Bringing up (vole) babies: A captive-breeding colony of an endangered small mammal in a university setting

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The University of California Davis (UCD) Teaching and Research Animal Care Services (TRACS) didn't even blink an eye when we told them we were creating a captive-breeding colony on campus to help recover the endangered Amargosa vole. Our team was then challenged to created best-guess protocols for how to transport and rear the little darlings; we worked with TRACS, UC Davis Campus Veterinarian, and the Institutional Animal Care and Use Committee (IACUC) to set up facilities, and then took the plunge. On July 12, 2014, 20 voles were removed from a marsh that was experiencing a major ecological collapse,, loaded into an SUV heading north to UCD, and introduced into their new home. A second batch of 12 voles just joined them in April 2016. Two years after we started the colony we fondly call "Tecopa North", we reflect how *much* we have learned about voles, what we would want to change, and how vital this colony is as insurance that the little frustrating bundles of furry joy will not go extinct.

Creating a captive-breeding colony is a big responsibility and it's a lot of work. We did it because Amargosa voles are only found in marsh habitats in the Mojave Desert, near the tiny town of Tecopa, and those marshes showed signs of severe impact from building and development, water drainage, and drought. Population models clearly indicated that there was an unacceptably high probability of extinction unless new habitat was made available. Creating new habitat takes time the voles didn't have, so for a while, a cohort of them would live in captivity. And you do this by working within an agency/academic/non-governmental organization (NGO) partnership called the Amargosa vole team, you get the best data available on the subject; then, the "stewardship agencies" (California Department of Fish and Wildlife and US Fish and Wildlife Service) work with the federal landowner, Bureau of Land Management (BLM), to give the "green light" to load-em-up. UCD IACUC assesses the plans and protocols and gives the approval for the facilities and any staff caring for the voles.

At first, CDFW veterinarian Deana Clifford, UCD veterinarian Janet Foley, graduate student Risa Pesapane, and scientific aid Austin Roy explored different caging, foodstuffs, environmental enrichment, water bowls, and husbandry protocols to make the voles comfy. How do we know a vole is comfy? When it exhibits normal behavior, maintains body mass, and successfully rears its pups. Giant challenge #1: voles are picky eaters. As far as we know, wild voles eat only 1 species of grass-like plant, a sedge called three-square bulrush. Graduate student, Stephanie Castle, has now amassed an impressive supply of bulrush plants at UCD greenhouse facilities, but we didn't have these on day 1, and voles can destroy a single plant of bulrush in a few short days. Even though we'd like them to stay as natural as possible (because some day they're meant to go back to Tecopa South), they were going to need to eat food we could buy. They did not love: fresh organic sprouts, alfalfa hay, cat grass (in pot or not), apples, potatoes, carrots, rodent chow, rabbit chow, sunflower seeds, green beans, you name it, they ignored it. Oh, oh-

what's this? They nibbled jicama! Then they devoured sweet potato. They lived, they washed their food in their water bowls, and they adjusted to rodent chow. Thank goodness!

Giant challenge #2: How do we get them to make new vole babies? ? Rodents can kill each other if they want, so we had to devise elaborate protocols for gently introducing pairs, watching for any sign of aggression, and making sure daddy didn't harm his mate or his pups. And when we put a male in a female's cage, she basically ignored him. She in one corner, he in another. No noise, no kerfuffle, nothing at all. But about 4 weeks later (1 week to get to know each other and 3 weeks to gestate), buried in a carefully constructed nest was a litter, was 4 tiny pink hairless blind pups! And if we disturbed them at all, the mom would bark threats at us, so we hung back and just just fed and watered and got out of her cage! Breeding successfully. Check!

Giant challenge #3: releasing voles back into the wild. We have tried on two occasions to release voles from the colony back into the wild (release video here). In nature, Amargosa voles seem to live only 4-6 months but a really successful dominant adult can survive a year or more. In order to understand what happens to voles after being released, a subset of those voles wore radio-collars to help us track their movements. Of released voles we knew to have died, we saw two different causes of death: aggression with other voles and a condition called hepatic lipidosis which often happens when quite chubby animals abruptly stop eating. The aggression angle is so interesting! In the wild, voles seem to stay within fairly large "home ranges" although, from day to day, they spend the majority of their time in a small core area within the home range. Older literature suggested family groups tend to share trails through the habitat, while unrelated voles tended to avoid other animals' home ranges. But our radio-telemetry data confirm that unrelated voles will wander into each others' home ranges at times. Physical exams of wild voles don't show the ear-tearing and face scarring we see in more aggressive animals. So there's little evidence of them fighting in nature. In fact, whether caught in a trap or handled in the colony, one feature that biologists often comment on is how docile these Amargosa voles are, especially compared to other California voles (which are wiggling, squeaking, BITING little brats). We've even wondered if their all-bulrush diet could help them evolve the kind of slow and steady behavior that cows show? But correction- there is one time our Amargosa voles get mean. When we move inside voles out into naturalistic mesocosms with natural light, abundant dirt, growing bulrush, and LOTS of room; a very calm vole can become extremely aggressive. So when we release colony-reared voles into the wild, it will need to be in very low numbers and spread far apart. They'll have to meet and greet on their own terms from the safety of their own territories, once they establish them.

We've also gained quite a lot of perspective on their diets. A few field camera images suggest voles *might*, every once in awhile, consume something that isn't bulrush. Feed analysis of bulrush tells us this food is barely nutritious enough to keep, well, any animal alive. Our attempts to get them to eat taught us they are highly wary of novel food but can learn to eat them. We've also deployed motion-activated cameras in the colony to "watch" them eat and snuck up on them to record short videos of eating behavior (watch video here); this way we could determine how they eat their food and which parts of the plants they like the most. Often they stretch their

bodies as high up the stalk of the plant as they can (it's easier if there's a little clump of plants they can climb) and then they chew the very tops with seeds and flowers if present. (And if possible, they can launch off the top and try to escape! Weeeeee). Or, they dig in the dirt and gnaw on the hard little rhizome. If a piece breaks loose, they hold it in their little paws and chew on it like corn on the cob. This also helps them control the growth of their teeth. With the input of nutritionists Andrea Fascetti and Jon Ramsey at UCD, we're now pursuing an exciting new lead, which is how the natural bacteria that live in a vole's stomach and intestines (their microbiome) could help them make the most of their obligate low-quality bulrush diet. Cows do it, rabbits do it, and apparently voles do too: they depend on bacteria to *ferment* the food, extracting every last possible bit of energy and nutrition from something people or dogs or voles lacking the right bacteria would starve if they tried to live on. Good grief- keeping a healthy *vole* colony means we also need to keep a healthy vole *microbe* colony. We're working on that now.

And finally, giant challenge #4: rolling with the punches and problem solving the little issues that come up on a daily basis in the colony. Voles sometimes get sick. We saw a few voles develop hard hairballs in their stomachs. As they get older, often times their teeth become deformed, which is now being studied by a team led by campus veterinary pathologist Denise Imai. We had a super-odd case where part of a placenta dislodged and started to grow in the vole's leg muscle. One vole had a fungus in its tail that spread to its spine and testicles. And Sarah and Nora, our veterinary student intern and our fearless colony manager, spearheaded colony-wide eradication of the tropical rat mite after it started to flourish in the colony. Now where did that come from!? Potentially the straw, which required sterilization techniques in an autoclave, first making sure it wouldn't burst into flames while being autoclaved!

One major benefit that has come from housing a captive colony of endangered voles is the opportunity to learn new things about their biology and behavior. Oh the things we've learned about voles! We now know their age when they can first mate, how often they can have a new litter of pups, the average number of pups per litter, and how fast the pups develop. We have learned that they build tunnels in their straw and shred the straw to make soft nest lining material. We've also learned how to incorporate interns into an endangered species captive breeding facility, by facilitating undergraduate colony caregivers' learning opportunities via mini-research projects. They have helped us to learn things like: a) how fast voles' necks grow and when they reach maximum circumference, so we can safely radio-collar voles in the wild, b) how they use water, c) how to determine age in a young vole is by change in coat color, d) whether certain coat color patterns and their little white beard are inherited, e) how long they can live, f) how to score aggressiveness once voles are moved to outside mesocosms, and g) how voles respond when given live plants and pots of dirt. You probably guessed what they did: shred, dig, and chew.

The BLM funded the sequencing of the entire genome at Purdue University of a vole from our colony. Using that genome and any traits we see in our captive voles, we can develop genetic tests to better understand many features of their biology. We are the proud "parents" of dozens of bouncing, bratty little Amargosa voles in a successfully reproducing insurance colony against

any disaster in the wild. As the vole team works to provide restored and newly upgraded wild habitat, our captive-reared voles will be prepared to get back to Tecopa South and start a new page in the vole history book.

About us: Janet Foley and Deana Clifford are veterinarians, founding members of the Amargosa vole team, and Co-Principal Investigators of the grants that fund the colony. Austin Roy is an environmental scientist at CDFW (a scientific aid at the time the colony was founded) and has done everything on the project from cleaning vole poo to trapping in the desert. Risa Pesapane is a PhD student who set up the colony and all its protocols on day 1, with help from Austin, graduated student Amanda Poulsen, and a host of very talented and committed undergraduate volunteers. Graduate student Stephanie Castle studies wetland ecology and restoration, and now vole nutrition. Nora Allan is a scientific aid at CDFW and our wonderful colony manager. Our captive colony and the research that we do receive generous support from Bureau of Land Management, California Department of Fish and Wildlife, UC Davis School of Veterinary Medicine, and US Fish and Wildlife Service, as well as donations from the public through our Indiegogo campaign (watch the campaign video).