

Adaptive Immunogenetic Variation in Endangered Salt Marsh Harvest Mouse Populations

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Adaptive immune system variation is frequently characterized in endangered species to assess the potential of limited populations to respond to disease. We have isolated the first adaptive immune system locus from the salt marsh harvest mouse, *Reithrodontomys raviventris*, in the major histocompatibility complex (MHC, Class II DRB). *R. raviventris* is endangered due to habitat limitation and disruption. Because *R. raviventris* is a low-dispersing species endemic to San Francisco Bay Estuary marshes, it is an ideal indicator species of local ecosystem health. Its response to wetland restoration and management can be used as a measure of success. Patterns and levels of functional genetic variation can serve as a metric to monitor this response. To measure baseline levels of genetic diversity, 12 sites were sampled across Suisun, San Pablo, and San Francisco Bays. *R. raviventris* individuals were sequenced at the functionally conserved mitochondrial cytochrome b locus (n=105) and cloned and sequenced at the functionally diversifying MHC locus (n=55). Patterns of genetic diversity show regional differentiation at both mitochondrial and MHC loci, particularly between northern and southern regions, corresponding to subspecies designations. Additionally, northern populations are functionally differentiated at the MHC locus, with unique alleles present in the more isolated sampling areas. Selection tests indicate evidence of positive selection acting on this locus (for antigen binding sites: dN/dS=3.43, p=0.02), suggesting that patterns of diversity may be due to local adaptation as well as genetic drift. Low allelic variation in southern populations may indicate limited adaptive potential. Genetic data can be used to prioritize conservation efforts and to aid habitat management and restoration design, determining if populations should be connected by habitat corridors or preserved as separate adaptive units.

Keywords: endangered species, conservation genetics, immunogenetics, population viability, *Reithrodontomys raviventris*

Poster Topic: Natural Resource Management: Endangered Species

Development and Use of an Environmental DNA (eDNA) Method for Determining Presence/Absence of Freshwater Mussels in the Tidally Influenced Delta

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Environmental DNA (eDNA) analysis is developing into a useful tool for detecting aquatic biota that are invisible to traditional sampling surveys. A rapid, cost-effective method, eDNA allows for surveying of rare or cryptic organisms, such as freshwater mussels, without harming the species. We recently developed an eDNA method that successfully detected California native freshwater mussels in the genera Anodonta, Gonidea, and Margaritifera. To evaluate the eDNA method in tidally influenced waters, we initiated a two part pilot study to characterize the distribution of freshwater mussels in the Sacramento-San Joaquin Delta. In the first component of the study we collected duplicate water samples at ten sites across the Delta, including a site near Stockton where freshwater mussels were previously documented, to determine: (1) a detection rate, and (2) mussel eDNA signal strength. No Gonidea or Margaritifera were detected, but Anodonta spp. was detected at four of the ten sites. The presence of shells and live specimens confirmed the presence of Anodonta at one location where eDNA was detected in both replicate samples.

Due to the non-uniform manner in which eDNA is distributed in the large, tidally influenced Delta it was necessary to refine the eDNA protocol to determine the number and location of sampling sites necessary to accurately determine mussel presence/absences. In the second component of the study we collected eight water samples along a single transect at each of the four sites where mussel eDNA was detected in the first part of the study. These results were used to develop a Delta specific eDNA sampling method.

Our results indicate eDNA is effective for determining mussel presence/absence in tidally influenced waters. Wastewater treatment plants in the Delta can use our eDNA sampling method to determine mussel presence/absence to comply with 2013 Environmental Protection Agency ammonia criteria.

Keywords: Environmental DNA, Freshwater Mussels

Poster Topic: Natural Resource Management: Endangered Species

Salt Marsh Harvest Mice (*Reithrodontomys raviventris*) Distribution, Abundance, and Population Trends in the East Bay Regional Park District

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The Salt Marsh Harvest Mouse (*Reithrodontomys raviventris*) is a state and federally listed endangered species endemic to the salt marshes of the San Francisco Bay Area. As a result of anthropogenic change, its pickleweed-dominated tidal marsh habitat has been greatly reduced. Habitat loss is the primary threat to the Salt Marsh Harvest Mouse. The East Bay Regional Park District manages 40 miles of shoreline, which includes viable Salt Marsh Harvest Mouse habitat. Using standard survey protocols, District staff, interns and volunteers surveyed five sites for Salt Marsh Harvest Mice between 2012 and 2016. During the months of May through August, greater than 100 Salt Marsh Harvest Mice were captured, with a total population index that approximates 2.5 (individuals/trap nights x 100). There was no significant correlation between captures and pickleweed height and coverage; however, populations differed significantly among sites. Continued monitoring is necessary to document their status, distribution, abundance and population trends to help inform conservation efforts for these and other special status species inhabiting tidal wetlands managed by the East Bay Regional Park District.

Keywords: Salt Marsh Harvest Mouse, endangered species, conservation & management

Poster Topic: Natural Resource Management: Endangered Species

Using DNA from Beetle Feces to Improve Cryptic Species Monitoring

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The Valley elderberry longhorn beetle (VELB) is an endangered subspecies inhabiting riparian habitat in the Bay Delta watershed. The loss of >90% of Central Valley riparian habitat has been a major cause of VELB decline. The USFWS monitors VELB abundance but this species is notoriously difficult to survey because adults are rarely encountered and larvae spend most of their lives embedded in the stems of their host plant, the elderberry (*Sambucus spp*). Surveys are limited to counting exit holes made by newly metamorphosed adults emerging from elderberry stems. However, this survey method is inadequate because VELB occupancy of elderberry is low and it is impossible to confirm visually whether an exit hole was produced by VELB or another species or subspecies. VELB overlap in some parts of its range with its non-endangered sister taxa, the California elderberry longhorn beetle (*Desmocerus californicus*; CELB), and exit holes in areas of overlap could belong to either subspecies. As part of a larger project to improve VELB monitoring protocols, we are developing a method that will assign exit holes to species using DNA from the larval frass inside. DNA extracted non-destructively from VELB and CELB museum specimens was used to screen 17 primer sets amplifying 7 putative DNA barcoding loci for single nucleotide polymorphisms (SNPs) that distinguish the subspecies. Once candidate loci are identified, we will genotype a larger number of VELB and CELB to ensure that the SNPs are truly diagnostic throughout each subspecies range. We will present the results of our work to date and describe how this tool can be integrated into the VELB monitoring program.

Keywords: Valley elderberry longhorn beetle, Fecal DNA, DNA barcoding, Monitoring

Poster Topic: Natural Resource Management: Endangered Species

Predation of Salt Marsh Harvest Mice in the Suisun Marsh

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Common predators of the salt marsh harvest mouse (*Reithrodontomys raviventris*) have never been identified. During thousands of hours in the Suisun Marsh, during a three year field study we made opportunistic observations of both nocturnal and diurnal predators. Through visual sightings, auditory observations, as well as observations of sign, including scat and tracks, we identified likely common predators of the salt marsh harvest mouse. We also observed confirmed predation events by a northern harrier (*Circus cyaneus*), a white tailed kite (*Elanus leucurus*) and a yellow bellied racer (*Coluber constrictor*).

Keywords: salt marsh harvest mouse, *Reithrodontomys raviventris*, predators, Suisun Marsh

Poster Topic: Natural Resource Management: Endangered Species

Attenuation of Unionid Mussel Environmental DNA in a River Environment

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Environmental DNA (eDNA) has recently emerged as a useful tool for detecting cryptic or rare aquatic organisms, such as imperiled unionid freshwater mussels. Recent EPA water quality criteria incorporate stringent ammonia toxicity criteria for wastewater receiving water bodies where mussels are present. Thus, there is an urgent need for wastewater dischargers to develop sampling strategies to determine mussel presence or absence in their receiving waters. To ensure a robust sampling design, the attenuation of the eDNA signal from its source is necessary to determine the distance between sample locations. To that end, we conducted a pilot study to evaluate the attenuation of eDNA concentrations downstream of caged mussels in a five-mile study reach in California's Cosumnes River. Collection of eDNA samples during a reconnaissance survey indicated that one unionid mussel species, *Margaritifera falcata*, was present in the lower Cosumnes River. Twenty specimens of another unionid mussel species, *Gonidea angulata*, which was not detected in the Cosumnes River eDNA samples, were collected from the Pit River and temporarily placed into a cage located at the upstream end of the study reach in the Cosumnes River. After allowing eDNA from *G. angulata* to be transported downstream for several days, duplicate eDNA samples were collected at 0.25-mile intervals downstream of the cage. All eDNA samples were analyzed using quantitative polymerase chain reaction (qPCR). Concentrations of *G. angulata* eDNA were highest near the cage and decreased over the five-mile study reach. Field blanks collected during sampling were negative, indicating a low likelihood of false-positive eDNA detections. The attenuation results will be used to inform sampling design for determining unionid mussel presence/absence in receiving waters for Central Valley wastewater treatment facilities.

Keywords: Environmental, DNA, unionid mussels, ammonia, criteria toxicity, attenuation, sampling design

Poster Topic: Natural Resource Management: Endangered Species

Analyses of Longterm Monitoring Data to Address Priority Data Gaps for Endangered Salt Marsh Harvest Mice, *Reithrodontomys raviventris*

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The federally and state endangered Salt Marsh Harvest Mouse (SMHM; *Reithrodontomys raviventris*) is the only mammal species in the world that is endemic to tidal marshes (Greenberg et al. 2006) in San Francisco Bay (SFB). Despite tidal marsh restoration efforts numerous threats to tidal marsh vertebrates remain with highly-fragmented, remnant marshes supporting much of the remaining endemic northern and southern subspecies.

The *Draft Recovery Plan for Tidal Marsh Ecosystems of Northern California and Central California* has identified that the SMHM may be at “risk of extinction due to vulnerability of small populations in the face of random naturally occurring events” (USFWS 2010). As a result, the Draft Recovery Plan called for scientific research efforts pertaining to survival and recovery in order to develop best management practices by the U.S. Fish and Wildlife Service (USFWS 2010). The California Department of Fish & Wildlife’s (CDFW) goals are aligned with the USFWS Draft Recovery Plan as outlined in the Strategic Plan to “...focus inventories, research, and resource assessment efforts on high priority habitats, species at risk ...” and to “direct activities toward maintaining, enhancing, and restoring wildlife”

Here, we directly address the critical, basic research needed “to fill in gaps in the understanding of the current distribution, density, and demographics of the salt marsh harvest mouse,” which received the highest priority for recommend actions over the next 5 years (USFWS 2010). We conducted population analyses from mark-recapture monitoring data to inform future standardized monitoring protocols and to address population variability over time in Suisun Marsh with detailed analyses at Crescent Unit. Trap session of 4 nights resulted in higher population estimates than with 3 nights of data. Preliminary mark-recapture analyses show that Hill Slough had the highest population of SMHM, followed by Peytonia, Crescent Unit, Bradmore Is. Reproductive demographics were skewed towards males.

Keywords: Salt marsh harvest mouse, population, distribution, demographics

Poster Topic: Natural Resource Management: Endangered Species