HUSBANDRY REPORT

Development of Husbandry Practices for the Captive Breeding of Key Largo Woodrats (Neotoma floridana smalli)

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The Key Largo woodrat is an endangered rodent endemic to the island of Key Largo in the Florida Keys. After several reports documented a steep decline in the population, the US Fish and Wildlife Service developed a recovery plan, including captive breeding and reintroduction. Captive breeding efforts were to be focused on providing animals for future reintroduction to protected areas on Key Largo. However, little was known about the husbandry needs or reproductive behavior of this elusive nocturnal species. In 2005, Disney’s Animal Kingdom® received 11 animals and began to systematically investigate methods of breeding Key Largo woodrats. Since the program’s inception, 30 pups have been born and successfully parent reared. In this report, we describe some of the husbandry techniques that have contributed to the success of the Key Largo woodrat captive breeding program at Disney’s Animal Kingdom®. The results obtained may be of use to other facilities maintaining woodrats and other rodent species. Zoo Biol 30:318–327, 2011. © 2010 Wiley-Liss, Inc.

Keywords: endangered species; rodent; management; captive breeding

INTRODUCTION

Endemic to Key Largo, Florida, the Key Largo woodrat (Neotoma floridana smalli) is a nocturnal rodent found only in two protected reserves, Crocodile Lake National Wildlife Refuge and Dagny Johnson Key Largo Hammock Botanical State

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Park. The US Fish and Wildlife Service (USFWS) listed the species as endangered in 1984, and recent survey estimates continue to show a dramatic decline in the wild population [McCleery et al., 2006]. Likely causes of the decline include habitat loss and invasive predators [USFWS, 1999; Greene et al., 2007].

A recovery plan was developed for the species [USFWS, 1999] and in 2005 the USFWS invited Disney’s Animal Kingdom® (DAK) to assist in the development of a captive propagation program. To date, Key Largo woodrats are maintained in only two captive facilities: DAK and Lowry Park Zoo (LPZ; colony established in 2002). DAK received 6.5 Key Largo woodrats from LPZ on August 4, 2005. This initial group included 3.3 wild-caught animals and 3.2 animals born at LPZ from wild-caught parents. The goal of the captive breeding program at DAK was to develop safe and reliable breeding techniques to produce progeny that would then be released in protected areas on Key Largo to augment the existing wild population [USFWS, 1999; Slack, 2003].

The development of a captive propagation program for this nocturnal species presented special challenges. Observations of wild Key Largo woodrats indicate that they may be solitary, with single males, single females, or females with pups most commonly encountered in recent field observations [J. Potts, personal communication]. In the wild, Key Largo woodrats attain sexual maturity with adult pelage and descended testes or patent vaginal openings at an average body weight of 200 g or approximately 5 months of age [Hersh, 1981]. Cycle lengths are estimated to be 4–6 days in length [range 3–8 days, Eastern woodrat, Neotoma floridana; Chapman, 1951] and gestation length averages 38 days [range 37–40 days; Alligood et al., 2008]. In captivity, sexual maturity, indicated by descended testes and reproductive behavior, has been observed in males at a body weight of approximately 200 g and as young as 80 days. Copulation has been observed year-round, indicating a lack of seasonal breeding in captivity for this species [Alligood et al., 2009].

Additionally, our video observations of wild Key Largo woodrats suggest that interactions between unrelated conspecifics are typically limited to reproductive encounters. Simulating these circumstances in captivity is challenging as females do not display any obvious physical changes associated with estrus. Encounters between male and female Key Largo woodrats when the female is not in estrus can include agonistic interactions, sometimes resulting in serious injury [D. Murphy, personal communication]. Furthermore, although many rodent species have high fecundity rates, wild female Key Largo woodrats typically produce an average of two litters per year with two pups per litter [Brown, 1978]. Given the challenges inherent in breeding successive generations of an endangered nocturnal rodent that cannot be socially housed and exhibits a low fecundity rate, we needed to implement new strategies to maximize our success.

**HOUSING AND HUSBANDRY**

Key Largo woodrats were housed in an outdoor compound (9.3 m × 4.7 m × 2.3 m) at DAK and were exposed to natural sounds and scents. The compound was protected by a double-layer secondary containment system composed of a snake-proof and rodent-proof 22-gage 1/4” mesh fence supported by a 1” square aluminum framework (Corners Limited, Kalamazoo, MI), and an outer chainlink fence. The compound also had a ceiling consisting of 3/4” foam insulation panels and a roof.
constructed from polycarbonate corrugated panels mounted on wood rafters. The perimeter of the compound was treated quarterly with Maxforce Ant Bait Granular (Bayer Environmental Science, Research Triangle Park, NC) and Amdro Granular Ant Bait (Ambrands, Atlanta, GA) to prevent ant infestation, and electricity and water were supplied to the compound. The temperature was controlled as necessary to approximate the climate in Key Largo. When the outside air temperature dropped below 16°C, the woodrats were given extra nesting material (e.g., Spanish moss); below 13°C, plastic tarps were lowered to insulate the interior of the compound; below 10°C, portable heaters were turned on. To cool the woodrats in hot weather, fans were turned on inside the compound when the temperature reached 24°C or higher, and ice packs were placed beneath each nest box (described below) when the temperature reached 29°C or higher to create a microhabitat within the enclosure.

Each Key Largo woodrat was housed in a separate custom-designed stackable enclosure (92.7 cm × 62.2 cm × 59.7 cm) (Corners Limited). The enclosures were designed as stackable units to most efficiently use the space in the compound (Fig. 1). Each stack held three enclosures and the compound had a 10-stack capacity. Each enclosure had a wire mesh floor that allowed urine and feces to fall through to a paper-lined tray below, promoting cleanliness and facilitating collection of urine and fecal samples for hormone analyses. Each Key Largo woodrat enclosure was fitted with a flange on each side, allowing the connection of any enclosure to either side of any other enclosure via 4” diameter wire mesh tubes custom made by DAK’s Night Husbandry Team (Fig. 2). The tubes had wire mesh exclusion doors at each end. One advantage of this design is that the woodrats did not need to be physically removed from their enclosures to be paired. This feature helped to minimize handling of the animals. Enclosures were also fitted with a 22.9 cm × 22.9 cm × 33 cm nest box constructed of 1/2” thick gray opaque marine plastic to provide a sheltered, darkened area for sleeping (Fig. 3). The dense plastic also provided excellent chew-resistant containment. Enclosures and nest boxes were fitted with brackets designed for mounting Weldex B (Cypress, CA) 3.66 mm infrared color bullet video cameras. The cameras were connected to a networked surveillance system, allowing us to observe the woodrats without disturbing them and to record footage for later review.

Fig. 1. Stackable enclosures.
We used this video surveillance system when monitoring for the birth of new pups and when conducting research on maternal care, pup development, and activity budgets [Alligood et al., 2008, 2009].

Enclosures were supplemented with vegetation (e.g., palm fronds, love grass, willow, Spanish moss, hay, and wood shavings) to allow the natural behavior of nest building. Woodrats were frequently observed to manipulate the nest material. Water was provided ad libidum (in bowls and bottles) and the woodrats received a daily diet composed of a variety of mixed vegetables (including carrots, green beans, corn, and sweet potatoes), romaine lettuce, Mazuri rodent pellets (Mazuri Rodent Pellets 5663, St. Louis, MO), and seed mix fed once per day at dusk. Additional Romaine lettuce was added to the diet in December 2005 to provide an alternative means of water consumption. Key Largo woodrats encounter very little fresh water in their natural environment and consumption of leaves may account for much of their water intake. Woodrats were typically observed to consume all vegetables and lettuce.
immediately upon provision and to cache the rodent block in their nest boxes. They received new environmental enrichment items, such as willow, pinecones, grapevine, cardboard tubes, and running wheels, twice per week. Keepers performed the husbandry routine in the early evenings, to be consistent with the natural nocturnal behavior of the woodrats. All cage cleaning, environmental enrichment, food and water replacement occurred between 17:00 and 19:00 h. When the keepers reached into an enclosure (e.g., to provide food, water, or enrichment), they wore protective gloves and used a soft plastic exclusion panel (Fig. 4). The panel was attached to the front opening of the enclosure with Velcro® (Velcro USA Inc., Manchester, NH) and had slits in the center allowing the keeper’s hand to enter the enclosure but preventing the animal from escaping. At the conclusion of the husbandry routine, selected woodrats were paired for breeding (see below).

The Night Husbandry Team developed protocols to safely remove woodrats from enclosures for close visual examinations, weighing, and transporting without directly handling the animals. The woodrats frequently spent time in the cardboard tubes provided in their enclosures for environmental enrichment. When an animal was in the cardboard tube, keepers were able to place end caps or hands with protective gloves over the open ends of the tube, securing the animal in the tube for safe removal. The animal could then be weighed while in the tube or transferred into a small clear plastic carrier for close visual examination or transport. Nest boxes were also used to safely remove animals from the enclosures. Keepers were able to secure a woodrat into the nest box by placing a wire guillotine door over the entrance to the nest box. A small clear plastic carrier was placed over the nest box entrance and the guillotine door was lifted, allowing the woodrat access to the carrier. Once the animal entered the carrier, the top was secured, containing the animal.

When anesthetization was required for medical procedures, woodrats were transported to the DAK hospital in their carriers. At the hospital, the top to the carrier was partially opened and a large mammal anesthesia mask was quickly placed over the woodrat. The mask opening was held flush to the bottom of the carrier to prevent isofluorine gas from escaping. Once anaesthetized, the animal could be handled for the medical procedure.

Fig. 4. Soft plastic exclusion panel.
The ability to determine the day of estrus is highly beneficial to breeding success. For many rodent species, individual stages of the ovarian cycle are examined in relation to changing blood hormone profiles and vaginal cytology to characterize cycles, estrus, and pregnancy. However, because our colony animals were potential release candidates, handling was avoided when possible. As an alternative, we evaluated the use of noninvasive hormone monitoring to examine reproductive function.

Urine and fecal samples were collected for enzyme immunoassay analysis of progesterone and estrogen metabolites in females to characterize ovarian cycles, identify estrus, confirm pregnancy, and test for seasonality. A pilot study was undertaken to determine the optimum time of day for sample collection for hormone analyses. Fecal and urine samples were collected three times during the active period (dusk, midnight and dawn) from four adult females for 3 weeks, to analyze possible circadian changes in excreted hormone concentrations. Similar to hormone patterns in other nocturnal and crepuscular rodent species where onset of activity cycle, breeding activity, and hormone secretion co-occur [Goldman, 1999; Challet, 2007], concentrations of excreted steroids in Key Largo woodrats were higher at dusk. Therefore, this time was chosen for all future sample collection. Daily urine and fecal samples were collected from five females for 1 year, between August 2005 and August 2006, for measurement of progesterone and estrogen metabolites. Three of these females bred during this period, with two females successfully giving birth. Fecal and urine samples with expected high hormone concentrations (pregnant) were assessed for progesterone, pregnanediol-3-glucuronide, estradiol, and estrone metabolite immunoreactivity in established enzyme immunoassay systems [Graham et al., 2001; Munro and Stabenfeldt, 1984; Munro et al., 1991]. Samples from known cycling, estrus, and pregnant females (determined post hoc when breeding was observed and births occurred) were subsequently tested. Fecal samples extracted in 80% methanol showed good immunoreactivity for progesterone metabolites with values reaching 300 ng/g feces, but analysis of daily samples failed to show cyclic patterns of fecal progesterone indicative of ovarian cycles or expected changes in hormone concentration or patterns during pregnancy. Although serial dilution validations of pooled samples indicated measurable concentrations of immunoreactive progesterone and estrogen at low sample dilutions, none of the tested immuno assay systems reliably indicated ovarian cycle activity, estrus, or pregnancy. This suggests that the Key Largo woodrat does not primarily metabolize reproductive steroids in urine or feces in a form recognizable to the antibodies used in the currently available antiseraums in established assays.

Because hormone analyses produced limited results, subsequent reproductive studies focused on individual and interactive behaviors of males and females before and during pairing as a means of predicting whether a pairing would result in copulation [Alligood et al., 2009]. Each Key Largo woodrat was listed in the AZA regional studbook, and breeding decisions were made in consultation with a USGS geneticist and approved by the USFWS. The enclosures of the male and female to be
paired were placed next to each other in the compound and the enclosures were then connected to each other. The female was given continuous access to the tube so that she could interact with the male through the exclusion door. New male–female pairs were connected for a 7-day “introductory period,” which allowed the animals to have visual and olfactory but not physical contact. After this period, the exclusion door on the male’s end of the tube might be opened for pairing.

Before pairing, keepers watched for male–female interactions through the exclusion door. The higher the percentage of female visits to the tube resulting in interactions with the male, the greater the chances that the pairing would be successful. During pairings, keepers watched for lordosis by the female and following by both animals. These behaviors were indicators that the pairing might result in copulation. Both before and during pairings, keepers listened for raspy vocalizations from the woodrats. These vocalizations, produced by both the male and the female, were strongly correlated with copulation [Alligood et al., 2009].

Keepers carefully observed the behavior of males and females before and during pairings, and recorded their observations. Using the detailed data collected by keepers across hundreds of pairings, we produced the first information on the copulatory pattern of the Key Largo woodrat [Alligood et al., 2009]. When a male Key Largo woodrat mounted a female, his hind feet remained on the ground and his front feet were placed on the female’s back. The copulatory pattern included thrusting, ejaculatory locks, and multiple ejaculations [pattern 3; Dewsbury, 1975]. This pattern differs from the eastern and white-throated woodrats, which do not exhibit thrusts (N. floridana, N. albigula; pattern 7) and the desert woodrat, which does not produce an ejaculatory lock (N. lepida; pattern 15) [Dewsbury, 1972, 1974a,b, 1975]. The average interejaculatory-lock-interval in Key Largo woodrats was significantly longer than that reported for other species [Alligood et al., 2009]. This finding influenced the development of our pairing protocol, which specifies that pairs should be left together for at least 15 min following each ejaculatory lock.

Breeding observations indicated that Key Largo woodrats copulated on up to three successive nights. Typically, nearly every copulation in rodents results in pregnancy [Kohn and Clifford, 2002]. In captive Key Largo woodrats, fewer than 15% of copulations have resulted in pregnancy. Infertile matings resulted in the female breeding back with the male within 1–2 weeks following the initial copulation. When females were paired daily with a male during this period, they copulated a second time on an average of 9 ± 3.5 days following the initial copulation night (N = 17 copulation series, N = 8 females). Ten of these copulations occurred between day 10 and 15, suggesting induced pseudopregnancy in the woodrats. The remaining seven copulations occurred between days 4 and 7 following the initial breeding night. This period is too short to represent a pseudopregnancy response (considered a lengthened luteal phase to allow for implantation) and may instead represent disruption of ovulation [Conaway, 1971].

**PUP REARING**

Following copulation, female Key Largo woodrats were observed via infrared camera on a “pup watch.” The observation period began 33 days after copulation and continued for 10 days. Thirty live pups have been born since the program began (see Table 1). Five stillbirths have also occurred. When live pups were born, the dam
and pups were observed on camera in their enclosure and nest box. With more than 1,488 hr of observation, we have gathered the first information on Key Largo woodrat pup developmental milestones, rates of pups' physical attachment to the dam, dam activity budgets, and interactive behaviors between dams and pups [Alligood et al., 2008]. The pups received care exclusively from their dam and were disturbed as little as possible by the keepers. All 30 (16.14) live births in our colony have been successfully dam reared. Based on our data showing declining nursing rates at approximately 25 days after birth, pups began receiving their own solid food diets at that time. The pups were weighed and physically examined at 19, 30, and 65 days after birth (see Table 2). When litters included more than one pup, the second and third pups each received a metal hoop ear tag (in opposite ears) at the 30-day exam. Following the 19- and 30-day exams, the pups were returned to their dam’s enclosure. At the 65-day exam, pups were anaesthetized to obtain weights, measurements, radiographs, and blood samples for DNA analysis. At this time, they were implanted with an AVID chip. Upon recovery from anesthesia, pups were placed in individual enclosures separate from the dam.

FIELD APPLICATIONS AND FUTURE PLANS

As an extension of our work with captive Key Largo woodrats, the team has been actively involved in field work on the island of Key Largo. Our work in the field has enabled us to apply findings from our research with captive Key Largo woodrats to their wild counterparts. We are now able to estimate the age of pups observed in the wild because of the information we have collected on pup developmental

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**TABLE 1. Sex, Parentage, and Generation of Key Largo Woodrat Pups Born at Disney’s Animal Kingdom®, 2006–2009**

<table>
<thead>
<tr>
<th>Year</th>
<th>Sire (accession #)</th>
<th>Dam (accession #)</th>
<th>Offspring (Males.females)</th>
<th>Generation (at Disney’s Animal Kingdom®)</th>
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</table>

*aBorn at Lowry Park Zoo.  
bWild caught.  
cBorn at Disney’s Animal Kingdom®.
milestones in captivity, and we have assisted the USFWS Key Largo woodrat working group with the development of reintroduction plans. Eleven Key Largo woodrats born and reared at DAK have recently been released on Key Largo as part of a pilot reintroduction. We are also applying information gathered in the field to the management of our captive colony. For example, we have discovered several Key Largo woodrat food caches in the wild. The DAK horticulture team has cultivated some of these plants so that the captive animals can be exposed to them in preparation for reintroduction to Key Largo. In addition, DAK’s nutritionist has analyzed the composition of food caches and fecal samples found on Key Largo to facilitate the development of captive woodrat diets that more closely mimic the diets of wild woodrats.

Over the course of the program’s development, DAK has had great success in developing innovative husbandry practices, utilizing technology to observe behavior, investigating behavioral processes in reproduction and development, and applying our findings in the field. As the program continues to move forward, we have plans for several areas of growth. As reintroduction plans develop, we will continue to focus on maintaining a healthy captive population that can serve as a source of potential candidates for release. We will also continue to examine interesting aspects of Key Largo woodrat behavior. We have developed an extensive new system of wire mesh tubes mounted from the ceiling of our Key Largo woodrat compound. The system provides a wide range of possibilities for configurations, including runways of varying lengths and stations for the placement of nesting material or food. This will allow us to create unique environmental enrichment and simulate natural situations to study behavior, such as social interaction and foraging. Based on the role of raspy vocalizations as a predictor of copulation, we are presently conducting further studies of the Key Largo woodrat vocal repertoire. As we learn more about this fascinating species, we will continue to apply the findings to our work in captivity and in the wild.

CONCLUSIONS

1. Applying Key Largo woodrat natural history and the findings of empirical studies, we developed safe and successful husbandry techniques for Key Largo woodrat propagation.
2. Infrared video monitoring has allowed systematic observation of captive Key Largo woodrat behavior with minimal disturbance.
3. Using mesh tubes to connect enclosures and cardboard tubes and nest boxes to catch woodrats has allowed us to minimize handling during pairings and transfers.
4. Thirty Key Largo woodrats have been born and reared at Disney’s Animal Kingdom® since the inception of the captive breeding program. Eleven of these have been released on Key Largo.
5. Our results may be of use to other facilities maintaining woodrats for exhibit and/or propagation.

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