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A RANDOM SAMPLING OF SALT MARSH HARVEST MICE IN A MUTED TIDAL MARSH

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Abstract: Successful conservation of an endangered species, such as the salt marsh harvest mouse (SMHM; *Reithrodontomys raviventris*), requires detailed knowledge of the species' habitat and microhabitat associations. Identifying critical habitat features, however, can be problematic. We designed and implemented a randomized sampling regime with a large sample size of sites ($n = 40$) to identify potential microhabitat requirements or habitat constraints affecting the localized distribution of the southern subspecies of SMHM (*R. r. raviventris*). We used the random trapping technique to determine SMHM distribution across a muted-tidal, predominantly pickleweed (*Salicornia virginica*) marsh at the Don Edwards San Francisco Bay National Wildlife Refuge, California, USA. We tested the null hypothesis that this species' distribution is not associated with pickleweed salinity, sympatric rodent species, or the site location (peripheral vs. interior). We found SMHM throughout the marsh and detected no association with interior versus peripheral sites. We detected no associations between SMHM and sympatric rodent species. Pickleweed salinities ranged between 200 and 899 mmol/kg Cl⁻. In the prebreeding phase, SMHM were randomly dispersed. However, in the breeding and postbreeding phases, the population was clumped and associated with sites having mid-range (500–699 mmol/kg Cl⁻) salinities in pickleweed. Managers and biologists attempting to conserve SMHM in managed marshes need to be concerned with maintaining this mid-range salinity level in pickleweed. We found that SMHM were absent from areas of low salinity (200–499 mmol/kg Cl⁻) and were rare in hypersaline areas (699 mmol/kg Cl⁻). Mid-range pickleweed salinities can be created and maintained in managed SMHM habitat only via fresh infusions of sea water on a natural tidal basis.

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Key words: California meadow voles, habitat preferences, *Microtus californicus*, pickleweed salinity, random sampling, *Reithrodontomys megalotis*, *Reithrodontomys raviventris*, salinity, salt marsh ecology, SMHM, San Francisco Bay, western harvest mice.

Successful conservation of an endangered species such as the SMHM requires detailed knowledge of its habitat and microhabitat associations. The failure to adequately examine and describe specific habitat requirements for special-status taxa may lead to misunderstanding a species' habitat requirements and could potentially result in management decisions that actually contribute to the extinction or commercial extinction of a species (Storer 1931, Weber 2002). For nonspecial-status species, poor management decisions can lead to the taxon becoming a candidate for either state or federal protection (Storer 1931, Weber 2002).

Salt marsh harvest mice are endemic to the salt and brackish tidal marshes of the San Francisco Bay (Dixon 1908, Fisler 1965), but suitable habitat for this species continues to decline. Habitat loss was the primary factor in the SMHM being listed as endangered by the U.S. Fish and Wildlife Service in 1970 (35 FR 16047) and the California

Department of Fish and Game in 1971. Approximately 810 km² (80%) of the historic tidal marshes inhabited by SMHM have been destroyed through filling, diking, or subsidence (Shellhammer et al. 1982, Goals Project 1999). Sixty percent of the remaining habitat is now diked (Nichols and Wright 1971, Shellhammer et al. 1988), resulting in highly altered, disjunct, and isolated patches of former habitat. These remnant diked and leveed marshes are subject to large variations in salinity depending on the amount of freshwater precipitation that collects in the diked areas and the degree of salt accumulation in the levee soils. Some marshes receive muted tidal flow, whereby seawater reaches the marsh via a (usually lengthy) indirect route that is controlled by tide gates (Goals Project 1999). These muted tidal marshes generally receive a fresh infusion of seawater only at the highest high tide.

Pickleweed is the dominant halophytic vegetation in most Bay Area tidal salt marshes (Mason 1957, Goals Project 1999). Numerous scientists have concluded that the optimal macrohabitat of SMHM is one dominated by pickleweed (Fisler 1965, Shellhammer et al. 1982, Bias 1994), especially when thick stands are heterogeneously

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mixed with other marsh species (e.g., alkali heath [*Frankenia grandifolia*]; Shellhammer et al. 1988). However, previous research has been restricted primarily to pickleweed-dominated habitats and pickleweed patches within entire marshes, particularly for studies on the southern subspecies.

A few studies have been conducted to examine salinity as a potentially important microhabitat requirement for SMHM. Fisler (1963, 1965), Zetterquist (1977), and Geissel et al. (1988) concluded that SMHM are well adapted to hypersaline environments. Pickleweed is the main diet component of the northern subspecies (*R. r. halicoetes*). Individuals of the northern subspecies will preferentially drink saline water only slightly less concentrated than seawater, even if offered fresh water (Haines 1944; Fisler 1963, 1965). In the laboratory, Fisler (1963, 1965) documented the dietary habits of SMHM and found that they ingest salt through both food and water. Salt is an integral component of the tidal salt marsh system as it is present across the habitat in the water, the soil, and the vegetation.

If a vertebrate drinks seawater, the salts are absorbed and the concentration of salt in the body fluids increases. If the salts are not excreted in a solution at least as concentrated as seawater, the body will become increasingly dehydrated (Schmidt-Neilsen 1990) and the organism will ultimately die. Therefore, since salinity intake is a limiting factor for most organisms (Schmidt-Neilsen 1990), including SMHM (Fisler 1965), investigating possible associations between salinity in pickleweed and the distribution of SMHM seemed appropriate. The salinity concentration within stands of pickleweed could vary on a microhabitat scale, and this variation could be associated with SMHM distribution within these stands.

Interspecific interactions also could potentially affect the distribution of SMHM within their preferred macrohabitat. Geissel et al. (1988) decided that competition exists between SMHM and California meadow voles (*Microtus californicus*). They concluded that SMHM are forced into lesser-quality habitat in the presence of high densities of California meadow voles (a.k.a. meadow voles), and that SMHM can only move into better-quality habitat when vole numbers decline.

Previous SMHM research has been frequented with a variety of logistic, methodological, and statistical problems. Historically small-mammal researchers have employed nonrandom trapping grids or transects as their sampling methodology (Shellhammer et al. 1982, Boonstra et al. 1987,

Bias 1994). In SMHM studies, trapping sites typically have been selected on the basis of characteristics believed to be important by the investigator but not verified as important (Rice 1974, Muench 1985, Johnson and Shellhammer 1988). Since little is known about the microhabitat preferences of SMHM within pickleweed-dominated marshes, researchers may introduce another form of bias by employing a nonrandom human perspective and choosing their study sites based on perceived optimal or marginal conditions or other criteria (Zetterquist 1977, Bias 1994, Geissel et al. 1988). Restriction of research to such areas reduces the inference of microhabitat preferences to specific areas within entire marshes and may further introduce bias in the findings. These potential biases make interpretation of the results ambiguous and can reduce the possibility of identifying actual microhabitat requirements within the larger macrohabitat.

In addition to nonrandom methodology, small sample sizes and pooling of data into a single data set have characterized past SMHM research (Rice 1974, Zetterquist 1977, Shellhammer et al. 1982). Pooling data across a study period to perform statistical analysis is a common practice with studies of endangered species that typically generate small sample sizes (Schroder 1987).

For our study, we attempted to circumvent some of the problems of identifying microhabitat requirements or habitat constraints affecting the localized distribution of SMHM. We employed a randomized design with a large sample size of sites ($n = 40$). Prior to undertaking this study, we successfully tested the feasibility of the random sampling design in a pilot study conducted at the New Chicago Marsh, Don Edwards San Francisco Bay National Wildlife Refuge, Alviso, California, USA, in spring 1996. In this study, we examined habitat and microhabitat components that might affect the distribution of SMHM. We quantified vegetation type at each sample location and investigated potential associations with California meadow voles, house mice (*Mus musculus*), western harvest mice (*Reithrodontomys megalotis*), salinity levels in pickleweed, and distance to habitat boundaries (i.e., site location).

STUDY AREA

New Chicago Marsh is a 142 ha, formerly diked marsh in the southernmost portion of the Don Edwards San Francisco Bay National Wildlife Refuge. It is one of the largest patches of remaining suitable SMHM habitat in the South Bay. The

area also is known to be inhabited by western harvest mice, California meadow voles, and house mice. Prior to 1994, the marsh was closed to seawater and only received water via freshwater precipitation. Under pre-1994 (diked) conditions, soil salinities increased and the soil pH became more basic than nearby tidal marshes (Eicher 1988). In the mid-1990s, the New Chicago Marsh was converted from a diked to a muted-tidal system (Goals Project 1999). The marsh was modified to allow tidal water to enter the system during the highest high tides via a manually operated tide-gate at the inlet, located approximately 1.5 km north of Triangle Marsh. During our study (1997), the marsh was dominated by pickleweed but reflected the alteration from tidal to diked marsh. Our study area consisted of a continuum of habitat types, ranging from one dominated by grass (introduced annuals and salt-grass [*Distichlis spicata*]) with no pickleweed, to one consisting of 100% pickleweed.

METHODS

Random Sample Point Selection

We divided a topographic map of New Chicago Marsh into 207 50×50 -m blocks. We randomly chose 40 blocks, and each 50×50 -m block was used as 1 trapping location (i.e., 1 block = 1 trapping site). In the field, we located each of the randomly selected 40 sample blocks and subsequently characterized each block as either interior or peripheral. Interior sites were in portions of the marsh that were flooded (or bounded by water on all sides) and were accessible only by kayak. Peripheral sites were located around the periphery of the marsh, which is contiguous with the inland landmass; these were accessible on foot (Fig. 1). As a result of the random draw and not by design, we identified 20 sites as interior and 20 as peripheral.

Live Trapping

We placed 3 noncollapsible Sherman live traps 5 m apart in a triangle formation in the middle of each 50×50 -m block for a total of 120 traps. Traps were baited at dusk with birdseed and crushed walnuts and supplied with cotton batting. We conducted sampling monthly from April 1997 through September 1997. We operated traps for 4 nights each month, for a total of 2,880 trap nights. All animals were released within 1 hr of sunrise.

We identified all captured rodents to species and then ear-tagged each individual with a

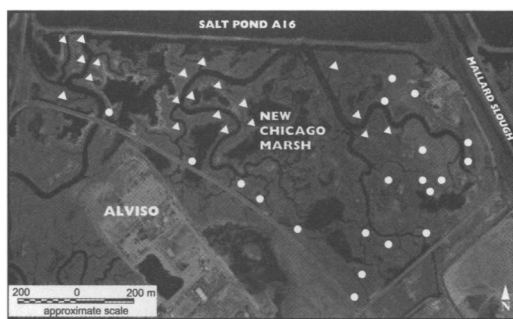


Fig. 1. Random trapping locations; New Chicago Marsh, Alviso, California, USA, spring 1997. Twenty sites characterized as interior, were accessed by boat (Δ). Twenty sites characterized as peripheral, were accessed on foot (\circ). New Chicago Marsh experiences muted tidal flow during high tides as water enters from the inlet 1.5 km north.

unique numbered tag. We sexed, measured, and weighed all captured animals. Salt marsh harvest mice were differentiated from western harvest mice using criteria developed by Fisler (1965) and refined by Shellhammer (1984).

Vegetative Composition

We determined plant species composition at each trapping site using the intercept line transect method (Brower et al. 1989). We measured horizontal coverage of vegetation to the nearest 5 cm. Descriptive statistics (percent cover and percent relative cover) were calculated to describe the overall vegetative composition of the marsh and the vegetative composition of each sample location. We determined presence and relative cover of pickleweed for all sites.

Pickleweed Salinity Analysis

On the final day of each sampling period, at each of the 40 sites and within the triangular area outlined by the trap placement, we randomly collected pickleweed by hand and subsequently analyzed the samples for mean salinity content. We bagged each sample, labeled it with the date and site location, and then stored the plant material in an iced cooler. Immediately following the conclusion of the morning's trapping activities, we transported the samples to the lab for processing. In the lab, we weighed the plant material, transferred it to paper bags, and then placed the bags in drying ovens to remove all water content. The plants were left until completely dry and then weighed again. We placed 10 gm of pickleweed from each sample in a centrifuge tube with 10 gm distilled water. This mixture was run through a

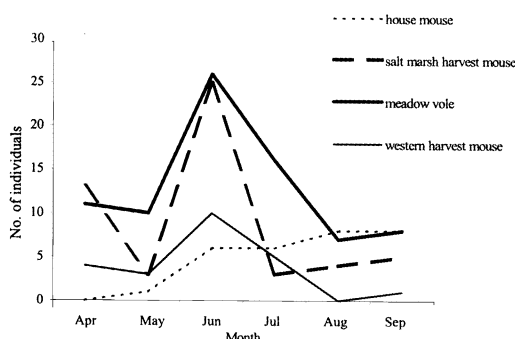


Fig. 2. Number of rodents captured in New Chicago Marsh, Alviso, California, USA, Apr 1997–Sep 1997. Only first-time capture data are included in the data set.

Cyclotron pulverizer, and we then centrifuged the resulting suspension to separate the supernatant from the cellulose. We tested salinity of the supernatant with an osmometer and expressed the results as mmol/kg of Cl^- in solution.

Preliminary Analysis of Pooled Salinity Data

To determine whether pickleweed salinity was related to the presence or absence of SMHM, we classified sites as to whether or not the mice were present. We performed an independent 2-sample *t*-test (Zar 1984, Tabachnik and Fidell 1989) on the pooled 6 months of survey data to determine whether mean pickleweed salinity differed between sites in which SMHM were present or absent. Only first-time capture data were used for all statistical analysis; recapture data were not analyzed for this study.

Distribution of SMHM

We subsequently divided the 6-month study period into 3 phases: prebreeding (Apr–May), breeding (Jun–Jul), and postbreeding (Aug–Sep). We performed a goodness-of-fit test to a Poisson distribution (Zar 1984, Tabachnik and Fidell 1989) on the capture data from each phase to determine whether SMHM distribution was random or patterned. For those phases in which a pattern was exhibited, we calculated the coefficient of dispersion (CD) to determine whether the distribution was clumped or uniform.

Associations Between SMHM and Microhabitat Variables

We used a 5-way hierarchical log-linear analysis (Zar 1984, Tabachnik and Fidell 1989) to test for associations between the presence/absence of

SMHM, pickleweed salinity levels, and the presence/absence of other rodent species. For this analysis, we used data from those time periods that illustrated a significant clumped or uniform distribution (i.e., breeding and postbreeding phases). Salinity data were ordinated into 6 levels: <399 mmol/kg Cl^- ; 400–499 mmol/kg Cl^- ; 500–599 mmol/kg Cl^- ; 600–699 mmol/kg Cl^- ; 700–799 mmol/kg Cl^- ; and 800–899 mmol/kg Cl^- . We characterized sites as to presence/absence of SMHM, western harvest mouse, meadow vole, and house mouse.

We performed an additional analysis to determine whether site location (peripheral or interior) affected associations between SMHM, pickleweed salinity levels, or the presence/absence of other rodent species. A 6-way hierarchical log-linear analysis was performed that included all variables from the previous log-linear analysis, plus site location.

RESULTS

Rodent Species

During the study period, 184 individual rodents were captured 440 times. We captured 54 SMHM (115 captures), 23 western harvest mice (63 captures), and 4 harvest mice that could not be identified to species (9 captures). Other rodents caught included 78 meadow voles (201 captures) and 29 house mice (61 captures; Fig. 2). Three shrews (*Sorex* spp.) also were caught.

Except for house mice, captures for all species increased through April and May, with greatest numbers captured in June (Fig. 2). Captures of SMHM declined in July, but increased again slightly through August and September. Meadow vole and western harvest mouse captures declined through July and August but rebounded slightly in September. House mouse captures increased gradually from 0 in April to 8 in September.

Vegetation Composition

We found that the New Chicago Marsh was predominantly pickleweed (63%) and alkali heath (20%). Bare ground also was prevalent with 11% relative cover across the marsh. Native saltgrass represented only 0.02% of all relative cover. The remaining 6% vegetative cover was a mix of mostly nonnative species: rabbit's foot grass (*Polypogon monspeliensis*), brass buttons (*Cotula coronopifolia*), seaside heliotrope (*Heliotropium curassavicum*), soft brome (*Bromus hordeaceus*), slender-leaved ice plant (*Mesembryanthemum nodiflorum*),

Table 1. Random trapping results data analysis for Jun–Sep 1997 at New Chicago Marsh, Alviso, California, USA. Significant associations for salt marsh harvest mice (SMHM), western harvest mice, and California meadow voles identified by 6-way log-linear analysis ($\alpha \leq 0.025$).

Association	P-value
Salinity*California meadow vole*site location	0.024
Salinity*SMHM	0.022
Site location*western harvest mouse	0.002
Site location* California meadow vole	0.021

sand spurrey (*Spergularia marina*), spiny sowthistle (*Sonchus asper*), and Australian saltbush (*Atriplex semibaccata*).

Pickleweed was present at 100% of trap sites. Relative cover of pickleweed at trap sites ranged from 2 to 100%, with an average relative cover of 63%. Seventy percent of trap sites had $\geq 50\%$ relative cover of pickleweed. Relative cover of pickleweed at SMHM capture sites ranged from 19 to 100%, with an average relative cover of 60%. Sixty-seven percent of all SMHM capture sites had $\geq 50\%$ relative cover of pickleweed.

Distribution of Salt Marsh Harvest Mice

Two of the 3 phases of our study period (breeding and postbreeding) showed a pattern to the population distribution of SMHM. Goodness-of-fit tests for the 3 phases indicated that the distribution of SMHM during the prebreeding phase was not different from random ($CD = 1$). On the other hand, the breeding ($CD = 1.407$) and postbreeding periods ($CD = 1.369$) were characterized by a clumped distribution.

Preliminary Analysis of Pooled Salinity Data

When we treated our study results as a single trapping effort, pickleweed salinity levels did not appear to differ between sites where SMHM were present and where they were absent. A 2-sample independent *t*-test indicated no significant difference ($P = 0.515$) between mean pickleweed salinity values in sites where SMHM were present and where they were absent.

Associations Between SMHM and Microhabitat Variables

The 5-way log-linear analysis for the breeding and postbreeding phases (those in which SMHM showed a clumped distribution), indicated that mid-range pickleweed salinity (500–699 mmol/kg Cl^-) was significantly related to the presence of both SMHM and meadow voles (Table 1). We found that the majority of SMHM and meadow

voles were captured at sites with mid-range salinity levels (500–699 mmol/kg Cl^- ; Fig. 3). Meadow voles and SMHM were absent at sites with the lowest salinity ranges (<500 mmol/kg Cl^- ; Fig. 3). We detected no statistically significant associations between the presence/absence of house mice ($P = 0.42$) or western harvest mice ($P = 0.63$) and pickleweed salinity values.

When we included site location (peripheral vs. interior) in the log-linear analysis, the results became more complex (Table 1). The 6-way analysis showed a significant 3-way association between mid-range-level pickleweed salinity, interior sites, and the presence of meadow voles (Table 1). We found no statistically significant relationships between house mice ($P = 0.25$), or western harvest mice ($P = 0.49$) and pickleweed salinity values. House mice were caught at low-salinity (399–699 mmol/kg Cl^-) sites. Western harvest mice were captured at sites with salinity levels between 500–699 mmol/kg Cl^- (Fig. 3).

Interspecific Interactions

We found no statistically significant 2-way or higher-order associations between meadow voles and SMHM ($P = 0.973$). At 14 of 20 sites where SMHM were captured, meadow voles also were present.

Site Location

Log-linear analysis showed no significant effect of site location on presence or absence of SMHM ($P = 0.23$). Through the 6-month course of our investigation, we captured SMHM at 25 of 40 sites located throughout the marsh (Fig. 4). Of these 25 sites, 58% were interior sites. During the peak

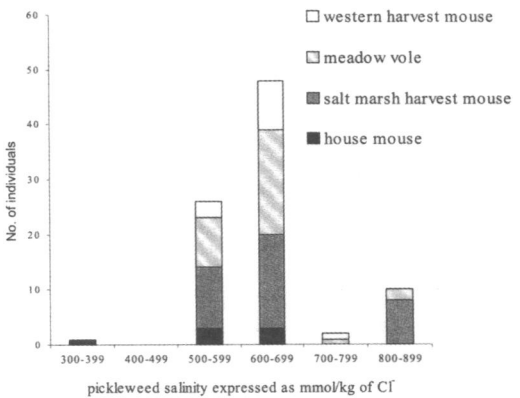


Fig. 3. Pickleweed salinity categories and total small mammal first-time captures per category for random trapping sites in New Chicago Marsh, Alviso, California, USA, Jun 1997.

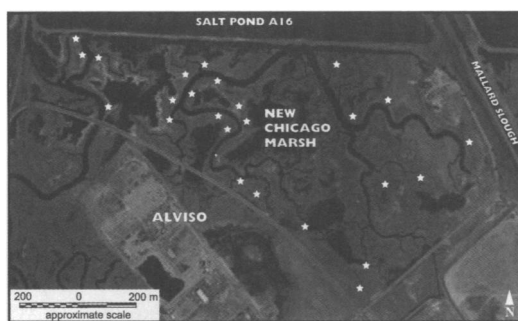


Fig. 4. Salt marsh harvest mouse capture locations (denoted ☆); New Chicago Marsh, Alviso, California, USA, Apr–Sep 1997.

capture month of June, 65% of the sites where SMHM were caught were interior sites.

We detected a significant effect of site location on the presence/absence of western harvest mice (Table 1). Western harvest mice were conspicuously absent from interior sites. Peripheral sites accounted for 92% of western harvest mouse captures.

The 3-way interaction between mid-range pickleweed salinity, interior sites, and the presence of meadow voles was found to be statistically significant (Table 1). Sixty percent of meadow vole capture sites were interior sites.

DISCUSSION

The distribution of SMHM displayed a clumped pattern during the breeding and postbreeding seasons, which in the absence of radiotracking was detectable only by random sampling the entire habitat. Wondollek et al. (1976) also found SMHM dispersion to be uneven within salt marshes, and concluded that most of their SMHM population preferred particular parts of the marsh although no statistical tests were conducted on their data. Bias (1994) found that habitat use differed seasonally among males and females.

The prebreeding phase showed no pattern to SMHM distribution, indicating that the mice were randomly distributed across the habitat. This suggests that during the prebreeding phase within pickleweed-dominated marshes, microhabitat resources important to SMHM are equally available throughout the habitat.

The presence/absence of pickleweed and the quality of that pickleweed strongly affect the distribution of SMHM. Over the last 20 years, a number of studies have indicated that SMHM use pickleweed as their primary habitat and that the

value of pickleweed increases with depth, density, and the degree of intermixing with other halophytic vegetation. However, the specific microhabitat requirements of SMHM have been more difficult to determine (Wondollek et al. 1976, Shellhammer et al. 1982).

Pickleweed height has been examined by many researchers and some preferences have been anecdotally noted at times, with researchers concluding that SMHM prefer pickleweed of a particular height (Wondollek et al. 1976, Geissel et al. 1988, Bias 1994). We think that the mice in these studies may have been keying on the salinity level within the pickleweed rather than the height of the plant, since height is an artifact of the salinity level of the plant. Geissel et al. (1988) found that the height of a pickleweed plant is inversely correlated to the salinity level within the plant.

Salinity in pickleweed is created through the influence of surrounding saline soils and water, but no relationship has been detected between water salinity and trap success of SMHM (Zetterquist 1977). In summary, past studies have followed the path of salt from water to soil to plant; and while the direction and components of the pathway have been clearly demonstrated, until our study, no association between one of those components and SMHM had been detected anywhere along this path.

We found a significant positive association between SMHM and meadow voles and mid-range salinity levels in pickleweed. All of our trap sites had pickleweed present, and although some SMHM were captured at sites with high pickleweed salinity, SMHM were absent where pickleweed salinity was extremely low. Kingma (Hayward State University, unpublished data) has since obtained similar results in a recently completed study on a restored marsh in the South Bay, finding a statistically supported association between SMHM distribution and mid-range salinity in pickleweed.

Despite similar capture rates, we detected no association between meadow voles and SMHM; whereas Geissel et al. (1988) concluded a competitive interaction existed between the 2 species. However, the latter study was restricted to a single grid located at the corner periphery of the marsh. Bias (1994) found that SMHM and meadow voles tend to occur in similar habitats. We found SMHM throughout the habitat, and we detected no significant association between the mice and interior or peripheral sites; whereas

western harvest mice were statistically associated with peripheral sites and meadow voles with interior sites. Again, in the absence of radiotracking data, these results are evident only with random sampling throughout the marsh.

Bias (1994) found that SMHM and house mice used overlapping, but different, habitats, and that house mice occurred in areas with the greatest grass cover. House mouse captures for our study were extremely low ($n = 29$), and therefore we treated house mouse data as descriptive rather than analytically. Low house mouse captures are reflective of the quality of the New Chicago Marsh habitat, because nonnative house mice tend to occur in higher numbers in highly disturbed marsh environments and areas in close proximity to human developments.

Site location could reflect a number of factors acting on SMHM population dynamics. Interior sites often are characterized by extensive waterways, flooding, floristic micropatterns, and predation pressures that differ from those associated with peripheral sites. Further investigation is needed to address the potential influences of the factors associated with site location on rodent populations.

MANAGEMENT IMPLICATIONS

The threat to SMHM continues to be habitat degradation (e.g., diking, desalinization, hypersalinization) as former tidal marshes become diked, and diked marshes become drying marshes from the lack of saline water flowing through them. Constituting 60% of the remaining habitat, managed salt marshes appear to be the key to long-term SMHM survival (particularly for the southern subspecies). Pickleweed grows extremely well in freshwater situations, and therefore monitoring managed marshes solely for pickleweed presence and abundance can be misleading. Managers and biologists working to conserve SMHM need to be concerned with monitoring and maintaining water salinity, water flow, and water levels, as well as pickleweed, within managed marshes. During the breeding and post-breeding phases, SMHM were associated with mid-range salinity (500–699 mmol/kg Cl^-) in pickleweed and avoided pickleweed of very low or extremely high salinity (200–499 mmol/kg Cl^-). Muted-tidal flows are necessary to properly manage diked salt marshes for SMHM conservation because pickleweed salinity is a direct result of the salinity of the surrounding soils and water. Lack of tidal flow results in either the desalinization or hyper-salinization of a diked marsh and

either of these extremes will result in a marsh that will not support SMHM populations for long-term conservation.

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