

Sound and Fury: Non-Biological Sound for the Selective Capture of Rabies-Infected Bats

A WDA Report from the Field

Denny G. Constantine (deceased)
1899 Olmo Way
Walnut Creek, California 94598, USA

ABSTRACT

Over two decades, accumulated observations and experiments showing that certain non-biological sounds may attract rabid bats led to exploitation of this behavior to capture rabies-infected bats. The exaggerated perception of sound was targeted as a stimulus to elicit attacks on sound sources attached to traps. Investigations on bats were carried out in California to explore the potential for selectively removing rabies-infected animals from environments shared with humans. Canyon bats (*Parastrellus hesperus*) were the focus because that species is the most common laboratory-diagnosed rabid-bat involved in attacks on people or animals in California. Sound sources were positioned behind the capturing wires of harp traps, and the traps were installed in desert areas where canyon bats were known to be abundant. Traps were successful when operated during both nighttime and daytime. A total of 101 rabid canyon bats and 15 rabid bats of six other species were captured during operation of these traps. The proportion of rabid bats caught with this technique far exceeded those achieved by use of non-selective means such as mist nets (0.5%) or harp traps without sound lures (0%). This technique may be useful for the collection rabies-infected bats and possibly other carnivores, may be useful as an adjunct to reduce risk of human exposure, and could help advance our understanding of the physiological effects of lyssa-viruses.

Key words: Rabies, non-biologic-like sound, sound-lure, trapping, bats, canyon bat, *Parastrellus hesperus*.

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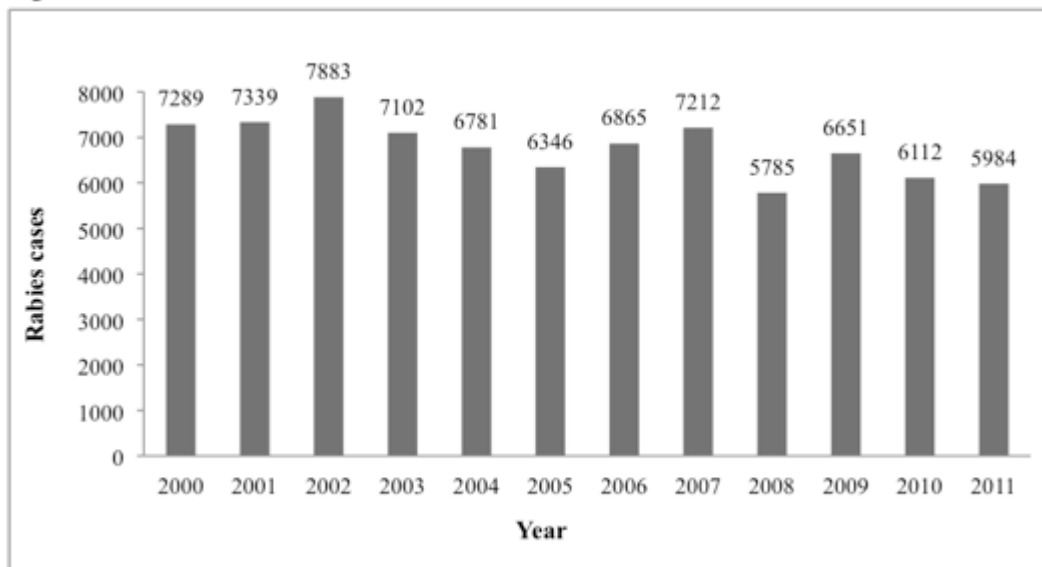
Milton Friend, David S. Blehert, and Rachel Abbott provided organization and help in the development of this publication. Appreciation is extended to hundreds of people for relevant cooperation and help during the last 20 years, representing friends, family, government agencies, landowners and managers. Although a complete listing is prohibitive a list of other significant contributors is presented as Appendix A. Note: As Dr. Constantine approached the end of his life he entrusted WDA with the data, images and information presented here. A week before his death he approved the major edits of this manuscript. David Jessup acted as an Associate Editor and Thierry Work acted as Editor in preparing it as the first in a new series of WDA Reports from the Field. We appreciate Denny's faith in WDA and his long life of contributions to wildlife disease investigations.

INTRODUCTION

Rabies has plagued society throughout recorded history (Wilkinson, 2002) and was first recognized as a disease of humans and dogs at the beginning of the Bronze Age in approximately 3000 BC (Koprowski, 2009). Despite modern advances in rabies-control and prevention, rabies remains a disease of high concern because of the dreadful course of the disease following onset of symptoms and the nearly universal fatal outcome. In the United States, preventive veterinary and

medical treatment has reduced human deaths from rabies to rare occurrences. Nevertheless, the number of laboratory-diagnosed cases in animals remains high (Figure 1).

Figure 1: Animal rabies cases in the continental USA between 2000-2011.



(Krebs and others 2001-2005; Blanton and others 2006-2011)

Because diagnosis requires collection and submission of adequate specimens from dead or dying animals, wildlife rabies is grossly under-reported and still reflects an unacceptable potential for human exposure to this zoonosis. Worldwide, as many as 100,000 humans are estimated to die from rabies each year (Rupprecht et al., 1995; Niezgoda et al., 2002).

Concern about rabies and the costs for treating potential human exposures to this virus have resulted in continuing exploration of means to combat this disease. Vaccination of dogs and cats, control of stray animals, and post-exposure treatment of people are long-standing approaches that have yielded substantial advances in rabies prevention and control in developed countries. However, some wildlife species, particularly several small carnivores and bats, often found in close proximity with human habitation, are major reservoirs for the rabies virus and pose unique challenges. Historically, local destruction of wild carnivore populations was commonly practiced during rabies outbreaks to temporarily reduce disease transmission potential by removing wildlife vectors. However, in recent years, questions about the effectiveness of these non-selective lethal practices, along with increasing public opposition to killing wildlife, have fostered the development of oral vaccination programs for wild carnivore populations as a rabies control measure (Johnston and Tinline, 2002; WHO CCRSR, 2004 ab; Shwiff et al., 2008).

Lethal control measures have historically not been applied to populations of insectivorous bats of North America because of the recognized value of these animals in insect control, and because methods to selectively attract rabid bats were unavailable. Nevertheless, growing concern about bat rabies (Niezgoda et al., 2002; Messenger et al., 2003) indicates that the development of effective tools to locally and selectively reduce numbers of free-flying rabies-infected bats would be desirable.

Bat rabies is characterized by a cycle wherein the virus is transmitted from bat-to-bat. Rarely, the virus is transmitted tangential to that cycle whereby a rabid bat infects a human, carnivore or other animal. For rabies control purposes, the basic cycle and tangential transmission are separate

entities. Disease control is most effective by interrupting the basic bat-to-bat transmission cycle, simultaneously preventing tangential transmission. However, until satisfactory methods of interrupting the basic transmission cycle between bats have been developed, the control of tangential transmission remains reactive: medicating exposed people (Manning et al., 2008; Rupprecht et al., 2010) and euthanizing or quarantining exposed animals (National Association of State Public Health Veterinarians, 2011).

This report presents findings from several decades of field investigations into the use of non-biologic sound as an attractant for the selective capture of rabid bats. The approach was developed based upon anecdotal cases suggesting that rabid bats might be attracted to sounds generated by humans (Figure 2).

Figure 2: Circumstances where rabid bats attacked people and animals in California during 1964-2005

Bat species	Attack circumstances
Canyon <i>Parastrellus hesperus</i>	<p>Canyon bat attacked and bit the wrist of a man driving a tractor.</p> <p>Canyon bat pursued, landed on and bit the bare back of a man riding a motorcycle.</p> <p>Canyon bat landed on and wrapped itself around the person's thumb then bit it.</p> <p>Canyon bat landed on a man's bare shoulder and was quickly away.</p> <p>Canyon bat landed on and bit the back of a boy.</p> <p>Canyon bat landed on and bit a crying child's bare abdomen during a diaper change.</p> <p>Canyon bat struck a parked automobile four times from which a person was target shooting.</p>
Free-Tailed <i>Tadarida brasiliensis</i>	<p>During the daytime a Brazilian free-tailed bat flew into a person's face but brushed it away.</p> <p>Brazilian free-tailed bat flew into a person's face at 1pm outside a hospital and brushed it away.</p>
Hoary <i>Lasiurus cinereus</i>	<p>Four separate incidents of individual hoary bats knocked down after repeated attacks on people.</p>
Myotis	<p>A California myotis was knocked down after repeatedly attacking a person's head.</p> <p>Long-eared myotis landed on a sitting man's mouth, biting him on his upper lip.</p> <p>A fringed myotis landed on a man's upper lip and was immediately brushed off.</p> <p>Fringed myotis was shot after repeated attempts to attack people over a 2 ½ hour period.</p> <p>Barking dog was attacked twice, 1-2 minutes apart, by a small-footed myotis.</p>
Silver-Haired <i>Lasionycteris noctivagans</i>	<p>Boy attacked at noon and bit on cheek by a silver-haired bat</p> <p>Boy attacked and bitten by a silver-haired bat on his forearm in his backyard.</p> <p>Silver-haired bat inflicted wounds on the head of an adult.</p>
Unknown	<p>A big brown bat struck the head of a person sleeping in bed and was brushed away.</p> <p>An unidentified bat attacked the neck of a person mowing a lawn.</p> <p>A child was attacked by an unidentified bat while riding his bicycle.</p> <p>A bat flew into the cab of a construction vehicle and bit the driver on his chest.</p> <p>A bat flew to and bit the lip of a playing child.</p> <p>After avoiding several attempted attacks, a man retreated indoors and was attacked again later stepping outside and he killed the bat.</p> <p>Barking dog was attacked and bitten on lip by a big brown bat.</p>

For example, a California teenager was laughing boisterously when a bat landed on her mouth and was brushed away. In another case, the driver of a backhoe was attacked by a bat. In many such cases of aberrant sound-induced bat-human interactions, when bats were recovered, they were found to be rabid. The overall annual monthly distribution of flying rabid bat attacks in California matched the pattern of bat abundance as reflected by their periods of activity, in contrast to seasonal hibernation or emigration. Although these attacks occurred in many parts of California, they were more common in desert areas where canyon bats predominate.

The hypothesis that certain sounds attracted rabid bats had not previously been explored, and the methodology described herein shows that rabid bats can be selectively captured using sound-based traps. This opens the possibility of capitalizing on rabies specific changes in the neurobiology of bats and possibly other carnivores to help reduce risk of human and animal exposure.

INVESTIGATIVE FINDINGS

Naturally occurring rabies-virus in free-ranging animal populations allowed for the investigation of behavioral differences between rabid and non-rabid animals and the use of sound as a lure for selectively capturing rabies-infected individuals. Desert areas of California were selected as field sites for the bat investigations because rabies was known to occur among insectivorous bats at these sites.

Figure 3: Documented cases of 85 flying rabid bat attacks in California during 41 years, 1964-2005

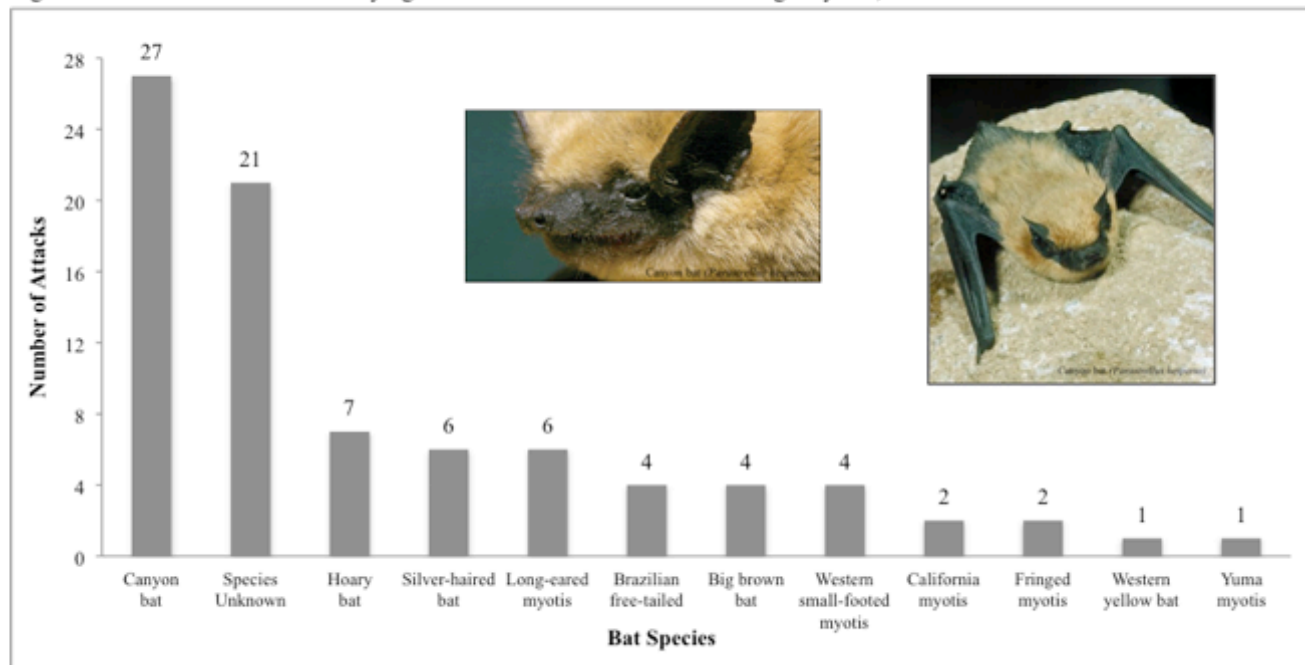


Figure 4: Geographic distribution of 85 flying attacks by rabid bats on people and animals in California during 1964-2005

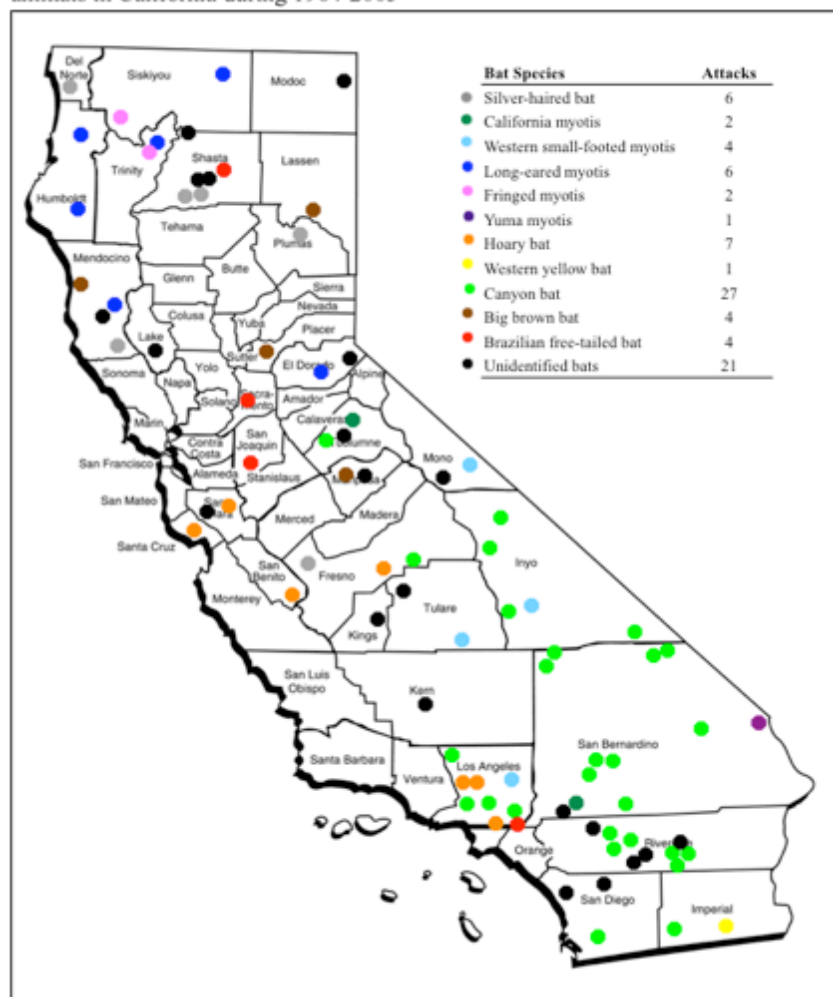


Figure 3 shows the species and Figure 4 shows geographic locations of 85 attacks of rabid bats on people and animals in California. An analysis of 82 of these cases shows that 42% of the attacks were by Canyon bats. This analysis also suggested that the majority, if not all of the bat attacks, were associated with sound produced by or otherwise associated with the victim. Thus, investigations were undertaken to test the hypothesis that rabid bats could be induced to attack sound sources associated with traps and be selectively captured and removed from a population. The experimental approach was guided by experience and knowledge gained by the author during more than 50 years of research on bat rabies (Constantine, 1967abc, 1970, 2009). The field work reported here was funded and performed by the author, who also performed field preparatory phases of the laboratory tests. Population Evaluations and Selection of Sound-Lure Trapping Sites From 1993 to 1999, bat populations at 32 potential trap sites in the southern California desert were selected and subsequently evaluated, based upon catch and release mist-netting over 48 nights (Figures 5 and 6).

Figure 5: Mist net bat capture and release sites evaluated during 1993-1999 for the selection of sound-lure trapping locations in California (in blue)

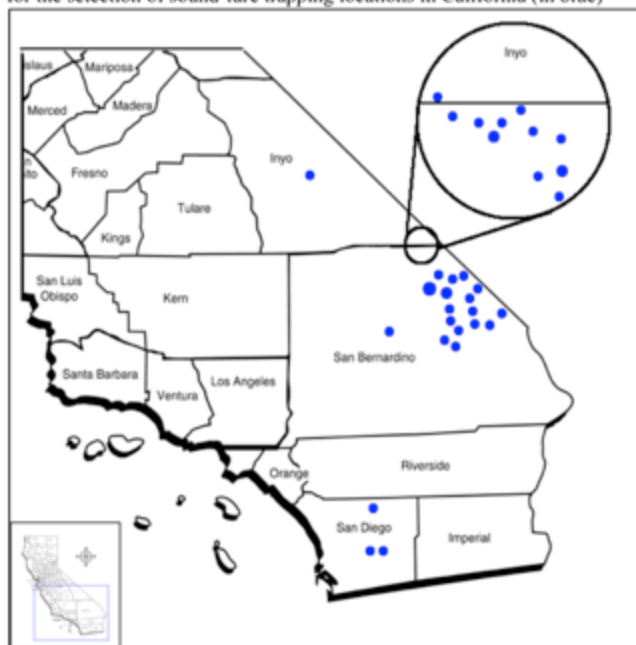
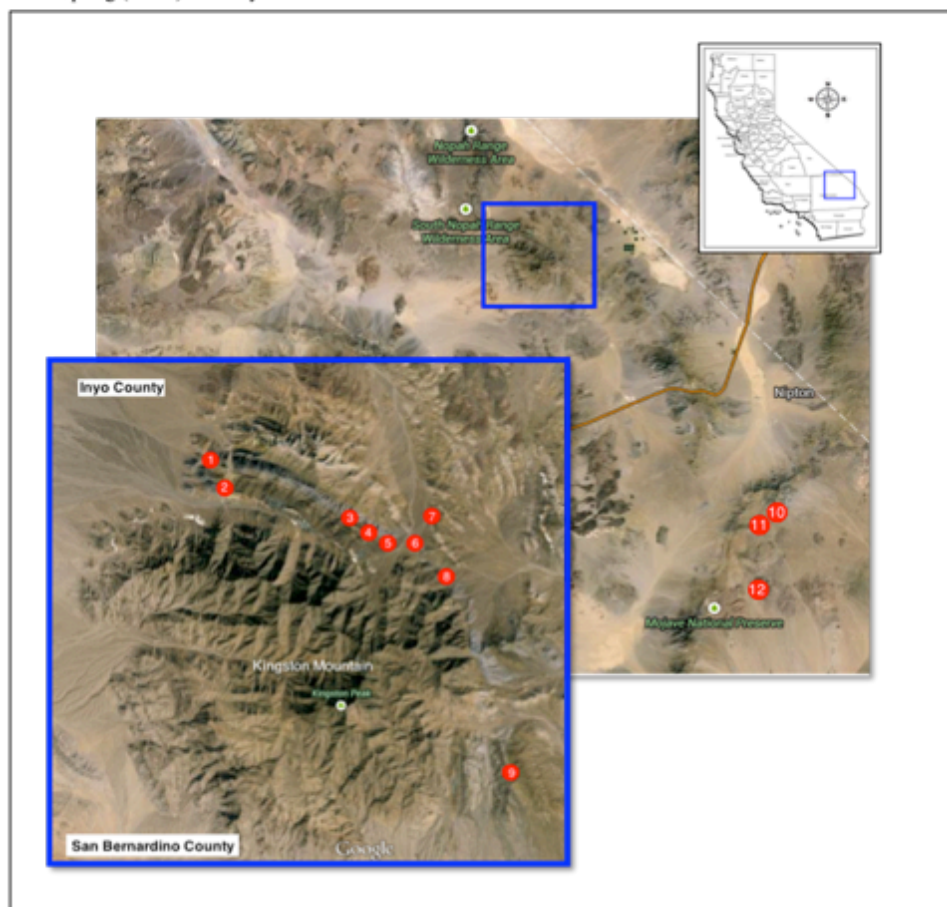


Figure 6: Northeastern San Bernardino County, bordering southeastern Inyo County and Nevada. Rabid bats were captured in sound-lure bat traps at the numbered Kingston Mountain cattle watering sites 1-9, but none were taken at similar sites 10-12. Greater numbers of bats were taken at dependable water sources, especially Crystal Spring (No. 1), Beck Spring (No. 2) and Canyon Pool (No. 5), the latter supplied from covered Horse Thief Spring (No. 4). Heavily shaded areas are between 1220 and 1829 meters elevation.



Of 1,776 bats netted, 1,067 were canyon bats. Mist netting catch per night ranged from 0 to 174 canyon bats at each site. Using the combination of bat abundance findings from all sources, sound-lure trapping sites were selected in the Mojave Desert of northeastern San Bernardino County (Figure 7).



Figure 7: a. Mist nets erected over cattle reservoir. b. Canyon bat caught in mist net.

Traps were placed near cattle water sources that are routinely frequented by bats.

Sound-Lure Trials

Preliminary Investigations. From 1993 to 2002, various sources of sound (Figure 8) were tested for their ability to attract bats and to differentially attract rabid bats.

Figure 8: Location, relative effort, differential success at attracting rabid vs non-rabid bats and comments on the source of the sound.

Year	Location(s)	Effort (in days)	Rabid vs. Non- rabid	Comments on Sound
1993-98	Various in CA	47	No rabid bats	As listed in preliminary investigations
1999	Various in CA	193	1 rabid	Commercial smoke alarm
2000	Kingston Mts.	13	21 – 0	48 kHz interrupted
2001	Mojave Desert	208	39 – 16	48 kHz interrupted
2002	Various in CA.	n/a	0	25-48 kHz interrupted

The location, relative effort, differential success at attracting rabid vs non-rabid bats and comments on the source of the sound are shown below. Two of these recorded sounds, a jackhammer and a motorcycle engine, had previously been associated with rabid-bat attacks.

Paired Trap Evaluations. During 2000 and 2001, individual large bat traps bearing 48 kHz sound units were deployed in the Kingston Mountains and matched with nearby equivalent traps bearing inactivated silent sound units. This controlled trial was done as proof of concept that sound units could attract and capture rabid bats. Trap pairs (Figure 9) were installed at 18 sites during 2000

and at six sites during 2001 for a total of 175 trap days. A total of 24 rabid bats (23 canyon bats and one big brown bat) were caught in sound-lure traps installed at cattle watering sites (Figure 10).



Figure 9: Test and control sound-lure trap pair installed in Joshua Tree habitat in the Kingston Mountain Range.



Figure 10: Small sound-lure bat trap attached to fencing for cattle and elevated to avoid contact with cattle.

No bats were caught in the silent control traps. In addition, 23 canyon bats were captured using mist-nets next to the cattle watering site where the majority of rabid bats had been trapped previously and subsequently in the Kingston Mountains. Laboratory tests performed to satisfy a health department concern that all bats in the area might have rabies disclosed that none of the bats caught in mist nets was infected with rabies. This suggested that the idea that sound preferentially attracts rabid bats could be valid.

Intermittent Versus Constant Sound. To contrast the attractiveness of intermittent (48 kHz ultrasound bursts of 0.1 sec duration separated by 0.1 sec of silence) versus continuous sound, small harp traps bearing sound units of either variety were deployed at previously successful rabid bat capture sites in the Kingston Mountains. Traps were operated from September 17-21, 2001. While trapping data from some locations was discarded due to trap vandalism, three rabid canyon bats were caught in intermittent sound traps during three trap days. No bats were caught during 13 trap days in traps emitting uninterrupted sound.

Filtering Rabid Bats from Multimillion Cave Bat Populations. During August 2007, while working at a Texas cave that houses millions of Brazilian free-tailed bats, the author stationed 48 kHz interrupted sound-lure traps in uninhabited sites within the cave distant from the noisy bats blanketing other ceiling areas, on the chance that infected bats beginning to experience rabies delirium might wander in flight within the cave and be lured into the traps. A concern was that practically all of the air space within the cave can be filled with flying bats as they enter into or depart from the cave, whereby healthy bats can blunder into traps, especially during migration periods or as young bats start flying. However, the effort yielded five rabid bats and ten healthy bats. Thus, 33% of the captured bats were rabid, contrasting with the usual 0.5% of rabid bats in this and similar cave bat aggregations, a statistically significant difference ($p < 0.001$).

DISCUSSION

Human concerns involving rabies and associated lyssavirus infections of bats have been elevated during recent decades as a result of global infectious disease emergence and resurgence (Niezgoda and others, 2002; Constantine, 2009). Carnivore rabies occurs as epizootics. In contrast rabies in non-vampire bats is largely a disease of seclusion that sporadically is recognized following human cases caused by rabies variants associated with bats. This lack of awareness as to the occurrence of rabies in carnivores and non-vampire bats likely contributed to the first laboratory-confirmed human exposure from the bite of a rabid insectivorous bat in the United States which occurred in 1953 (Venters et al., 1954). It also likely contributed to a greater study and understanding of the evolution and biology of carnivore-born rabies than that of rabies in insectivorous bats.

Findings reported here provide methodology, use of 25-48 kHz interrupted sound lure traps, for enhancing bat rabies investigations and possibly for combatting rabies in bats. Clearly, sound-lure traps were effective in selectively capturing free-flying rabid insectivorous bats within desert areas of California. The unprecedented capture of 116 rabid bats by this means supports further application and exploration of this technique as a means for enhancing rabies surveillance in other free-living bat populations, and for the selective removal of rabid bats as a component of bat rabies prevention and control efforts. This technique may be useful for the collection of other lyssavirus-infected bats from nature and as a tool to further study of this group of viruses (Constantine, 2009).

In desert canyon bats, rabies is infrequent infecting ca. 0.5% of mist-net captured individuals. The high percentage of rabid bats trapped within the first two days of sound trap operations is especially striking considering that rabid bats were trapped over an extended period of time

following trap placement. The idea that rabid bats can be depleted from a population using sound traps merits confirmation. There also appeared to be a seasonal pattern to trapping of rabid bats. For instance, successful traps usually contained a single rabid canyon bat, but in August and September sometimes contained two or three. Six traps during those months each contained two bats, and two traps each contained three bats. During a more recent (August 2006) exploratory trial in Riverside County, three smaller bat traps set at three previously successful sites collectively caught ten rabid canyon bats during the first 24 hours. The first two traps, each bearing single 25 kHz sound units, captured two and three rabid bats; the third trap, bearing a single 48 kHz sound unit, captured five rabid bats (Figure 11).



Figure 11: Site bordering a water reservoir and sound-lure trap installation where five rabid canyon bats were captured within 24 hours of trap activation.

After 10 days the three traps had captured a total of 27 rabid canyon bats. Bat traps usually were checked twice daily, thereby providing limited data on time and intervals between rabid bat captures during daytime. Daylight hours are when most reported bat attacks on humans occur, and rabid bats have been captured between 1330 and 1610.



Figure 12: Sound-lure harp traps used to capture rabid Canyon bats. From left to right, an early model using a smoke alarm, a later model with a 25 kHz sound unit, and a later model with a 48 kHz sound unit.

Figure 12 provides a comparison of an early model sound-lure harp trap with later model 25 kHz and 48 kHz versions.

A basic question is whether or not sounds made by captured rabid bats attract additional rabid bats into traps or if familial or other factors play a role. Bat-to-bat voice attraction is a reasonable possibility influencing capture, as healthy bat utterances are known to attract healthy bats (Constantine, 1961b). However, only rabid bats were captured by the sound-lure traps. Conversely, perhaps calls of rabid bats repel healthy bats due to a learned response associated with previous attacks. Some insight relative to the question posed is gained from the aggressive interactions observed between rabid bats and sound-lure traps.

Rabid animals that attack sound-lure traps likely would attack other noise sources, including other animals and humans. Six of the 11 species of rabid bats reported to have engaged in flying attacks on noisy humans, animals, or machines in California, were captured in sound-lure traps in this study. Bat researchers have worked for years at and in caves harboring large numbers of Brazilian free-tailed bats, some of which were likely infected by rabies virus, without experiencing an attack. Thus, it was not anticipated that this species would be attracted to sound-lure traps. However, researchers usually avoid making loud noises while in those caves. Also, noise emanating from those large bat concentrations could drown-out human voices. In the absence of background noise from within bat colonies, rabid Brazilian free-tailed bats were captured by sound-lure traps, suggesting there may be other unique applications for this technology if adapted to prevailing circumstances.

Bats that attack and are caught in sound-lure traps essentially are self-submitted samples of rabies. In contrast, mist-netted bats seldom have rabies. In addition, netted samples may be biased by pregnant, heavy lactating, or very young bats most of which are likely to be rabies-negative. Catch rates by either method change with time of year and resulting transitions within bat populations. For example, by June female canyon bats are giving birth. By July, females are still lactating and mature males have moved to higher elevations for summer. Since canyon bats do not reproduce until their second year (Hayward and Cross, 1979), younger males may stay at low elevations. By August, the year's crop of young bats starts to fly. By September, young bats become difficult to differentiate from mature bats, and some bats may disperse to higher elevations to fatten prior to winter. Mating occurs by late September and may occur intermittently until March (Hayward and Cross, 1979). These transitions are reflected in sound-lure trap captures of rabid bats and mist-net trap results within the Kingston Mountain and Riverside County areas. During that trapping, rabid canyon bats initially attacked sound-lure traps in relatively large numbers throughout the warm months. However, the catch rate then quickly declined, probably as rabid individuals were filtered from the population.

Prevention of attacks by rabid animals through their selective removal from environments shared with humans is one of the objectives explored here. This approach provides another potential means for rabies control through the reduction of a source of rabies exposure and transmission. Although reported flying non-vampire rabid bat attacks on people are rare, averaging only two reported attacks annually in California, secondary aspects of bat rabies remain important. The removal of rabid bats may lessen the more common pathway of bat rabies transmission to domestic cats and other animal species that can contract rabies when they capture or investigate rabid bats. These contacts then lead to secondary human exposure.

Programs for sound-lure trap removal of rabid bats could not only reduce potential human and domestic animal exposure (Constantine, 2009), but could also contribute to the development of healthier bat populations. For example, after the removal of 43 rabid canyon bats from the Kingston Mountains in 2000 and 2001, no further sound-lure bat trapping was undertaken in that area until June 2007. At that time, three of the smaller size sound-lure traps were individually

installed for three successive 24-hr periods at three former prime trapping sites. Surprisingly, no bats were caught. The same procedure was repeated in October 2010 and August 2011, when two and seven rabid canyon bats were trapped, respectively, indicating that rabies had returned. No similar reduction in the number of rabid bats captured occurred in western Riverside County. Sound-lure traps captured 16 rabid canyon bats during one week in 2001 and 3 during three weeks in 2002. When revisited in 2006, 27 rabid canyon bats were caught in sound-traps in the same area, during ten days. This tool then may provide insight on the “attack” rate of rabies in bat populations. Developing a better understanding of how quickly rabies re-establishes in a rabies-depleted populations might provide insight on emergence of lyssaviruses in chiroptera.

Clearly, non-biologic sound used as described may be useful for the collection of both rabid canyon bats and other bat species. Other lyssaviruses may induce similar behaviors in other bat species and sound lures could prove useful in their study and management. The neurobiology of rabies may differ from species to species, but attraction to certain sounds may be a fundamental effect of rabies on the mammal brain. In addition to bats, sound-lure trapping of carnivores may have merit for combatting rabies, especially among major host species such as skunks, raccoons, and canids.

The selective removal of rabies-infected domestic dogs in developing countries could save many of the 100,000 people estimated to die of rabies annually and reduce the need for an even larger number of persons to have anti-rabies treatment following dog bites each year (Rupprecht et al., 1995; Niezgoda et al., 2002), and thus reduce the attendant high public health costs. Worldwide rabies control, prevention, treatment, death losses and health costs remain a major concern. The use of non-biologic sound deserves further investigation as an addition to those tools and programs now in use to combat the deadly effects of rabies and other lyssaviruses on humans and animals.

APPENDIX A

The author thanks: Anna J. Wong, as leading microbiologist of the rabies section, California Department of Health Services Viral and Rickettsial Diseases Laboratory, performed relevant rabies diagnostic tests from 1999 through 2004 and volunteered much weekend work to help prepare traps and equipment for field trips; Charles E. Rupprecht, Chief, Rabies Section, Centers for Disease Control and Prevention, helped in many ways and arranged for characterization of virus isolates; William R. Rainey and Elizabeth D. Pierson helped identify relevant instrumentation, generated electronic sounds, and gave invaluable help and suggestions; Michael G. Antriasian and the author's son, Kenneth G. Constantine, provided expert aircraft pilot and mechanic services throughout California; Gerald P. Mulcahy, California Department of Fish and Game, participated in early field work in eastern Riverside County, and Joseph M. Szewczak, University of California White Mountain Research Station, did likewise in Inyo County, David Fjelline and Robert McCurry contributed equipment and lore relating to skunks; EME Services' Thomas Tracy Allen gave invaluable insights regarding ultrasound equipment and theory; Texas State Health Services Zoonosis Control's Thomas J. Sidwa offered extensive cooperation, and James S. Wright facilitated detailed field support; Merlin D. Tuttle and the staff of Bat Conservation international gave affirmative support. Government agencies provided permits, cooperation, or help: Nevada state and county health officials Arthur F. DiSalvo, Donald S. Kwalick, and Claire Schmultz; Arizona Game and Fish Department personnel; Texas Parks and Wildlife's Becky Beard; California's 58 county governments, particularly public health departments and animal control staff that obtain specimens and basic data; Donna Murrill and staff of the Butte County Health Department Laboratory provided extensive epidemiological data. Other infectious disease or laboratory directors and managers kindly tested specimens taken locally during field activities and

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