Feature Article

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The Brown Tree Snake (*Boiga irregularis*) as a Laboratory Animal

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Researchers have not viewed reptiles as biomedical research animals in the past, yet they have come to realize the advantages of using reptiles over traditional mammalian species, such as rats and mice[1,2]. Indeed, using nondomesticated research animal models such as reptiles has lead to recent advances in the fields of endocrinology, animal behavior, toxicology, anatomy, physiology, neurobiology, and reproductive biology[1-9].

Advantages Over Mammalian Vertebrates

The increased use of reptilian species for biomedical studies is evidenced in the literature. Greenberg *et. al.* have implicated reptiles in biomedical studies investigating such diverse topics as physiological stress, chemoreception, ovulatory cycling, and developmental-communicative disorders and learning[1]. In that review, the authors stated that, "...several unique qualities of reptiles may provide models useful for research on a diverse array of problems of biomedical interest..." The *Biology of the Reptilia* series, now in its nineteenth volume, has provided a wealth of information concerning biomedical studies using reptilian models[2].

There are several advantages in using reptiles for biomedical research, especially for addressing questions at the organismal level. For example, reptiles, especially snakes, depend primarily on their chemical senses of olfaction and vomerolfaction to detect chemical stimuli from their environment[6]. They also display highly stereotypical behaviors that are often triggered by specific environmental cues, such as reproductive behaviors. These characteristics make reptiles predictable and consistent experimental animals when compared to other vertebrates, which tend to be multisensory in nature and have more complex, less stereotypical behaviors. These attributes not only provide less variability in experimental results, but also facilitate the creation of an environment in the laboratory that is biologically meaningful to the reptile of concern. Thus, it is relatively easy to maintain physically and behaviorally healthy reptiles in the laboratory[4,10-11].

The brown tree snake (*Boiga irregularis*) is a reptilian species that is now of great concern to biologists because of the ecological disaster it has caused on the Pacific island of Guam (**Fig. 1**). Although much research on the brown tree snake is being applied to controlling its population and limiting its dispersal to other islands[12,13], several studies have provided valuable knowledge of its physiology[14-16]. Work in our laboratories focuses on the reproductive biology of the brown tree snake, as well as its behavior, endocrinology, and chemical ecology[17].

The pressing need to control the brown tree snake as a pest species on Guam and on other Pacific islands has compelled our laboratories, and others, to begin laboratory-based studies addressing both applied and basic research concerns. Due to a variety of logistical problems involved with collecting snakes and performing our experiments on Guam, it was necessary to house a captive population in our laboratories. As the brown tree snake and other rep-

tile species become more common in laboratory research, there is an increasing need for established husbandry techniques.

There are well-established husbandry guidelines for common laboratory animals, such as avian and mammalian species[18]. In contrast, there has been less attention focused towards establishing husbandry guidelines useful to caretakers of animal facilities housing reptiles such as snakes[19]. Snakes differ from mammals and birds in many fundamental ways, reflected in the different physical and biological requirements of snakes in captivity[10-11,20-23]. Here, we describe the husbandry practices we have established for this tropical, arboreal snake.

FIGURE 1. A brown tree snake in its cage.

Biology

The brown tree snake, a rear-fanged colubrid, is native to the eastern and northern coastal areas of Australia, Papua New Guinea, and the Solomon Islands, where it is considered an unremarkable species[24]. Its primary habitat is in the tropical rain forests of these areas where they are nocturnal, spending the night foraging for an unspecialized diet of lizards, small mammals, birds, and bird eggs[25,26]. These snakes range in size up to three meters and can administer venom—considered mildly toxic to humans[14,27]—through grooves in their rear fangs. Snake victims have reported swelling, discoloration, and bleb formation at the bite site[28].

The brown tree snake was introduced accidentally to the Pacific island of Guam during or after World War II. Like many other Pacific islands, no native snake species existed on Guam. Over the next 50 years, this species flourished on Guam; a recent study found densities of brown tree snakes estimated to be nearly 13,000 snakes per square mile[27,29].

As Guam's brown tree snake population increased, it became a serious menace to humans on the island. These snakes climb power lines to search for prey and, in many instances, cross wires, causing power outages. This has been a major problem for the island (there have been 1200 snake-caused power outages from 1978-1994), and has caused severe losses in revenue and equipment[30,31]. These snakes will also enter houses, are very aggressive towards people if forced into a defensive position, and will repeatedly strike at the threat[32]. As a result, approximately one in a thousand visits to a Guam hospital is due to brown tree snake bites[33]. These snakes also prey on pets and small domestic animals, such as rabbits and chickens[34].

On Guam, the most devastating effect of the colonization of brown tree snakes has been on the avifauna of the island. The dense brown tree snake population has caused a dramatic decrease in the number of birds on the island[35,36], with ten of twelve species of native forest birds being completely eliminated. Three of these species were endemic to Guam and are now extinct[36]. The remaining species can be found on snake-free neighboring islands. With such a major loss in prey, the snakes now survive on introduced rats and lizards, small domestic animals, and the endangered Mariana fruit bat[25,37,38].

Unfortunately, the brown tree snake problem is not limited to Guam. Individual brown tree snakes have appeared on many immediately surrounding islands such as Wake Island, Diego Garcia, Okinawa, as well as Oahu, Hawaii[27]. Current management and research efforts are focused on controlling the spread of the brown tree snake, while controlling the population levels on Guam itself[12,13].

Husbandry

We have maintained a population of brown tree snakes in our laboratories at the National Institutes of Health, the National Zoo, and Oregon State University for the past six years. During this period, we have found them to be a hardy and durable laboratory animal. With proper handling and special consideration for their biological and behavioral needs, the brown tree snake is successfully maintained in the laboratory. We have developed a husbandry system that properly maintains the brown tree snake in laboratory-simulated tropical conditions.

The ambient temperature of our snake facility is held between 25°-30°C. A humidifier maintains the room humidity at 75-80%. Air flow is limited to preserve the humidity and to prevent cooler air from entering the room. Fluorescent bulbs, controlled by a tork clock, provide light.

The snake cages we use were designed specifically for housing arboreal snakes[39]. They are constructed of sturdy Plexiglass so that we can make unimpeded observations and clean the cages easily (**Fig. 2**). There is adequate room inside the cage for the brown tree snakes to climb vertically along tree branches provided in every cage. Newspaper bedding is easily removed when soiled, and water bowls placed on the cage floor provide humidity and water for the snakes to

soak in before shedding. We spray cages daily using a pressurized tank and hose to keep the cage humidity high.

We regularly clean the walls of the cages and the branches inside with a moist sponge sterilized in a 5% solution of bleach, and then thoroughly rinse with water between uses to prevent transfer of any diseases or parasites between cages. The newspaper bedding on the floor of the cage is changed every 2-3 days, and the drinking water is changed when soiled, or every 2-3 days. Every six weeks, we sterilize each cage and all of its contents with an iodinated cleanser (Betadine (10%), The Purdue Fredrick Co., Norwalk, CT) to prevent the growth of parasites.

Unlike most laboratory animals, it is important to the health of snakes that there are some feces left in the cage during daily cleaning. Snakes rely heavily on pheromonal communication and are most comfortable in an environment that contains their own odors. In fact, it has been found that snakes placed in newly cleaned cages will defecate sooner than snakes placed in uncleaned cages to presumably provide these chemical signals to their environment[40].

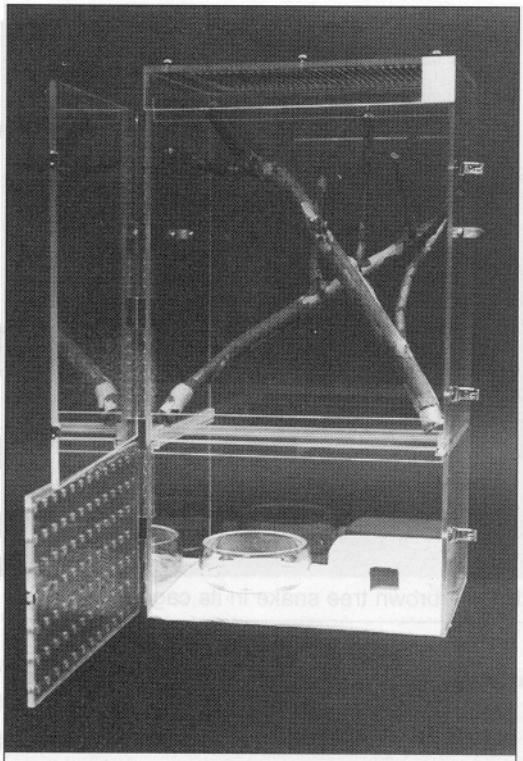

FIGURE 2. The cage setup used to house our captive colony of brown tree snakes. The cages were specifically designed to house arboreal reptiles.

In their natural habitat, the brown tree snake spends the day in small cavities where it can hide and stay humid. We have provided two transparent plastic hide boxes to facilitate this behavior, one attached to the roof of the cage and the other on the floor. We use sterilized moss and lichen as bedding; these hold water very well and keep the humidity level high inside of these shelters. The bedding in the hide boxes is changed every 2-4 weeks as it becomes soiled.

The brown tree snake can be aggressive when placed in a defensive situation. It will repeatedly strike at the caretaker if disturbed, although it will generally stay calm and will not strike if it is resting in its hide box. The caretaker must use caution when the snakes are aggressive to avoid being bitten, and also to make sure the snakes do not injure themselves by attempting to bite each other, or by accidentally striking the cage wall. When proper handling techniques are followed, bites or injuries are rare.

We feed our snakes a diet of live mice and freshly killed or frozen mice, chicks, and chicken embryos every two weeks. The different food sources are alternated to provide a balanced diet. Care is taken to make sure all frozen food is completely thawed, since partially frozen food can rot in the snake's gut. We do not supplement our snakes' diet with vitamins and have not noticed any deleterious effects. Any uneaten food is removed as soon as possible or within 2-4 hours after being presented to the animals.

Diseases

We have experienced only limited problems with disease or pathogens in our laboratory colonies of tree snakes. These animals are remarkably healthy, and apparently resistant to most commonly encountered pathogens. We have only experienced rare cases of infectious stomatitis (mouth rot), usually noticed when the animal appears to be "curling its lip." Closer inspection reveals small petechial hemorrhages along the gum line[41]. We have had success in treating this condition by applying a 3% solution of hydrogen peroxide to a cotton swab, and cleaning out the buccal cavity. Affected animals will normally resume feeding within two weeks.

Our only experience with a lethal pathogen involved a condition in which numerous skin ulcers developed on the ventral surface of the snakes. Multiple soft brown raised foci measuring up to 2 cm in diameter appeared in the skin over the ventral surface of the body. In many of these foci, the keratinous layers of the epidermis were separated from the underlying epidermis and there were areas of hemorrhage in the dermis and subcutis. This multifocal necrotizing dermatitis contained high numbers of bacteria and fungi, including an unusual species of fungus tentatively identified as a *Geotrichum* species. Bacteria identified were strains of nonhemolytic *Staphylococcus*, *Enterococcus*, *Achromobacter* and *Streptococcus*, and *Pseudomonas aeruginosa*. Death in these snakes probably resulted from shock and fluid loss associated with these skin lesions. We encountered this problem only once, and could not ascertain the source of the problem.

Conclusion

Many laboratories are now engaged in research directly applied to resolving the brown tree snake problem. In the process, we are discovering valuable basic knowledge, and it is becoming a well-characterized reptilian species for research. With the current trend towards the development of alternative animal models, a need for proper husbandry techniques has been established for this and other reptile species. The guidelines here, while intended specifically for the brown tree snake, are also of general use to any laboratory considering the use of snakes as models for biomedical research.

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