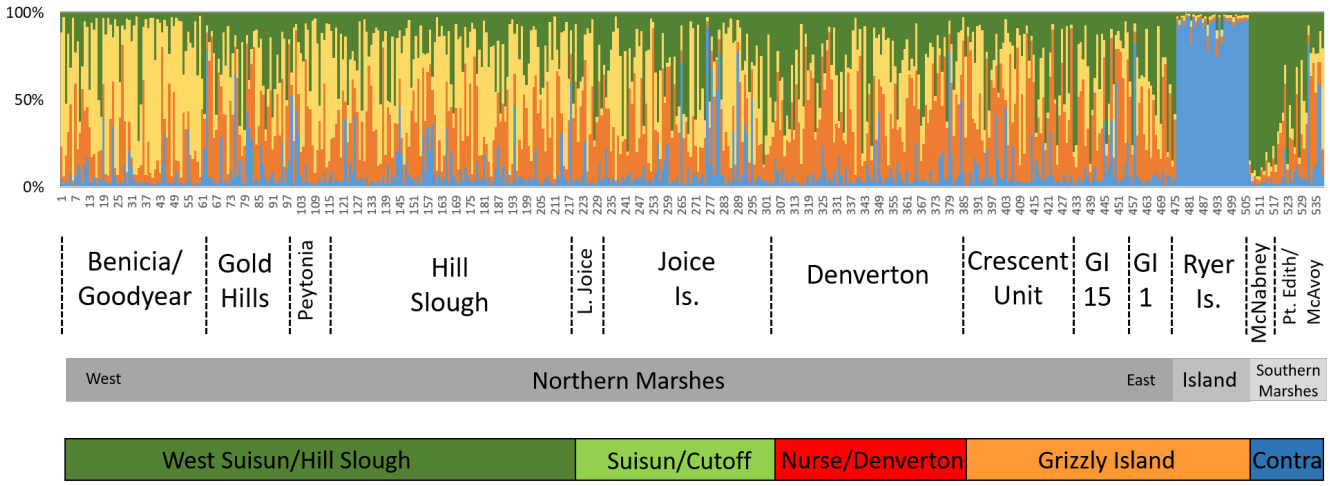
The tree of SMHM populations identified the population on Ryer Island as the most distinct, followed by McNabney Marsh (Figure 2). Both populations were on long branches indicative of increased genetic distance from other populations. Both populations from the Contra Costa County shoreline to south of Suisun Bay (McNabney Marsh and Point Edith) were most closely related to one another. Although genetically distinct from all other sampling locations, Ryer Island branched off from locations that were part of the Grizzly Island Management Unit, which may indicate an ancestral relationship or a degree of connectivity.



Note: Distance is Nei's D_A. The value at the node of Point Edith and McNabney Marsh is bootstrap support based on 200 replicates.
Source: Data compiled by University of California, Davis in 2019

Figure 2. Population Tree of Salt Marsh Harvest Mouse Sampling Locations across the Suisun Bay Area Recovery Unit, Based on Locations with More than 10 Individuals

The presence of population structure within SBARU was also assessed using all 538 SMHM individuals, without prior assignment to populations. Increased support was identified from K = 2–4, after which point the support dropped. Individuals from Ryer Island formed a discrete cluster at all K values from 2 to 10 (Figure 3). At K = 3, individuals from McNabney Marsh separated, along with a portion of individuals from the neighboring Point Edith. At K = 4 (the value with the highest support), discrete populations were resolved at Ryer Island, McNabney Marsh (along with a portion of neighboring Point Edith), and to a lesser extent, Benicia/Goodyear, which were the sites in the southwestern extreme of the northern marshes.



Note: This graphic represents all 538 salt marsh harvest mice genotyped at 16 microsatellite loci. Each bar represents an individual animal, with the percentage of their ancestry assigned to one or more genetic clusters. This graphic represents K = 4, which was the best supported number of genetic clusters, as estimated in the program Structure. Sampling sites are arranged in order from west to east, first considering the main northern marshes, then Ryer Island, and finally the southern marshes.

Source: Data compiled by University of California, Davis, in 2019

Figure 3. Salt Marsh Harvest Mouse Population Structure across the Suisun Bay Area Recovery Unit.

Because of the strong signal at Ryer Island relative to other sites, the analysis was run again without this location. The highest support was identified at K = 2, where the majority of the genetic makeup of individuals from McNabney Marsh and Point Edith was assigned to one cluster, and a large portion of Benicia/Goodyear was assigned to another. This broadly supported the findings of the full data set. The relationship among the northern marshes (excluding McNabney Marsh, Point Edith, and Ryer Island) was also examined. The highest support was for K = 2. This analysis did not partition out any discrete populations, thus indicating little population substructure. However, individuals farthest to the west (in Benicia and Goodyear) were primarily assigned to one genetic cluster, while those farthest to the east were primarily assigned to the second, potentially indicating isolation by distance (IBD).

3.6 LANDSCAPE GENETICS: UNIVARIATE ANALYSIS

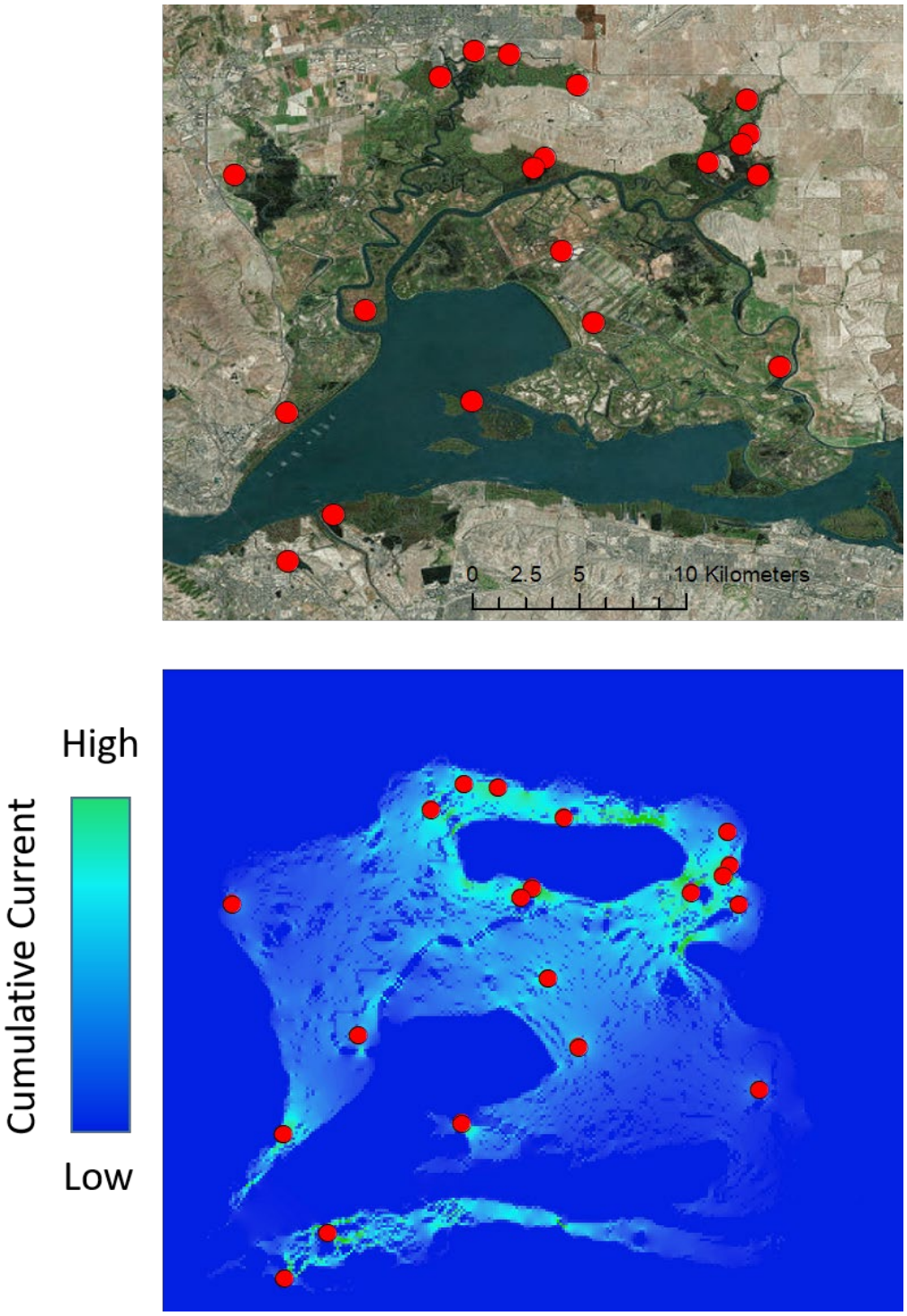
The role that elevation and vegetation cover play in explaining the observed population subdivision among SMHM was examined. AICc was used to assess support for a range of resistance values for each cover type and for elevation. Across the full bay data set the following resistance values were identified for each parameter: Grass (IBD-10), Urban (500-10), Water (100-200), Wetland (IBD-2), and Elevation (500-200) (the highest ranked resistance value is listed first for each variable). There was no improved explanatory power over IBD across all

resistance values for Barren, Crop, or Woodland, indicating that these cover types did not affect the genetic distances among mice at this scale. Both Cropland and Woodland were located primarily at the periphery of the study site. For the northern marsh, the following resistance values were identified for each parameter: Grass (IBD-10), Urban (5; but the full range within 2 AIC units), Water (IBD-10), Wetland (IBD-2), Elevation (10; but the full range was within 2 AIC units). Barren, Crop, or Woodland did not affect the genetic distances among mice within the northern marsh.

3.7 LANDSCAPE GENETICS: MULTIVARIATE ANALYSIS

The supported values from the univariate analyses were selected for mixed model analyses. Barren, Crop, and Woodland were excluded based on the results of the univariate analyses. In cases where AICc did not discriminate among a small range of R_{max} values, the R_{max} value with the highest R^2 was chosen. For the full Bay-wide data set, eight multivariate models containing Elevation (500), Urban (500), and Water (200), as well as IBD were tested. Models that contained Urban with Elevation were removed because Urban was indicated to be an uninformative variable. The highest performing model included both Elevation (500) and Water (200), while the second only contained Water (Table 4). These two models accounted for more than 99% of the AICc weight (Table 5). For the northern marsh data set, 16 multivariate models containing Elevation (10), Grass (10), Urban (5), and Water (10), as well as IBD, were tested. The best supported model in the northern marshes was IBD (Table 5). The next four highest models all included single variables that were flagged as being uninformative.

The best supported model for the full Bay-wide data set (Elevation [500], Water [200]) was used to create a combined resistance surface in ArcMap. Based on the combined resistance surface the program Circuitscape was used to generate a cumulative current map of SMHM connectivity (Figure 4). Based on the top performing model the current map indicated connectivity among SMHM sampling locations across the northern marshes, and that these populations were separated from populations on the Contra Costa Shore by Suisun Bay and Grizzly Bay. The current map indicates connectivity between Ryer Island and Grizzly Island, across the narrowest part of the water channel. In this model, the Potrero Hills appear to act as barrier, while the low elevation area to the north appears to facilitate connectivity between the marshes of Hill Slough and Denverton.

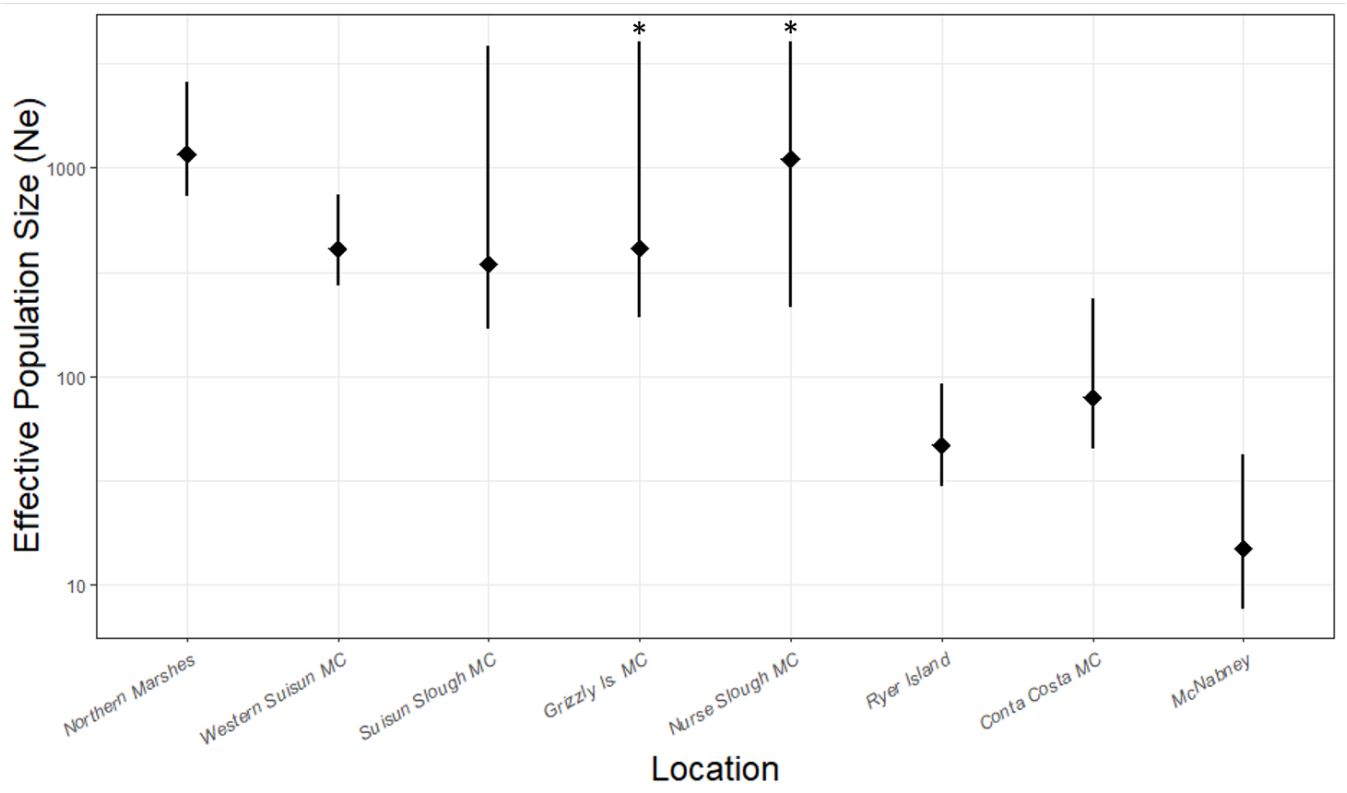


Note: The current map was generated in the program Circuitscape based on the best supported model that contained Elevation (500) and Water (200). Darker blue indicates areas that with higher resistance that may restrict gene flow. Areas shaded in light blue to green have higher current density, and may facilitate gene flow (higher connectivity). Narrower areas of conductance tend to have higher current, while broader areas tend to have more diffuse current. Source: Data compiled by University of California, Davis, in 2019

Figure 4. Cumulative current map modelling connectivity among 20 Salt Marsh Harvest Mouse trapping locations within Suisun Bay Area recovery Unit, CA.

3.8 GENETIC EFFECTIVE POPULATION SIZE

Among the genetically distinct populations identified in this study, the highest Ne was located within the northern marshes (Figure 5). The populations of Ryer Island and Contra Costa had similar Ne, and both were significantly smaller than the northern marshes. All four marsh complexes within the northern marshes had similar and overlapping Ne estimates. The population at McNabney Marsh had the lowest Ne among all population examined within the SBARU.



Note: Ne estimated for genetically distinct populations identified in this study, as well as for marsh complexes (MC) identified in the recovery plan. Diamonds indicate the mean value, while the horizonal bars indicate the 95% confidence intervals. The asterisks indicate where the upper 95% confidence interval encompassed infinity. The Y axis is on a log 10 scale.

Source: Data compiled by University of California, Davis, in 2019

Figure 5. Genetic effective population size (Ne) of Salt Marsh Harvest Mouse populations within Suisun Bay Area Recovery Unit, CA.

4 DISCUSSION

Understanding how populations of endangered species are subdivided and which landscape features impede gene flow aids conservation practitioners in making informed management decisions. This study used a genetic analysis of 538 SMHM from across the SBARU to show the occurrence of multiple populations that were separated by both open tracts of water and uplands. In addition, a large diverse population was identified across the northern marshes of Suisun Bay. These and other findings are discussed in greater detail below.

4.1 SUBSTRUCTURE AND DIVERSITY

Multiple lines of evidence resolved the population subdivision among SMHM across Suisun Bay. Distinct populations were identified at Ryer Island, along the Contra Costa County shoreline (Point Edith and McNabney Marsh), and across the northern marshes. Cluster analysis indicated that the mice on Ryer Island formed a discrete genetic unit. In addition, this population was placed on the longest branch in the population tree, indicating that it was highly differentiated from all others. This status was also corroborated by the highest average pairwise F_{ST} values across all locations examined. Although genetic drift can accelerate the divergence observed through such measures in very small populations, the heterozygosity levels on Ryer Island were comparable, if slightly on the low side, to those values in other locations. Additionally, analysis of genetic effective population size indicated that this population had similar diversity to animals from the Contra Costa County shoreline, despite being restricted to a much smaller geographic area. Moreover, the highest number of private alleles was observed on Ryer Island. Taken together, these results indicated that the SMHM on Ryer Island formed the most distinct population identified, and that this pattern was best explained by long-term isolation (rather than small population size and accelerated genetic drift). The clustering of Ryer Island with three locations from Grizzly Island in the population tree is consistent with Ryer Island having been colonized from or most recently connected to the population in Grizzly Island, as would be expected given its proximity. Indeed, this is supported by the current map (Figure 4), which indicates movement across the narrowest stretches of water connecting Ryer Island and Grizzly Island.

As expected given their separation from other populations by the Sacramento River, the SMHM populations from the Contra Costa County shoreline at Point Edith and McNabney Marsh together composed the second most differentiated unit within Suisun Bay. These locations grouped together in the population tree and, to some extent, in the structure analysis. Nevertheless, the McNabney Marsh population was also somewhat differentiated from its nearest neighbor at Point Edith based on this analysis and its location on a long branch in the population tree. The genetic diversity (i.e., allelic richness and heterozygosity) of the McNabney Marsh population also was on the low end of the range observed. The population also had the lowest genetic effective population size in the study. In contrast to that found at Ryer Island, McNabney Marsh had a very low number of private alleles, which is consistent with recent isolation. Taken together these results indicate that isolation and small population size have resulted in genetic drift and a reduction of genetic diversity, which has led to this population becoming genetically distinct.

The SMHM from the northern marshes of Suisun Bay were distinct from those along the Contra Costa County shoreline and on Ryer Island. Based on the inhabited acreage and density of mice (USFWS 2013), these marshes likely hold the highest remaining numbers of SMHM across the species' range. Given that, they are of particular concern for the persistence of the species as a whole. Although the analysis did identify some differentiation among sites in the area, the pairwise F_{ST} values were substantially lower than when considering Ryer Island or McNabney Marsh. The population tree indicated that populations that were geographically close to one another were generally closely related genetically, which is consistent with the populations being isolated by distance. The cluster analysis

indicated some differentiation between the sites at southwestern extreme of the northern marshes (Benicia and Goodyear) from sites farther to the east, but generally provided support for isolation by distance. The cumulative current map indicated high connectivity among sites within the northern marshes, with the possible exception of areas of higher elevation, and wider expanses of water. Estimates of genetic diversity were generally similarly high across the area, with no indication of a systemic loss of diversity. Most locations had only a small proportion of private alleles, indicating that genetic diversity was largely shared across the area. The small number of locations with elevated proportions of private alleles tended to be more geographically distant from another sampled sites. The genetic effective population size within the northern marshes was the highest in the study. Indeed, when broken into the four constituent marsh complexes, each of these had high and over-lapping estimates, indicating that they support genetically healthy populations of salt marsh harvest mouse.

4.2 DIVERGENCE TIMING

Although different populations of SMHM were identified within the SBARU, the timing of divergence among them is unknown. Understanding the timing of divergence among the populations identified would allow researchers to better appreciate how valuable they are from a conservation standpoint. This is especially true considering that population divergence can occur on a short time frame because of genetic drift, especially when populations are small, go through a bottleneck, or stem from a small number of founders (as was likely the case for the McNabney Marsh population). Populations that are relatively recently differentiated are unlikely to have had sufficient time to evolve new adaptations, which are of particular interest when considering the preservation of the species' evolutionary potential and ability to cope with environmental change (USFWS 2013). Previous genetic analyses on 60 SMHM from Suisun and San Pablo Bays identified shared ancestry across the northern subspecies of SMHM (Statham et al. 2016). However, a study examining a better representation of individuals from Suisun Bay, and focusing on a rapid evolving but clonally inherited marker such as the mitochondrial D-loop region, or on nuclear markers with low rates of homoplasy (e.g., single nucleotide polymorphisms from genotyping by sequencing methods), would provide a clearer picture of the phylogenetic relationship and the timeline of interest among SMHM populations within the SBARU.

4.3 LANDSCAPE GENETICS

Landscape genetic analysis allows scientists and managers to attempt to explain the causes of subdivision within a species. This study followed a casual modeling approach, that allowed the genetic and landscape data to identify which variables influenced population substructure. This approach was used to examine the role of landscape variables in explaining SMHM population substructure at two different scales: across the full bay and within the northern marshes.

The full-bay data set allowed an assessment of the impact that large stretches of open water have on causing population subdivision. The northern marshes data set allowed an assessment of the role played by landscape features in explaining more nuanced population subdivision within the species. The full-bay data set identified both Water and Elevation as the most predictive landscape features restricting gene flow (and therefore animal movement). This finding indicates that SMHM in other locations that are isolated by elevation and/or water, which include several additional islands within Suisun Bay, likely represent genetically distinct populations. This result can help prioritize study sites for future efforts to find and identify distinct populations of the species. Many of the remaining marshes of central San Francisco Bay are fragmented and isolated by both elevation (and associated urban features) and water. Thus, if any of these locations retain relict populations of the species, they would be

isolated and susceptible to inbreeding and to extirpation because of stochastic events, with limited opportunity for natural recolonization.

Within the northern marshes, geographic distance was the best predictive landscape variable. This finding was in keeping with other analyses (e.g., cluster analysis and the population tree). Neither Urban (primarily small roads at this scale), Elevation, Water, nor Grass appear to explain the observed genetic distance within the northern marshes. Previous research indicates that the species' movements through open habitats were not restricted (USFWS 2013 and references cited therein). SMHM are known to swim well (Shellhammer et al. 1982), although the degree to which they do this in sloughs or open water is unknown. The species is sometimes found in grasslands at the upper edge of marshlands, but the degree to which this habitat is used is unclear (USFWS 2013 and references cited therein). Potentially none of these landscape variables substantially restrict SMHM movement and gene flow at the scale of the northern marshes. Alternatively, the anthropogenic changes to habitat (e.g., converting to grassland, or building roads) may not have occurred long enough ago to have resulted in detectable population differentiation (Landguth et al. 2010).

One of the main evolutionary factors affecting the differentiation of populations is genetic drift. Genetic drift can rapidly affect small populations, where the reproductive success of a small number of individuals affects the genetic makeup of a descendant generation. In contrast, large populations will take substantially longer to become genetically differentiated as a result of drift. The population size of SMHM in the northern marshes is likely to be large enough that it is not substantially affected by drift. Taken together, at the scale of the northern marshes, the landscape variables likely represent porous barriers, and/or that insufficient time has passed for their effect to be detectable.

4.4 MANAGEMENT IMPLICATIONS

The recovery plan (USFWS 2013) states that it is necessary to maintain well-distributed populations throughout the range for the recovery of the species. The results of this study demonstrate that SMHM are well distributed across the majority of the SBARU. One exception is the eastern end of the Contra Costa County shoreline marsh complex, where trapping was conducted at two adjacent locations (Bay Point and McAvoy Harbor) and only three SMHM were caught. Considering that SMHM from Point Edith and McNabney Marsh are genetically distinct from those elsewhere, additional work should be conducted to establish whether SMHM are present at other sites within the Contra Costa County shoreline marsh complex, and to determine their relationship to other populations and their genetic diversity. The habitat of the Contra Costa County shoreline marsh complex is narrow and highly fragmented; it would be useful to determine whether anthropogenic barriers are restricting gene flow and animal movement.

The results of this study also demonstrate that SMHM are present on Sherman Island, however only a single SMHM was caught there. This location is at the farthest eastern extreme (along with Collinsville) of the species' range and the farthest up the Sacramento–San Joaquin Delta. With the rising sea level, the mice from this location will likely play a role in the eastward range expansion of the species. Therefore, further study of these mice is warranted.

The recovery plan also states that the range of genetic variation must also be maintained, and that an inventory is needed of the genetic variation, population subdivision, genetic effective population size, and levels of gene flow (USFWS 2013). Research conducted for this study indicated that genetic diversity was similar across much of the SBARU, except in a small number of more isolated or peripheral locations, such as Ryer Island and McNabney Marsh. This research also serves as an inventory of the genetic effective population size and major population

subdivision within the SBARU. Distinct populations were identified at Ryer Island, the Contra Costa County shoreline marsh complex, and the northern marshes of Suisun Bay (encompassing the vast majority of four marsh complexes). The genetic relationship of additional island sites beyond Ryer Island could not be assessed.

One of the criteria for downlisting or delisting the SMHM is that VHAs within marsh complexes must be connected to allow them to function as one large population over time (USFWS 2013). Across the northern marshes (Figure 2), SMHM were effectively part of one large population, and barriers to gene flow were not detected. This may be because the changes to marsh habitat do not pose substantial barriers to mouse movement. Alternatively, it may be because the population size is so large that any recent barriers have not had sufficient time to give rise to distinct gene pools.

4.5 FUTURE WORK

The identification of a distinct population of mice on Ryer Island raises important questions about the presence of other unique island populations in Suisun Bay (and indeed elsewhere throughout the species' range). A single SMHM was caught on Sherman Island. In the future it would be worth targeting Sherman Island and other islands (e.g., Roe Island, Freeman Island, Snag Island, Browns Island, Winter Island). This work also provides insight regarding the potential for natural recolonization of other isolated marshes throughout the San Francisco Bay estuary. In addition, an analysis focusing on the timing of separation of Ryer Island and other island populations would allow researchers to determine whether these populations are differentiated on an evolutionary time scale.

5 REFERENCES

Andrews, S. 2010. FastQC: A Quality Control Tool for High Throughput Sequence Data. Available: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Accessed June 2016.

Aylward, C. M., J. D. Murdoch, and C. M. Kilpatrick. In prep. *Multi-scale Landscape Genetics of American Marten at Their Southern Range Periphery.*

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67:51.

Carlsson, J. 2008. Effects of Microsatellite Null Alleles on Assignment Testing. *Journal of Heredity* 99:616–623.

Castoe, T. A., A. W. Poole, A. P. J. de Koning, K. L. Jones, D. F. Tomback, S. J. Oyler-McCance, J. A. Fike, S. L. Lance, J. W. Streicher, E. N. Smith, and D. D. Pollack. 2012. Rapid Microsatellite Identification from Illumina Paired-End Genomic Sequencing in Two Birds and a Snake. *PLoS One* 7:e30953.

Chen, C., S. S. Khaleel, H. Huang, and C. H. Wu. 2014. Software for Pre-processing Illumina Next-Generation Sequencing Short Read Sequences. *Source Code for Biology and Medicine* 9:8.

Clarke, R. T., P. Rothery, and A. F. Raybould. 2002. Confidence Limits for Regression Relationships between Distance Matrices: Estimating Gene Flow with Distance. *Journal of Agricultural Biological and Environmental Statistics* 7:361–372.

Cushman, S. A., K. S. McKelvey, J. Hayden, and M. K. Schwartz. 2006. Gene Flow in Complex Landscapes: Testing Multiple Hypotheses with Causal Modeling. *American Naturalist* 168:486–499.

Do, C., Waples, R. S., Peel, D., Macbeth, G. M., Tillett, B. J. & Ovenden, J. R. (2014). NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. Molecular Ecology Resources. 14, 209-214.

Excoffier, L., and H. E. L. Lischer. 2010. Arlequin Suite Ver 3.5: A New Series of Programs to Perform Population Genetics Analyses under Linux and Windows. *Molecular Ecology Resources* 10:564–567.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies. *Genetics* 164:1567–1587.

Fisler GF (1965) Adaptations and speciation in harvest mice of the marshes of San Francisco Bay. University of California Publications in Zoology 77:1-108.

Hernandez, L. D., Z. T. Lounsberry, P. W. Collins, R. W. Henry, S. D. Newsome, and B. N. Sacks. 2014. Development and Characterization of 15 Polymorphic Microsatellite Markers for North Pacific Albatrosses Using Paired-End Illumina Shotgun Sequencing. *Conservation Genetics Resources* 6:491–493.

Holleley, C. E., and P. G. Geerts. 2009. Multiplex Manager 1.0: A Cross-Platform Computer Program that Plans and Optimizes Multiplex PCR. *Biotechniques* 46(7):511–517.

Kalinowski, S. T. 2005. HP-Rare: A Computer Program for Performing Rarefaction on Measures of Allelic Diversity. *Molecular Ecology Notes* 5:187–189.

Landguth, E. L., S. A. Cushman, M. K. Schwartz, K. S. McKelvey, M. Murphy, and G. Luikart. 2010. Quantifying the Lag Time to Detect Barriers in Landscape Genetics. *Molecular Ecology* 19:4179–4191.

Langella, O. 1999. Populations 1.2.31 population genetic software. Available: http://bioinformatics.org/~tryphon/populations/. Accessed December 2018.

Locke, M., E. Baack, and R. Toonen. 2007. STRand (version 2.2.30) on the WWW for general users. University of California, Davis, Veterinary Genetics Lab: Informatics.

McRae, B. H., B. Dickson, T. H. Keitt, and V. B. Shah. 2008. Using Circuit Theory to Model Connectivity in Ecology and Conservation. *Ecology* 10:2712–2724.

Park, S. D. E. 2001. "The Excel microsatellite toolkit." *Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection*. PhD thesis, University College Dublin, Ireland.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* 155:945–959.

Reponen, S. E. M., M. J. Statham, L. Thompson, and B. N. Sacks. 2014. Microsatellite Primer Development for the Salt Marsh Harvest Mouse (*Reithrodontomys raviventris*) and Cross-amplification in the Western Harvest Mouse (*R. megalotis*). *Conservation Genetics Resources* 1–3.

Rice W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225

Roffler, G. H., M. K. Schwartz, K. L. Pilgrim, S. L. Talbot, G. K. Sage, L. G. Adams, and G. Luikart. 2016. Identification of Landscape Features Influencing Gene Flow: How Useful are Habitat Selection Models? *Evolutionary Applications* 9:805–817.

Rozen, S., and H. J. Skaletsky. 2000. Primer3 on the WWW for general users and for biologist programmers. In *Bioinformatics Methods and Protocols: Methods in Molecular Biology*, eds. S. Misener and S. A. Krawetz, 365–386. Totowa, NJ: Humana Press Inc.

Sambrook J., D. Russell D (2001) Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor Laboratory Press, MD.

Shellhammer, H. S. 1982. Reithrodontomys raviventris. *Mammalian Species, American Society of Mammalogists* No. 169:1–3. Washington, DC.

Shirk, A. J., E. L. Landguth, and S. A. Cushman. 2018. A Comparison of Regression Methods for Model Selection in Individual-Based Landscape Genetic Analysis. *Molecular Ecology Resources* 18:55–67.

Smith K. R., M. K. Riley, L. Barthman-Thompson, I. Woo, M. J. Statham, S. Estrella, D. A. Kelt. 2018. Toward Salt Marsh Harvest Mouse Recovery: A Review. *San Francisco Estuary and Watershed Science*. 16: 1-24.

Statham, M. J., S. Aamoth, L. Barthman-Thompson, S. Estrella, S. Fresquez, L. D. Hernandez, R. Tertes, and B. N. Sacks. 2016. Conservation Genetics of the Endangered San Francisco Bay Endemic Salt Marsh Harvest Mouse (*Reithrodontomys raviventris*). Conservation Genetics 17:1055–1066.

Takezaki, N., and M. Nei. 1996. Genetic Distances and Reconstruction of Phylogenetic Trees from Microsatellite DNA. *Genetics* 144:389–399.

Tucker, J. M., F. W. Allendorf, R. L. Truex, and M. K. Schwartz. 2017. Sex-Biased Dispersal and Spatial Heterogeneity Affect Landscape Resistance to Gene Flow in Fisher. *Ecosphere* 8:e01839.

Untergasser, A., I. Cutcutache, T. Koressaar, J. Ye, B. C. Faircloth, M. Remm, and S. G. Rozen. 2012. Primer3—New Capabilities and Interfaces. *Nucleic Acids Research* 40(15):e115.

Vázquez-Domínguez, E., and S. Espindola. 2013. Characterization of ten new microsatellite loci from the endangered endemic rodent, *Reithrodontomys spectabilis*. *Conservation Genetics Resources* 5:251–253

U.S. Fish and Wildlife Service. 1970 (October 13). Appendix D—United States List of Endangered Native Fish and Wildlife. *Federal Register* 35:16047–16048.

———. 2013. *Recovery Plan for Tidal Marsh Ecosystems of Northern and Central California.* Region 8, Sacramento, CA.

USFWS. *See* U.S. Fish and Wildlife Service.

Waples, R. S., and C. Do. 2008. LdNe: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources* 8:753–756.

Williams, P., and P. Faber. 2001. Salt Marsh Restoration Experience in San Francisco Bay. *Journal of Coastal Research* 23:203–211.

This page intentionally left blank

6 TABLES

Table 1 Newly Developed Microsatellite Loci for the Salt Marsh Harvest Mouse

Locus	Plex	Dye	Sequence (5'-3')	No. Alleles	Allele Size Range (bp)
Rrav43	3	6-FAM	F: TTGTCTTGCTCCCACTCAGT	8	260–284
			R: TCAGCTGGTGGAACTCTGAA		
Rrav49	3	PET	F: GCAGGGGCAGATATGAAACC	18	187–231
			R: CTATCTTTAGGGATATGATAAGCCA		
Rrav57	3	VIC	F: GCAGGCTCCAAAGCTACAAA	4	180–196
			R: ATTCACCGGTAAGGGTAGGG		
Rrav61	3	NED	F: AAATCCAATCCATCCATCCA	5	137–157
			R: CACCATTTTCTGGCCTCTGT		
Rrav46	4	6-FAM	F: GTTCTCAGGACGGACTGTGG	4	219–241
			R: CTCCCAACATGGTGTGTTGC		
Rrav44	4	VIC	F: TACTGGCATCGGAAGTCAGG	8	192–208
			R: TCTCTACTTAGCGTCCCGCC		
Rrav47	4	6-FAM	F: CTTGTGCATCAAAGGATGGC	4	144–156
			R: TGCTCTGAGTTCTAGGCCAG		
Rrav51	4	VIC	F: GAATAGTCTATAGGCAGTCCGGG	5	127–139
			R: CCCTGACCTCCACATACACA		
Rrav62	4	PET	F: CCACTCAGCCAGCCATTAAA	11	182–222
			R: TTTGCCAAGGACTCAAGGAT		

Notes:

bp = base pairs; No. = number

An annealing temperature of 62 degrees Celsius was used for all loci.

Source: Data compiled by University of California, Davis, in 2019

Table 2 Genetic Diversity Summary Statistics of Salt Marsh Harvest Mouse from Sampling Locations across Suisun

Location	n	A	SD	Ar	SD	Pr	SD	He	SD	Ho	SD
GIWA, Goodyear Sl.	58	5.9	3.4	4.5	1.9	0.08	0.18	0.65	0.04	0.61	0.02
GIWA, Gold Hills	34	5.6	3.1	4.6	2.1	0.03	0.08	0.62	0.05	0.59	0.02
Peytonia Sl. ER	16	4.8	2.2	4.4	1.8	<0.01	0.00	0.63	0.04	0.55	0.03
Hill Sl. Ponds1+2	80	5.5	3.0	4.3	1.7	<0.01	0.00	0.64	0.04	0.60	0.01
Hill Sl. WA Pond 4	10	4.1	1.5	4.1	1.5	<0.01	0.00	0.63	0.04	0.62	0.04
Hill Sl. WA Pond 8	15	4.7	2.5	4.4	2.1	<0.01	0.01	0.63	0.04	0.62	0.03
SRCD Lower Joice Island	12	4.9	2.4	4.7	2.2	0.06	0.25	0.63	0.05	0.57	0.04
GIWA. Joice Island Unit, Tidal	52	5.7	3.0	4.5	1.8	0.01	0.05	0.64	0.04	0.62	0.02
GIWA. Joice Island Unit, Managed	20	5.1	2.1	4.4	1.6	0.04	0.11	0.63	0.05	0.60	0.03
CWA Denverton Control	27	4.9	2.5	4.1	1.9	<0.01	0.00	0.64	0.04	0.66	0.02
CWA Denverton Tidal	16	4.8	2.4	4.2	1.8	<0.01	0.00	0.61	0.05	0.55	0.03
CWA Denverton 3	11	4.7	2.2	4.6	2.1	0.01	0.04	0.65	0.05	0.64	0.04
DWR Bradmoor Island West	10	4.0	1.9	4.0	1.9	0.03	0.09	0.65	0.04	0.66	0.04
DWR Bradmoor Island East	12	4.1	2.0	3.9	1.8	<0.01	0.00	0.58	0.06	0.51	0.04
GIWA, Crescent Unit	48	5.6	3.0	4.4	1.8	0.07	0.12	0.64	0.04	0.61	0.02
GIWA Pond 15	25	5.1	2.4	4.3	1.7	0.03	0.10	0.63	0.04	0.58	0.02
GIWA Pond 1	15	4.5	1.9	4.1	1.6	0.02	0.08	0.60	0.04	0.58	0.03
Ryer Island	31	4.2	1.6	3.8	1.3	0.13	0.31	0.59	0.03	0.58	0.02
MVSD McNabney Marsh	12	3.9	1.8	3.8	1.7	0.01	0.04	0.60	0.04	0.51	0.04
Point Edith WA	17	4.6	2.2	4.2	1.8	0.01	0.02	0.63	0.05	0.60	0.03

Note:

This table shows only locations with at least 10 individuals represented. A = average number of alleles per locus; Ar = Allelic richness rarified for 10 diploid individuals; He = expected heterozygosity; Ho = observed heterozygosity; n = number of individuals; Pr = number of private alleles rarified for 10 for 10 diploid individuals; SD = standard deviation.

Source: Data compiled by University of California, Davis, in 2019

Table 3 Pairwise F_{ST} Values among 20 Salt Marsh Harvest Mouse Sampling Locations

Location	GD	GH	PY	HS1+2	HS4	HS8	LJ	JIM	JIT	DC	DT	D3	BIW	BIE	CU	GI15	GI1	R	MN
GD																			
GH	0.024																		
PY	0.033	0.043																	
HS1+2	0.010	0.018	0.022																
HS4	0.025	0.035	0.026	0.003															
HS8	0.016	0.009	0.031	0.005	0.011														
LJ	0.016	0.030	0.024	0.021	0.031	0.028													
JIM	0.012	0.020	0.023	0.010	0.034	0.026	0.013												
JIT	0.014	0.011	0.033	0.005	0.015	0.008	0.027	0.008											
DC	0.031	0.023	0.037	0.018	0.019	0.024	0.028	0.026	0.006										
DT	0.014	0.006	0.028	0.004	0.014	0.001	0.021	0.012	0.001	0.003									
D3	0.015	0.023	0.022	0.003	0.016	0.010	0.014	0.014	<0.001	0.007	<0.001								
BIW	0.020	0.025	0.050	0.020	0.010	0.014	0.020	0.038	0.008	0.014	0.014	0.021							
BIE	0.046	0.034	0.065	0.030	0.047	0.044	0.029	0.047	0.021	0.015	0.016	0.026	0.033						
CU	0.017	0.018	0.026	0.021	0.032	0.017	0.024	0.022	0.014	0.017	0.010	0.025	0.031	0.042					
GI15	0.017	0.029	0.034	0.016	0.023	0.027	0.029	0.009	0.011	0.014	0.005	0.003	0.029	0.037	0.015				
GI1	0.031	0.043	0.038	0.028	0.063	0.042	0.024	0.034	0.018	0.030	0.033	0.014	0.060	0.038	0.037	0.031			
RI	0.097	0.102	0.120	0.094	0.120	0.101	0.115	0.095	0.084	0.107	0.102	0.085	0.098	0.119	0.095	0.088	0.091		
MN	0.078	0.096	0.098	0.072	0.090	0.083	0.075	0.067	0.062	0.084	0.079	0.056	0.090	0.120	0.079	0.077	0.082	0.138	
PE	0.030	0.023	0.052	0.026	0.040	0.021	0.031	0.026	0.016	0.016	0.008	0.025	0.027	0.023	0.026	0.024	0.039	0.096	0.064

Notes:

BIE = DWR Bradmoor Island East, BIW = DWR Bradmoor Island West, CU = GIWA Crescent Unit, D3 = CWA Denverton 3, DC = CWA Denverton Control, DT = CWA Denverton Tidal, G = GIWA Goodyear, GD = GIWA Gold Hills, GI1 = GIWA Pond 1, GI15 = GIWA Pond 15, HS1+2 = Hill Slough WA Ponds 1+2, HS4 = Hill Slough WA Pond 4, HS8 = Hill Slough WA Pond 8, JIM = GIWA Joice Island Managed, JIT = GIWA Joice Island Tidal, LJ = SRCD Lower Joice Island, MN = MVSD McNabney Marsh, PE = EBRP Point Edith WA, PY = Peytonia Slough ER, RI = Ryer Island.

The vast majority of pairwise comparisons were significant at $\alpha = 0.05$.

Source: Data compiled by University of California, Davis, in 2019

Table 4 Highest Ranking Landscape Resistance Models in Each Salt Marsh Harvest Mouse Study Site Ranked by AICc and ΔAICc

Study Site	Model	AICc	ΔAICc	W	Wcum
Full Bay	Water + Elevation	-1142.87	0.000	0.926	0.926
	Water	-1137.80	5.073	0.073	1.000
	IBD	-1125.51	17.362	0.000	1.000
	Elevation	-1125.14	17.737	0.000	1.000
Northern Marshes	IBD	-847.749	0.000	0.882	0.882
	Urban	-842.327	5.421	0.059	0.941
	Elevation	-840.847	6.902	0.028	0.969
	Water	-839.959	7.790	0.018	0.987
	Grass	-838.365	9.384	0.008	0.995

Notes:

ΔAICc = change in Akaike Information Criterion, corrected for small sample size; AICc = Akaike Information Criterion, corrected for small sample size; IBD = Isolation by Distance; W = weight; Wcum = cumulative AICc weight

Top models were those where AICc weight (W) contributes to 99% of the cumulative AICc weight (Wcum).

Source: Data compiled by University of California, Davis, in 2019