Care and Husbandry of the World's Only Flying Mammals

Bat husbandry aims to achieve optimal conditions for health, flight, reproduction, and social interactions.

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Bats are the only mammals capable of powered flight (Figure 1). By conquering the skies, bats diversified into a variety of niches, and with >1200 species, bats now represent nearly 25% of all mammals. Evolutionary adaptations to various niches have resulted in a diverse range of body sizes from the 2.0g fingernail-sized bumblebee bat to the 1.2 kg giant golden-crowned flying fox with an impressive two-meter wingspan. Adaptations for flight, such as elongated bones and compliant wing membranes, make bats a unique animal model for studies of skeletal biology, locomotion, sensory biology, and wing membrane wound healing. Bats have also been recognized as unique models for reproductive physiology and neurobiology. Because bats are nocturnal and the majority use laryngeal or tongue-click echolocation to orient and hunt in total darkness, they are excellent animal models for understanding the neural mechanism of hearing. Unlike humans who only hear sounds between 0.020 - 20 kHz, the auditory system of bats ranges up to <200 kHz. Beyond having a greater range of sensitivity to sounds, bats are also incredibly resilient to hearing loss. They can be exposed to sounds as loud as 140 decibels of sound pressure (i.e., louder than a rock concert) without associated hearing loss.

Figure 1. *Carollia perspicillata*

Figure 2A-B. *Carollia* are leaf-nosed bats (A). Individuals can be identified with wing bands or microchips (B). Photo credit, Dr. Christopher J. Vinyard.

Husbandry of these unusual and complicated creatures requires creativity and attention to species-specific needs. Authors from the Northeast Ohio Medical University (NEOMED), SUNY Downstate, and McMaster University formed a collaboration aimed at optimizing the health and enrichment of bats as laboratory animals. This article highlights unique aspects of bat husbandry that achieve optimal conditions for health, flight, reproduction, and social interactions of two small-bodied bat species.

Veterinary Procedures

Quarantine and acclimatization procedures are specific to the institution, bat source (e.g. wild-caught or captive bred), and necessary experiments. Wild-caught bats are acclimated for 1-2 weeks on arrival as part of up to a 90-day quarantine period. Quarantine allows for the treatment of parasites, observation for development of subclinical conditions, and clinical vaccinations. At NEOMED, wild-caught bats are given topical treatment with kitten flea powder (0.1% pyrethrin + 1% piperonyl butoxide) for external parasites (lice, mites, fleas), and when not precluded by experimental use they may also be treated with Ivermectin (4 micrograms, subcutaneous, 2 weeks apart). Wild-caught bats are also given two rabies vaccinations (50 μL, killed virus vaccine, ~4 weeks apart), whereas 1 vaccination is given to captive-bred animals arriving at the facility. At McMaster University, wild-caught bats are given a cutaneous (topical) application of selamectin (10-20 μL of Revolution® purple for dogs; 120 mg/mL) for ectoparasite treatment. At both NEOMED and McMaster, individual bats are identified with colored, numbered, plastic split-ring forearm bands and subcutaneous passive integrated transponder (PIT) tags. Bats that appear ill and die (or are euthanized) during quarantine are usually submitted to a public health laboratory for rabies testing.

All personnel who care for or handle bats for experimental procedures are required to receive pre-exposure rabies prophylaxis and wear personal protective equipment (PPE) appropriate for the work being performed. Examples of typical PPE include leather gloves for handling bats, lab coat or gown, hair net, foot nets, surgical mask, and face shield or eye goggles. Injuries to humans who work with bats are extremely rare; nevertheless, when humans are bitten by a bat they should wash the bite wound thoroughly with soap and water and report the incident to the appropriate health authority (e.g., institutional safety officer or occupational health professional, local public health authority) who will evaluate for post-exposure rabies prophylaxis.

Seba's Short-Tailed Bat (*Carollia perspicillata*)

Carollia perspecillata (Family Phyllostomidae) are leaf-nosed bats commonly used as a model for studies in mammalian reproduction, skeletal biology, auditory neurobiology, and evolutionary developmental biology (Figure 2A-B). Our colony was originally captured on the island of Trinidad to form a long-term colony at SUNY Downstate in Brooklyn, NY. A sub-population of this colony (n=30) was transferred to NEOMED where they reproduce successfully with females having a single pup at a time.

At NEOMED, *Carollia* are housed in three large walk-in flight-cages (4 × 1.5 × 2.1 m; l × w × h) with multiple roosting areas in each cage (Figure 3). Frames are made of PVC pipe with ceiling and side walls covered by a 1.5 cm plastic mesh. The room mimics a tropical habitat with temperatures near 27°C (80°F) with 60-80% humidity on a 12-hour light/dark cycle. For enrichment and to offset potential male-male conflicts, each cage has several wicker baskets (changed 2-3 times per year), a large, opaque nestbox containing hanging wooden panels, and grapevine wreaths (Figure 4).

Carollia are classically considered frugivorous (fruit eater) but are healthiest with multiple nutrient sources. We allot 32g per day of a puree or smoothie of peaches, peach nectar, monkey chow, and multivitamins for each individual. The diet is supplemented with freshly diced fruits including bananas, apples, mangos, melons, plums, and pineapple with whole fruits occasionally hung from ropes. To avoid bats plunging into food or water, pedestals along the walls support a tray-like table with two recessed food dishes and a chicken-watering device.

Carollia are easily stressed and prefer little human interaction, but are not aggressive. Thus, we minimize handling time within the flight cages. Flying *Carollia* can be caught with a soft fishing net and handled with leather

Figure 3. *Carollia* are housed in a large enclosure that supports flight at NEOMED.

Figure 4. Baskets and wreaths are hung to establish multiple roosts and minimize conflict.

Figure 5. *Eptesicus fuscus*

gloves. During flight cage cleaning and maintenance, the colony is moved into smaller cat-cages for a maximum of 5 days, handled as little as possible, and are given an abundance of fresh fruits treats to minimize stress.

Big Brown Bat (*Eptesicus fuscus*)

Eptesicus fuscus (Family Vespertilionidae) is one of the most common species of insectivorous bats in North America used in scientific research (Figure 5). *Eptesicus* is a model for studies in echolocation and sound production, neural mechanisms of hearing, mammalian wound healing, reproduction, and development. Most of the *Eptesicus* in the McMaster University colony were pregnant females that were originally wild-caught in the spring and subsequently gave birth to single or twin pups in captivity. As best as possible, our captive research colony emulates the structure of natural maternity colonies in the wild.

The McMaster *Eptesicus* husbandry facility is a combined indoor and outdoor enclosure (~2.5 × 8.3 × 2.7 m) nested in a building and private courtyard. The indoor portion of the caging system is divided into three sections: a vestibule for investigator entry and equipment (2.5 × 1.5 × 2.7 m), a quarantine cage that houses bats freshly caught from the wild (2.5 × 1. 5 × 2. 3 m), and an established colony cage that houses bats that have passed quarantine (2.5 × 1. 5 × 2. 3 m). Bats within the established colony cage can pass *ad libitum* through a narrow hole in an external wall to a large outdoor flying area (2.5 × 3.8 × 2.7 m) with a metal roof.

The vestibule lies between the two indoor cages and has separate access to each cage. A window in the vestibule provides bats with seasonal photoperiod cues. The temperature of the entire facility varies with ambient conditions. A wash down heater with a thermostat within this vestibule keeps the indoor winter colony temperature above freezing. The vestibule also contains PPE and environmental monitoring equipment, supplies for daily care, and a container (e.g. cooler with cage) for transporting bats.

Both cages and part of the outdoor flying area are lined with ¼" stainless steel wire mesh to prevent animal escapes and allow bats to freely crawl within the facility. The height of the mesh ceiling is lower in the cage areas to keep bats within human reach. Each cage contains multiple roosting areas: a folded bath towel suspended from the wire mesh and a hollow wooden roost (Figure 6A-D). Plastic vines suspended from the ceiling of each enclosure are moved randomly at regular intervals and thus serve as an echolocation environmental enrichment. Other enrichment is provided by wooden stumps and tree branches for roosting and behavioral staging. All wood is autoclaved prior to entry into the facility. The towels are cleaned/changed once per year when all bats are removed for one day to census and clean the colony. During cleaning, we scrub the caging mesh with a wire brush and a solution of Virkon disinfectant, rinse with water, and then air dry.

As *Eptesicus* are insectivores, they are fed a diet of mealworms (*Tenebrio*) obtained from a commercial supplier. A black light within the outdoor enclosure attracts wild insects to allow hunting

Figure 6A-D. The indoor enclosure for *Eptesicus* (A) at McMaster University. Attached to the sides of cages are trays of meal worms (B), a water tray with a plexiglass insert (C), a hollow wooden roost (D), and a bath towel for roosting. Garlands are also hung for echolocation enrichment.

Figure 7. The outdoor flight enclosure for *Eptesicus* at McMaster University contains branches and stumps for crawling and roosting, and a black light (not shown) to attract insects.

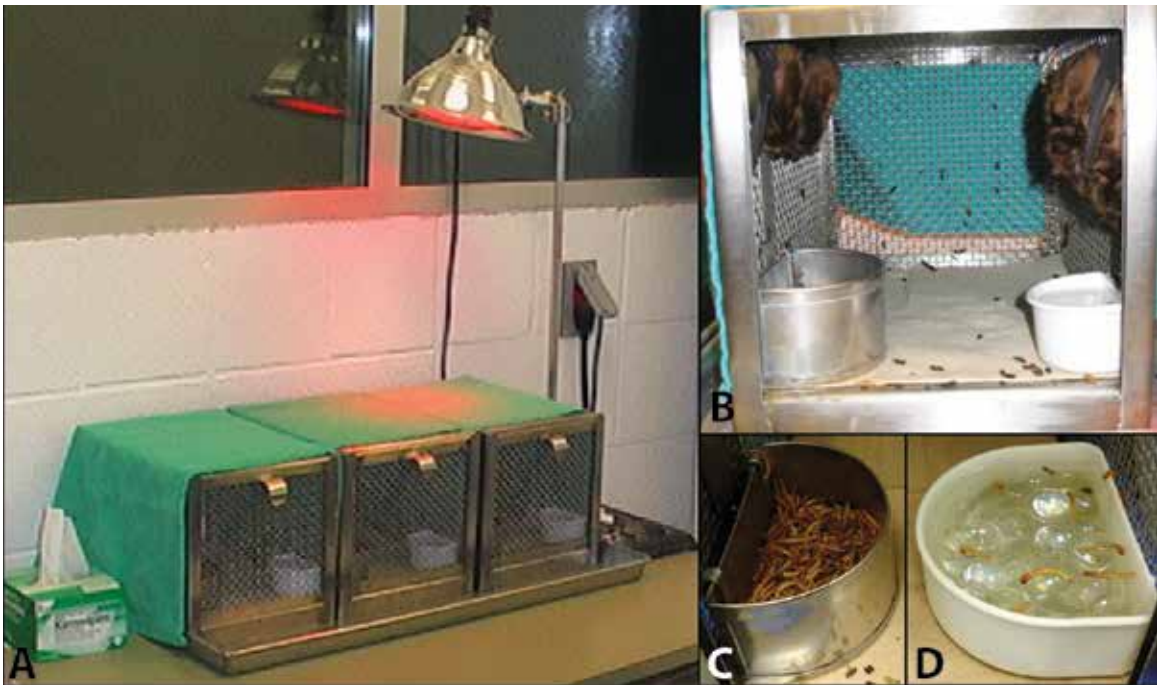

Figure 8A-D. Small cages for *Eptesicus* are kept at the desired temperature by a heat lamp (A, B) and contain mealworm (C) and water containers (D). To prevent drowning, the water container is partially filled with large glass beads.

enrichment (Figure 7). Superworms (*Zophobas*) are also provided as dietary environmental enrichment. Although nutritionally complete, we fortify the mealworm substrate with wheat semolina, powdered milk, calcium powder, Miner-All, Vit-All, spirulina and supplement with small amounts of freshly diced fruits or potatoes. In captivity, *Eptesicus* consume approximately 2-4 g of mealworms per day, depending on the season and the animal's reproductive status. New bats freshly caught from the wild must be individually trained to eat mealworms from a dish and this is somewhat laborious; fortunately, females that give birth in the lab teach their pups how to eat in captivity. Food/water trays in each cage are changed/cleaned daily and each water tray contains a plastic insert or large glass beads to prevent bats from falling in and drowning (Figure 8A-D).

While *Eptesicus* commonly live in manmade dwellings, they prefer minimal human interaction and are not aggressive. As a colonial species, *Eptesicus* prefer to interact with conspecifics, adapt well to captivity, and can be trained to work for food rewards, but can become obese when housed singly and/or not given space to fly and exercise. Although we have had success using towels, some captive colonies of *Eptesicus* cannot free claws caught in the loose threads. In this case, high thread-count sheets can be substituted.

Conclusion

Optimization of protocols extends to several species of bats besides the taxa discussed here. These efforts thereby set the stage for other investigators to elucidate the remarkable mechanisms shaping the genetic, physiological, and biomechanical attributes of the world's only flying mammal.

Animal use protocols and enrichment programs were approved by the NEOMED and SUNY Downstate Institutional Animal Care and Use Committee or the Animal Research Ethics Board of McMaster University. Animal care and use at NEOMED and SUNY Downstate is accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care, International (AAALACi), and at McMaster University it is accredited by the Canadian Council on Animal Care (CCAC).

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