Successful care and propagation of the endangered amargosa vole (*Microtus californicus scirpensis*) in captivity

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The Amargosa vole (*Microtus californicus scirpensis*) is a highly endangered rodent endemic to a small stretch of the California portion of the Amargosa River basin in Inyo County's Mojave Desert. Although the Amargosa vole has survived in this naturally fragmented ecosystem for thousands of years, recent habitat degradation due to land development, water drainage, and marsh exploitation has further isolated the species and reduced its available habitat. As part of a conservation effort to preserve the species, a captive breeding population was established in 2014 to serve as an insurance colony and as a source of individuals to release into the wild as restored habitat becomes available. Here we provide information about appropriate care and husbandry for the Amargosa vole. We also provide recommendations for housing and disease management to preserve natural behaviors and defenses in captive-born animals.

**KEYWORDS**
captive breeding, captive management, conservation, husbandry

**1 | STATEMENT OF THE PROBLEM**

The Amargosa vole (*Microtus californicus scirpensis*) is a highly endangered rodent endemic to a small stretch of the California portion of the Amargosa River basin in Inyo County's Mojave Desert. Unlike other, more widespread subspecies of *Microtus californicus*, the Amargosa vole only inhabits patches of marsh habitat that are surrounded by harsh desert, limiting dispersal and keeping animals in small, isolated subpopulations (Cudworth & Koprowski, 2010; Neuwald, 2010; U.S. Fish and Wildlife Service, 1997). The average lifespan of a wild Amargosa vole is 3–4 months (Klinger, Cleaver, Anderson, Maier, & Clark, 2015) though six individuals have been noted to surpass 12 months of age in the wild, with the oldest surviving over 37 months. The Amargosa vole's habitat requirements include permanent standing water and vegetation dominated by Olney’s three-square bulrush (*Schoenoplectus americanus*) which is their main source of food and shelter (McClungaghan & Montgomery, 1998). Voles can swim, climb, and burrow through stacked bulrush litter that has accumulated over the tops of living bulrush plants. They consume bulrush rhizome, stalks, and seeds when available (McClungaghan & Montgomery, 1998). Amargosa voles can breed year-round but have higher reproductive success when bulrush emerges from winter senescence in spring (Clifford, Foley, Roy, & Allan, 2016; Cudworth & Koprowski, 2010). Because the Amargosa vole diet is so restricted and bulrush is low in nutrients and high in fiber, it is likely that they require extensive capabilities to ferment their food (Hammond & Wunder, 1991; Lovegrove, 2010).

Although the Amargosa vole has survived in this naturally fragmented ecosystem for thousands of years, recent habitat degradation due to land development, water drainage, and marsh exploitation has further isolated the species, and reduced its available habitat (Neuwald, 2010; U.S. Fish and Wildlife Service, 1997). Due to an increasing risk of extinction, the Amargosa vole was listed as endangered by the state of California (California Department of Fish and Game, 1980) and by the US Fish and Wildlife Service (Nowak, 1984). As part of a conservation effort to preserve the species, a
captive breeding population was established at the University of California, Davis. The colony serves as an insurance colony and as a source of individuals to release into the wild as restored habitat becomes available. Effective management and care are crucial to the success of the captive propagation program.

2 | DESCRIPTION OF THE PROCESS

2.1 | Housing and husbandry

The captive colony was founded with 10 male and 10 female juvenile Amargosa voles from the Mojave Desert in July 2014 with six additional male and six female voles added in April 2016. After 2-week quarantine, voles were housed in indoor cages or outdoor naturally landscaped pens (mesocosms). Indoors, temperatures were between 18 °C and 24 °C with a 12:12 hr day: night cycle. Cages were solid plastic with wire bar lids (Ancare, Bellmore, NY). Voles were housed individually in rat cages (26.7 × 48.3 × 20.2 cm) or in two- or three-vole groups of breed pairs or same-sex littermates in larger guinea pig cages (50.8 × 40.64 × 21.59 cm). A brick was placed on each cage lid for escape prevention (Figure 1). Bedding was rice or wheat straw at least 10 cm deep to mimic natural bulrush litter and to allow for burrowing and nest-building (Figure 2). Straw was autoclaved prior to use. Cages were spot-cleaned daily with soiled straw replaced. To minimize stress to animals, natural odors were allowed to accumulate and cage bottoms were replaced monthly. Voles received compostable cups (16 oz. Ingeo Clear Round Deli Containers, Boulder, CO) filled with potting soil for enrichment. The cups effectively encouraged natural digging behaviors.

Outdoor housing was used to help transition captive voles to natural conditions. Chain link runs (214 × 214 × 305 cm) with attached roofs and floors protected against large predators. Each run was completely covered with 1.3 cm wire mesh to prevent animal escape and access by small predators (Figure 3). Four 560 l structural foam stock tank mesocosms (147.3 cm × 99.1 cm × 63.5 cm) were inside each run (Rubbermaid, Winchester, VA). Mesocosm drains were covered with 1.3 cm wire mesh and gravel to prevent animal escape while allowing water to drain. Mesocosms contained potting soil with growing bulrush collected from Tecopa, CA and were covered with tight-fitting 1.3 cm screen with a latched wooden door to prevent animal escape (Figure 4). Voles were protected from heavy rain with a Plexiglas or wood overhang in one corner. Straw and burrowing opportunities provided additional protection from exposure. Behavior and activity level were monitored by setting camera traps (Reconyx, models PC 900 Hyperfire and RC60, Holmen, WI) in each mesocosm. Camera images were reviewed twice per week. Any indication of poor health or aggression was noted and appropriate management actions taken. Male voles housed outdoors were observed to become aggressive toward each other, even if raised together indoors without previous incident; however female siblings could be safely housed together outdoors indefinitely. For this reason, only female voles were housed in groups outdoors.

2.2 | Diet

Maintaining captive voles on a bulrush-only diet presented a significant management challenge because voles destroyed living bulrush very quickly and growing sufficient bulrush in greenhouses was impractical. Voles rejected bulrush when first moved into captivity and were maintained on commercial pelleted diet, which was rodent chow (Teklad 2018, Envigo, Hayward, CA) initially but transitioned to a high-fiber rabbit chow (LabDiet 5326–3, Stewart’s Feed Service INC, Lawrenceville, GA) to more closely mimic a high-fiber wild diet. In indoor housing, 3–4 pieces of chow were placed directly in the cage daily with ad libitum water in an open 12 cm × 5 cm bowl to allow natural water use behaviors, although voles regularly defecated and stored straw in their bowls. In outdoor housing, animals received 2–3 pieces of rabbit chow daily in addition to planted vegetation and water bowls which were scrubbed and refilled as needed.

FIGURE 1 Indoor Amargosa vole caging for (a) singly-housed animals and (b) group-housed animals in either breed pairs or same-sex sibling groups of two or three. A brick was placed on top of each cage lid to prevent animal escape.
2.3 Breeding and reproduction

We aimed to preserve as much genetic diversity within the colony as possible and maintained 95–110 animals at a time. Breed pairs were chosen to avoid mating closely related animals. Each colony founder was paired with a mate captured at least one trap line (10 m) away to prevent inbreeding. This was based on evidence from Pearson (1960) that families of California voles remain faithful to their established runways. Captive-bred individuals were paired based on pedigree data and additional wild individuals were brought into captivity 2 years after colony establishment to ensure continued genetic diversity. We preferred that animal ages be staggered; thus we employed a "trickle-breeding" approach to prevent a large cohort of newborn voles at any one time (Figure 5).

Female and male California voles are known to reach sexual maturity as early as 3 weeks and 6 weeks of age, respectively (Hatfield, 1935). Captive Amargosa vole females were paired with mates as early as 45 days old (mean 184 days, range: 45–417 days), but typically not older than 9 months. Females were almost always paired with older males. Mates were placed into a new cage during pairing to avoid potential territorial aggression but separated if aggression was observed. Only two cases of physical aggression were noted between breed pairs, with the females as the aggressors in both cases.

Some females gained notable mass and girth while pregnant, while others did not. Copulatory plugs were only conspicuous within a few hours of mating. Increased visibility of nipples could indicate pregnancy in primiparous females just prior to parturition, but the change appeared to be permanent and was not a useful indicator for multiparous dams. Gestation length was observed to be 21 days (Hatfield, 1935). If only one litter was desired from a pairing, the male was removed from the breeding cage after 20 days to prevent consummation of the female’s post-partum estrus (Greenwald, 1956). If more than one litter was desired in close succession, the male was left in the cage. Males did not become aggressive toward offspring.

The breeding cage was left mostly undisturbed for 10 days after pups were born to avoid stress to the dam and potential pup abandonment. Food and water were provided, but spot cleaning was minimal. Females displayed protective behavior when pups were younger than 14 days old and if a pup was uncovered by bedding, the dam would pull it back under protective cover. At 20–21 days after birth, pups were weaned, removed from the parental cage, marked with uniquely numbered metal ear-tags (Monel 1005–1, National Brand and Tag Co., Newport, KY), and housed in groups of up to three same-sex litter-mates.

2.4 Medical practices

Retro-orbital bleeding was used for Amargosa voles. Topical selamectin (60 mg/ml) and 7.4% permethrin-impregnated cotton balls (MiteArrest, Boston, MA) were effective for treating mites. However, Amargosa voles appeared to be sensitive to anesthetic drugs and we used approximately 0.2 ml ketamine/xylazine (1:9 ratio) to anesthetize a 100 g Amargosa vole, less than half of published doses for most small mammals.
2.5 Common diseases

Animals were assessed for health at least once per month during physical examinations. Mass, body condition score (Ullman-Culleré & Foltz, 1999), mentation (level of alertness), hydration, breathing, ear/eye/nose appearance, and presence of ectoparasites were evaluated. Voles euthanized due to health conditions were submitted to a pathology laboratory to undergo comprehensive disease testing and also to serve as sentinels for the colony. Although infectious disease screening was conducted regularly with sentinel voles, we did not aim to eradicate common pathogens and parasites unless they caused severe morbidity or were not present in wild Amargosa voles. This allowed the voles to retain natural defenses against pathogens. Amargosa voles developed tumors, abscesses, and various other diseases in captivity, the most common being molar apical elongation (MAE), which resulted in ocular discharge, abnormal mastication, dyspnea, abnormal mentation, weight loss, and death (Imai et al, in press).

3 DEMONSTRATION OF EFFICACY

Captive-bred Amargosa voles easily transitioned from indoor housing to more natural outdoor housing, which was promising for captive release attempts. Animals tunneled, chewed on bulrush plants, and built nests (Figure 6). As they do in the wild, Amargosa voles successfully bred year round in captivity (Cudworth & Koprowski, 2010). From June 2014 to August 2017, 285 voles were reared in captivity. Nearly 80% (57/72) of pairings produced pups and the average litter size was 2.88 pups (range: 1–6). Approximately 25% of paired females became pregnant within 7 days of pairing, and half within 14 days. Most pup mortalities occurred within the first 24–48 hr after birth and pups that died were often cannibalized by the dam. Average lifespan was 327 days in captivity (range: 37–1,034) and the majority of captive vole deaths were due to euthanasia for medical conditions. The diet transition to high-fiber rabbit chow potentially delayed onset and prevalence of MAE in the colony. Before the diet transition the average age of MAE onset was 5.3 months, ranging from 1.7–11.2 months. One year after the transition, only one animal under 12 months of age had developed MAE. Other diseases found in the colony were not attributed to life in captivity.

4 CONCLUSION

Amargosa voles can be successfully maintained in captivity. Non-standard laboratory bedding and water containers are recommended to encourage natural behaviors, and special care must be taken when administering anesthetic drugs. Otherwise, these animals are docile, easy to breed, and adjust well to commercial caging and food.

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