Final Report

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National Zoo's Conservation and Research Center

Security Population of Virginia Big-eared Bats (*Corynorhinus townsendii virginianus)*

Background

In October 2009, Smithsonian Institution's Conservation and Research Center was awarded a US Fish and Wildlife Service (US FWS) grant to establish a captive colony of federally endangered Virginia big-eared bats for the following reasons:

> "There are only 15,000 Virginia big-eared bats remaining in a few caves in West Virginia, Virginia, Kentucky and North Carolina. White-nose syndrome has already infected some of the caves in this area, and if it continues, this bat subspecies could likely become extinct. The National Zoo has developed a multidisciplinary team of scientists, veterinarians, nutritionists and curators who are working with the U.S. Fish and Wildlife Service and West Virginia Division of Natural Resource to establish this insurance population of Virginia big-eared bats, thereby buying time to determine the cause of, and cure for, this disease. Bats in this population may eventually be needed to re-establish the subspecies in the wild. Virginia big-eared bats have never been kept or bred before in captivity, so lessons learned from this project will be of broad interest to agencies and organizations if white-nose syndrome in wild bat populations makes it necessary to consider captive breeding of other bat species." (Joint FWS and NZP press release, October 26, 2009)

As part of the above-referenced US Fish and Wildlife funded project, Smithsonian Institution's Conservation and Research Center (CRC) in Front Royal, VA, contracted with Singleton Consulting (SC)

to provide expertise in establishing and ensuring a healthy, stable captive population of Virginia big-eared bats (VBEB). To further ensure excellent care of these federally endangered animals, the US FWS captive care protocol called for two CRC animal care staff to attend Bat World Sanctuary's (BWS) week-long captive care workshop, which focuses solely on care of insectivorous bats.

Singleton Consulting was selected to assist with this project because of our ten years of experience with eight species of captive bats. In addition SC has trained with and assisted BWS with captive care projects for various insectivorous bat species.

Corynorhinus townsendii virginianus is a new species to captive propagation, but several behavioral and environmental characteristics are known from field research. These include:

- A proclivity for stress responses; wild colonies have been known to abandon roost sites upon human disturbance; handling has caused sudden death during mist netting and field surveys (Stihler, pers. comm. Nov., 2009)
- These are relatively small bats with extremely dense pelage
- These bats are cave dependant year round and prefer to roost in open areas and not in tight crevices
- These bats are slow flying gleaners and hawkers and have been observed flying at very slow speeds close to and among vegetation (Stihler, pers. comm., Nov. 2009)

With this background knowledge of VBEB, natural history provided by West Virginia Division of Natural Resources (WV DNR) and FWS field personnel, a care protocol in place, prior captive care experience and expertise provided by SC, and intensive training provided to CRC staff by BWS, a solid plan was in place to assure the health and viability of captive VBEBs.

Scope of Work

The agreed upon scope of work (SOW) was to provide professional services related to bat capture techniques and husbandry for the purpose of establishing a secure population of Virginia big-eared bats at the National Zoo's Conservation and Research Center (NZP), to include but not be limited to:

1) Assisting with final enclosure set up/modification.
2) Reviewing and editing caretaking protocols.
3) Providing daily care for bats (diet preparation, feeding, cleaning, behavioral observations, handling, and medical treatments).
4) Instructing NZP staff on all aspects of bat husbandry during times set by the bat husbandry supervisor.
5) Keeping detailed daily records in MS Word. Records were to include (but not be limited to) health status (weight, medical treatments, injuries, abnormal behavior, deaths), daily routine (diet preparation, feeding, cleaning, behavioral observations, handling), enrichment, and pen modification and design. Daily records were to be sent electronically to curatorial staff via e-mail.
6) Communicating pertinent information to veterinary, nutrition and curatorial staff.
7) Work was to commence with SC arrival at CRC on November 3, 2009 and end on November 21, 2009, by which time it was anticipated that incoming bats would be stable and CRC staff would be able to maintain bats' health without further assistance from SC.

The above scope of work was approved prior to SC arrival at CRC by Supervisor, Warren Lynch.

Twenty female Virginia big-eared bats (VBEB) and twenty male VBEB were captured at School House Cave in West Virginia on November 9, 2009 by West Virginia Department of Natural Resources and US

FWS personnel with assistance from CRC staff and SC. Bats were processed at Bat World Nova's satellite facility in Cabins , WV, approximately 15 minutes from School House Cave.

Each bat was weighed, the forearm was measured, wing punches were taken from each wing to collect genetic material, and the bat was assessed for hydration level and a unique identification mark (nontoxic acrylic paint) was applied to the ear and elbow. Initial health findings such as, injuries, wing tears and scars were also documented for each bat. Bats were then transported by USFWS to the CRC.

Housing

Recommendations by BWS and SC for proper cage design were not taken into consideration. Upon SC arrival at CRC, the final construction of the bat flight enclosure was inspected and suggested modifications to remove sharp edges and possible escape portals were recommended. Most, but not all escape portals were modified prior to SC's departure on Nov. 21st. However, the cage contained several structural defects and shortcomings, including design flaws that allow bats to roost out of reach of caretakers, open spaces where bats could escape into other areas of the flight cage and the absence of a padded floor. Long term durability of the cage will likely be poor because it lacks a structural exterior frame. Cage mesh is too large (1/4" mesh rather than the recommended 1/8"), which may allow wings and feet to slip through the cage and cause injury, entanglement, self-mutilation and death (Lollar, 2002). Access doors and interior doors are of poor quality and are cumbersome to maneuver, particularly with a bat in hand.

It was strongly recommended that the room temperature in the bat flight room be constant and consistent at or around 72 degrees Fahrenheit. Bats were captured just as they were entering winter torpor. As such periods of extended torpor are undoubtedly accompanied by profound metabolic changes, it is important that seasonal cues be masked and even artificially reversed. During SC's participation in the project, room

temperatures fluctuated from approximately 60 to 80 ° F, sometimes in less than ten minutes. Humidity levels were also not consistent, and as a result bat's skin became visibly dehydrated.

Water sources were confusing to the bats in that water pans were moved, changed, or emptied with in the first three weeks into the project. SC recommended keeping the placement of water sources consistent. However, on two occasions mealworms were placed in the water pans instead of water and new water pans were installed very late at night. For several days thereafter, the original water pans stayed empty in the flight cage on the floor next to the new water source. SC noted that during night observations several bats tried to glean food or water that was not available from the empty water pan.

Feeding

Bats were weighed before each feeding and each bat was fed by hand each morning and evening from November 9th to November 17th,despite the fact that during that time several bats were observed to be self-feeding on mealworms. SC advised against continuing to hand-feed bats that had the ability to self-feed, as this would result in bats relying on proffered food and raise the stress levels of stress-prone bats that should be handled as little as possible. On November 17th, eight days after arrival, the nutritionist finally approved allowing bats showing signs of self feeding to skip a hand feeding session. From November 17th to the 21st (SC's departure), most bats were still being hand fed each morning and evening. In addition, bats were weighed twice daily. Weights and notes were entered into data sheets for comparison to entire colony.

Each bat was examined for weight trends and feeding responses. Dehydrated bats were given fluid therapy before or after feeding sessions. There were at least six people present during each feeding session. This level of interaction was strongly recommended against. In fact, it is established that bats in rehabilitation and captive care normally exhibit daily fluctuations in weight (Lollar, pers. comm., Nov. 2009). The negative aspects of handling (stress, fear, risk of physical injury) must be weighed against the

information gained (See **Figure 1** and **Figure 2**). Feeding should be the most pleasurable experience of a newly captive animal and is a powerful tool to transition formerly wild animals into captivity. Creating stressful conditions around feeding is contraindicated and may result in anorexia and other manifestation of poor health.

Figure 1 Photo taken of bat on day of capture

Figure 2 Photo taken of same bat after two weeks in captivity at the CRC

Nutrition

Upon arrival at CRC, bats were offered blended diet (mealworms, water, and baby food only), whole mealworms and mealworm viscera. Mealworms and other insects were not gut loaded or hydrated according to SC recommendations. All recommended supplements were strictly prohibited by the nutritionist on staff. However, approximately four days after bats arrival, feeder mealworms were fed produce, which made them more palatable and nutritious to the bats. A recommended supplement (calcium, vitamins) used in either the blended diet or as a top dressing for feeder insects was not approved for use by the nutritionist.

A positive feeding response should have been observed upon initial feeding, however, the large size and preparation of the mealworms selected and the mealworm water and baby food blend were not readily accepted. SC believes this is in part due to palatability issues. Viscera and moisture were lacking in most worms because they were gut loaded with a dry commercial medium and hydrated with damp paper toweling, which was ultimately eaten by the mealworms.

Analysis and documentation for recommended dietary components were made available to CRC's nutritionist, but SC saw no evidence of nutritional comparisons. If such comparisons were done, results were not shared with SC.

It was recommended that a highly palatable, easy-to-digest diet be available should a bat become emaciated, stressed or refuse to eat. Such diets are readily available as in the blended diet referenced above or an enteral/oral diet such as Vital HN, both of which have been used in veterinary management of debilitated bats. However, when a liquid was required to properly feed emaciated or debilitated bats, none was available, despite the fact that such a diet had been previously approved by the nutritionist.

Handling

Proper bat handling techniques were encouraged for all personnel and veterinarian staff. Mishandling of the bats resulted in broken fingers soiled fur, skin infections, eye infections, bruised legs, bruised testicles, anorexia, capture myopathy and death. See **Figure 3** and **Figure 4**.

Figure 3 Eye infection as a result of improper feeding techniques and mishandling of bat

Figure 4 Bruised left leg as a result of mishandling of bat

It was strongly suggested that bats potentially suffering from capture myopathy not be placed in an incubator. As it was already suspected that these bats are susceptible to capture myopathy (CM) (Sturges, pers. comm., 2009) and that this particular species had died during handling in field studies (Sthiler, pers. comm. 2009). Cautionary principles should have come into play. CM-suspect bats would have been better served by cooling and fluid therapy as heat is contraindicated in the handling of CM prone species (Dunn, Miller and Wildlife International, 2009).

Stress and pain are clinical conditions and need to be treated as such. Methods to reduce stress, such as minimal handling, a quick transition to self-feeding status, and fewer staff handling bats were

recommended by SC and the care protocol developed by FWS. Reduction of stress levels would have resulted in more bats that readily self-fed, fewer bats with health issues, and minimal to no deaths.

Veterinary Care

Because SC lacked veterinary certification, the scope of work did not include all possible veterinary outcomes. However, several recommendations were made based on proven trends and known practices in veterinary management of bats in rehabilitation and captive colonies. Recommendations for veterinary care given by SC were based on methods used by wildlife and clinical veterinarians who have treated bats in captivity for over twenty years.

During the initial three weeks of the project, several veterinarians handled the bat colony. At times, two or more vets were present during feedings. In the absence of the veterinarian, a technician with no prior bat handling experience was given authority to treats bats if needed.

In several cases of life threatening emergency, SC was not allowed to intervene and evaluate the bat in order to potentially save its life. Even in cases of mortality SC was only reluctantly granted permission to examine bats. No pathology, toxicology or necropsy results were discussed with SC.

The use of Revolution ® (selamectin) is recommended for the treatment and prevention of ecto- and endoparasites (Lollar, 2002). However, fipronil was selected by CRC vets. Application did not seem consistent from bat to bat, and the dosage appeared excessive and left the bats' pelage wet. Selamectin, unlike fipronil, is labeled as a miticide and effective dosage is minimal and has been successfully used for many years in large rehabilitation and zoo facilities.

Bats developed ulcerative dermatitis because food was left adhered to the face and chin after feeding sessions. Healthy self-feeding bats are not known to develop such adhesions. Food adhesions are a result of improper feeding methods, overly stressed animals that are not grooming adequately, inappropriate food being offered (too large insects) and/or inadequate post-feeding cleaning. Dermatitis was treated

with a hydrogen peroxide lavage followed by a topical ointment that contained steroids. SC strongly recommended that the hydrogen peroxide lavage be stopped because such treatment prohibits the growth of healthy tissue (Saffle, 1995). SC also strongly recommended the topical treatment with an oil-based ointment be halted because such ointments collect debris and may further complicate the infection. In addition, wildlife has a tendency to groom such topicals off and ingest them (Hiss, pers. comm., Sept 2007). As toxicity levels for many of these medications are unknown for bats, in particular, caution was urged. See **Figure 5** and **Figure 6**.

Figure 5 Illustrates ulcerative dermatitis as a result of improper feeding methods and inadequate grooming techniques post-feeding

Figure 6 Shows food encrusted on bat's muzzle, chin and chest

In addition, Craig Stihler of WV DNR had already relayed to the staff that this species of bat avoids getting wet. Given their extremely dense pelage, it is reasonable to assume that these bats cannot effectively groom once waterlogged. Other densely-furred bats have been known to suffer from matting and skin infections if they are wetted and not thoroughly dried and groomed. In fact, the appearance of these bats' pelage was consistent with improper drying and grooming within days of arrival.

Despite the fact that SC is a fully trained lab technician and wildlife biologist with prior bat handling experience, no one but the veterinarian and one team member from the CRC was allowed to perform subcutaneous injections of fluids. While this may adhere to CRC veterinary protocol, poor subcutaneous injection techniques caused the bats to cry out on several occasions. On two occasions the needles entered the gloves of the caretaker holding the bat during injection, which constitutes a health risk to employees as well as to bats.

One bat presented with what appeared to be an eye infection and eyewash was selected as the method of treatment. The eye flush was applied in an excessive amount, caused unnecessary pain and stress, and likely resulted in the bat becoming wet in the process. SC was not permitted to examine the bat after the eye wash was performed. Ophthalmic ointment rather than eyewash is the recommended treatment for eye infection in insectivorous bats (Lollar, 2002).

Enrichment

Environmental enrichment for bats in captivity is essential to their psychological well being, stress reduction, and ultimately survival and reproduction, as has been well established for other captive wild mammals (Shepherdson, et. al., 1998; Lollar, 2002; Hiss, 2008). Several recommendations were made for enrichment to elicit natural foraging behaviors, such as providing the bats with an enclosure that more closely resembles their natural habitat and providing more feeding stations and an alternate prey item, such as crickets. On only one occasion did the supervisor instruct caretakers to offer crickets, but the crickets offered were not the recommended size. Consequently, the small crickets escaped from the enclosure.

As VBEBs are known to glean from vegetation, CRC personnel assured site inspectors that silk foliage would be used to add interest and gleaning areas to bats (Sturges, pers. comm. 2009) and SC also advised the addition of such enrichment, however it was not added to the enclosure prior to SC's departure.

It was also recommended that all bats be allowed to roost together as a colony and not separated by gender. Colonial bats form strong social bonds and allowing them to roost in self selected groups may ease the effects of a stressful period. Allowing the bats to roost as they normally would in the wild can help reduce stress levels during transition from the wild to captivity (Johnston, 1997).

Conclusion

Although bats have been understudied in captivity, the recommendation of SC and BWS are supported by thousands of hours of research and published literature. Zoo and exotic veterinarians have very little to no experience with insectivorous bats. Insectivorous bat nutrition is also understudied; however, insectivorous bats have been maintained in rehabilitation and educational facilities for years. Diets and treatment methods developed by such facilities have proven successful for maintaining insectivorous bats for numerous years. Many rehabilitation methods have been developed or reviewed by practicing or consulting veterinarians or nutritionists. The fact that the USFWS protocol for this project required personnel to train at BWS, the nation's largest and most respected bat rehabilitation facility, stands as testament to the validity of time-tested rehabilitation methods and expertise in veterinary and nutritional care.

Despite this, SC's on-site recommendations were met with resistance or outright hostility. Communication with the client was difficult or nonexistent during the three week period. SC was not introduced to the team of Primary Investigators who were responsible for making all decisions for the bat colony in a timely manner. Additionally, and most significantly, SC was not invited to any daily meetings or conferences to discuss colony progress or provide further recommendations to help the project succeed. In general, SC opinions were not solicited and SC recommendations for daily care, diet, feeding, cleaning, behavioral observations, handling and medical treatments were, for the most part, disregarded.

The agreed upon Scope of Work was also disregarded. SC was not given the opportunity for final protocol discussion, was unable to offer further staff training, and was not included in daily record keeping. To address the lack of communication, SC attempted to address caretaking staff prior to feeding sessions, but input and advice was met with resistance. Had SC been included in daily staff meetings, recommendations would probably have been accepted in a more positive manner and effective communication would have been possible.

A total of five bats died during the consultant's tenure, two female bats and three male bats, and another three bats have died since SC's departure, representing a 20 % loss in just five weeks. Such high losses in formerly healthy bats are unprecedented in rehabilitation facilities. SC believes that all but one of those deaths could have been avoided by following the handling and feeding advice of the paid consultant and by caretakers adhering to the methods they learned at BWS's workshop.

In an effort to stop the losses, Emergency Recommendations were submitted November 20th (see Appendix A). While there was no meaningful response to the Emergency Recommendations, we notice the rate of mortality has slowed and we are hopeful this project is now on the right track.

It is unclear why the client accepted initial recommendations by SC and facilities such as BWS, but decided against implementing those recommendations once the project began. We suspect generalizations were made from general mammalian care and accepted zoo practices rather than established methods for the long term care of insectivorous bats provided by non-zoo facilities.

This project represents a unique opportunity to help conserve a federally endangered species, an arena in which CRC excels, and an opportunity to raise public awareness of White-Nose Syndrome, one of the greatest wildlife disease threats to arise in recent history. There is no reason to expect that this project will be anything but successful if the recommendations of Singleton Consulting and established, proven methods for maintaining bats in captivity are followed. These animals are too precious to leave their fate

to the whims and fancies of ‘experts’ who have no experience maintaining captive native insectivorous bats in healthy, reproducing colonies.

We do wish to take this opportunity to commend the bat caretaking team and supervisor at CRC. The team’s dedication to the project was exceptional. Team members often worked after hours or used personal time to complete tasks and all put in outstanding effort during a stressful, but very exciting, project launch.

Appendix A:

Immediate Recommendations, submitted to Warren Lynch November 20, 2009

Please see the following information outlining the immediate recommendations by Singleton Consulting and Bat World Sanctuary. Disregard of the following recommendations will likely lead to further VBEB fatalities.

- Bats are succumbing to capture myopathy because bats are being mishandled and over-handled. Recommendations on correct handling techniques have been intentionally ignored by the staff. Immediately decrease handling of these bats, and further, the personnel involved in the deaths of bats should not be allowed to partake in any further care of these animals. Proper bat handling techniques must be encouraged for all personnel and veterinary staff working with this colony. Broken fingers, soiled fur, skin infections, eye infections, bruised legs, bruised testicles, anorexia, capture myopathy, and death are unacceptable in a project this critical.
- Use of the incubator must cease immediately. Well-informed veterinarians know that the use of heat is contra-indicated on animals suffering from capture myopathy. Instead, cooling and fluids are recommended for bats that succumb to capture myopathy.
- The temperature and humidity levels in the bat room must be stabilized. Inconsistent temperatures will contribute to the deaths of more bats.
- The staff and veterinarian must be absolutely sure that bats are not wet or damp when released after handling.
- Handling techniques must be refined so that bats do not become dirty or caked with food during hand-feeding sessions. Bats should never be released when dirty or the subsequent deaths of more bats will result.
- The use of hydrogen peroxide must stop immediately. Knowledgeable veterinarians are aware that it should never be used on any skin wounds beyond initial treatment. Continued use of this product

will delay wound healing and prevent new cell growth, increase the likelihood of more infection as well as stress from over-handling, and death. Use Novolsan or Chlor-a-Flush in place of peroxide.

- It is absolutely critical that an enriched, easily assimilated, palatable diet is used. The recommended diet for these bats can be found in "Diagnostic and Treatment Update for the Rehabilitation Insectivorous Bats."
- It is strongly recommended that the staff consult with Mark Finke, PhD Nutritional Scientist, for further diet recommendations for insectivorous bats.
- Energel and Vital HM powder must be kept readily available should a bat refuse to eat.
- Medium to large sized crickets are critical in providing an additional food source (not an alternate food source!). Crickets will better provide a natural foraging strategy and enrichment to these bats.
- Additional feeding stations and food trays filled to the maximum capacity must be in place to allow every bat to fed and decrease the possibility of a bat not having the opportunity to feed.
- Allow the males and females to roost together as they would naturally do in the wild.
- Add enrichment items such as silk foliage and fake trees to create a simulated natural habitat environment critical for the physiological health and well-being of bats in captivity.
- Create detailed daily record keeping templates to populate upon entry/exit of bat room to increase communication between veterinary staff and bat caretaker(s). Initial observations while colony is being established can not be re-created or recalled. Lessons learned may be overlooked causing subsequent future captures to also fall prey to unnecessary mistakes and shortcomings.
- Secure a copy of the companion manual "Diagnostic and Treatment Update for the Rehabilitation of Insectivorous Bats" and have it available along with the "Captive Care and Medial Reference for the Rehabilitation of Insectivorous Bats" book in the bat room.

Appendix B: Final VBEB plan, signed (attached)

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VIRGINIA BIG-EARED BAT (*Corynorhinus townsendii virginianus*)

PLAN FOR CONTROLLED HOLDING, PROPAGATION, AND REINTRODUCTION

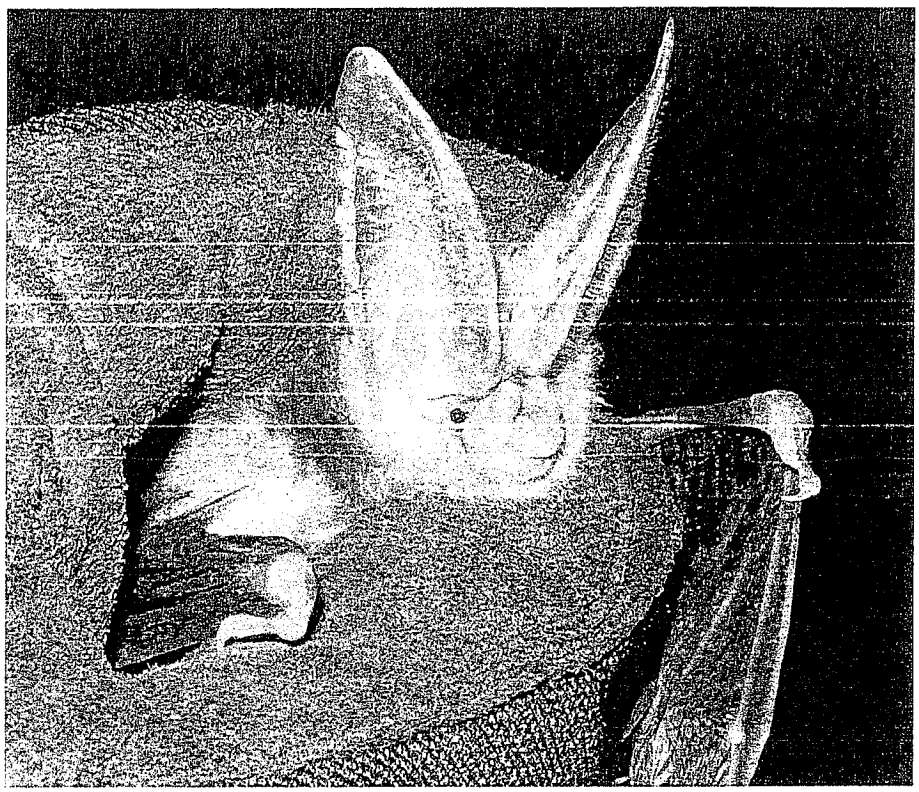

Photo courtesy of Craig Stihler, WVDNR

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APPROVAL:

Lead Field Supervisor, West Virginia Field Office U.S. Fish and Wildlife Service

Approve Deborah Carter Date 8/21/2009

Acknowledgements: The West Virginia Field Office would like to thank all the individuals who contributed their time and expertise towards the development of this plan. Those individuals are listed in Appendix A

Background

Life History

The Virginia big-eared bat (*Corynorhinus townsendii virginianus*) (VBEB) is a medium-sized bat that has brownish fur, long ears, weighs less than 0.5 ounces, and is approximately four inches in length from head to toe. They inhabit caves and mines in both summer and winter. During the winter they hibernate in clusters that may contain many hundreds of individuals. During the summer they use caves for maternity sites and roosting. They emerge each night to forage for moths and other insects among woodlands, forest edges, old fields, and hay fields. Mating begins in autumn. Sperm are stored in the female reproductive tract through the winter, and fertilization occurs shortly after arousal from hibernation. When the females arrive at their maternity sites they are pregnant and have one young per pregnancy. Young are born around mid-June, and by mid-July the young begin to leave the cave at night to forage. Most bats leave the maternity cave by late September. Although they may use different caves during the summer and winter periods, no long-distance migrations are known, and movements of up to 20 miles have been documented between summer roosts and hibernacula (Stihler 1994; Stihler 1995; C. Stihler, personal communication). The bats return year after year to the same hibernation and maternity sites (C. Stihler, personal communication). Bats that use different maternity caves may mix together in the same hibernation site and vice versa. Banding data collected by the West Virginia Division of Natural Resources (WVDNR) documented that bats roosting in four separate summer roosts hibernate primarily in a single roost in Pendleton County, West Virginia. Some individuals from two of the four summer roosts were also found in another hibernaculum approximately one mile away (C. Stihler, personal communication).

Population and Distribution

The range-wide population of VBEB is currently estimated to be approximately 15,000 bats. Four genetically-distinct sub-populations of VBEB occur in northeastern West Virginia/northwestern Virginia, southern West Virginia, eastern Kentucky, and western Virginia/North Carolina (Piaggio 2009). Throughout this range, there are only 13 caves that have been documented to support groups of more than 20 hibernating VBEB, and only eight that have supported groups of more than 100 individuals. There are only 17 caves known to be used as maternity sites and five other caves that are known to support summer bachelor-colonies composed of more than 20 individuals. The northeastern West Virginia/northwestern Virginia region, which encompasses Tucker, Pendleton, and Grant counties, West Virginia and Highland County, Virginia, supports the largest population segment. Over 60% of the range-wide population hibernates in these counties. Caves in this region also support approximately 77% of the range-wide maternity population (Service 2008b). In addition, all five caves currently designated as critical habitat under the Endangered Species Act occur in this region (44 FR 61290-61292). The area encompassed by all the hibernation and maternity caves in this region is approximately 30 miles long and 36 miles wide. There are only three caves located outside of West Virginia that support more than 100 hibernating VBEB (Service 2008a). These caves are located in Tazewell County, Virginia; Avery County, North Carolina; and Lee County, Kentucky.

Threats

The VBEB was listed as endangered under the Endangered Species Act (87 Stat. 884, as amended; 16 U.S.C. 1531 et seq.) in 1979 due to their small population size, limited distribution, and vulnerability to human disturbance. Since the time of listing, recovery efforts have been focused on purchasing important VBEB habitats, and working with private landowners to implement protective measures such as gating cave entrances and restricting access to caves during times that VBEB are present. These measures have been extremely successful, and numbers of hibernating VBEB have increased approximately 450% since 1984, when the recovery plan was finalized (Service 2008a, Service 1984).

During the winter of 2006/2007, a condition named "white-nose syndrome" (WNS) was first noted among bats hibernating in caves near Albany, New York. The syndrome was named because affected bats appeared to have a white substance on their muzzles and wings. By winter 2007/2008, WNS had spread over 130 miles to the north and 80 miles to the south and was known to occur in approximately 25-30 caves and mines. Almost all of the known bat hibernacula within 80 miles of the original cave were affected. As of late winter 2008/2009, WNS had spread to caves located over 500 miles from the original site, and had affected caves in eight other states including Vermont, New Hampshire, Massachusetts, Connecticut, Pennsylvania, New Jersey, West Virginia and Virginia (Butchkowski, 2009; Hicks et al. 2009).

Although the causes of WNS and the mechanism of spread are still being investigated, it appears to be assciated with a newly described fungus *Geomyces destructans* (Gargas et al. 2009). WNS has caused large-scale mortalities of bats in affected caves, with 55-64% mortality being documented within one year of detection, and up to 97% mortality within two years (Hicks et al. 2009). It is currently estimated that over 1 million bats have died as a result of WNS (Hicks et al. 2009). Affected species include little brown (*Myotis lucifugus*), northern long-eared (*M. septentrionalis*), big brown (*Eptesicus fuscus*), and tricolored bats (*Perimyotis subflavus*), as well as the federally-endangered Indiana bat (*M. sodalis*). To date no species of hibernating bat known to be present within affected caves and mines has shown evidence of being resistant to the effects of WNS.

In February 2009, four caves within the 30 x 36 mile range of the northeastern West Virginia/northwestern Virginia VBEB subpopulation were documented to be affected by WNS, including one maternity cave designated as critical habitat, and one hibernation site that supports approximately 125 VBEB (C. Stihler, personal communication, 2009; J. Wallace, personal communication, 2009). If current trends regarding the rate of WNS spread continue, WNS can be expected to be in all major VBEB caves in this subpopulation, including all caves designated as critical habitat, within the next year (winter 2009/2010). Further, WNS would be expected to occur in the range of the other genetically-distinct VBEB subpopulations within the next 1-2 years. Although no VBEB mortality associated with WNS has been documented to date, in the absence of data suggesting otherwise, it is prudent to assume that VBEB could experience similar mortality rates if they are susceptible. As a result, large-scale mortality of VBEB in the northeastern region of West Virginia is likely during the winter 2009/2010.

Justification for Captive Propagation

A number of factors combine to make VBEB extremely susceptible to the risk of extinction and/or local extirpation from WNS. These include:

- the extremely limited number of caves known to support the subspecies (only eight hibernacula range-wide that support groups of more than 100 individuals);
- the concentration of the largest sub-population (containing 60-77% of the range-wide population) within a restricted geographic range (a 30 x 36 mile area);
- the documented emergence of WNS within this area including within the fourth largest VBEB hibernaculum for that sub-population and also in another cave that is one of the five caves designated as critical habitat;
- the proximity of the remaining sub-populations to other known WNS-affected caves (30-60 miles to western Virginia/North Carolina, 60 miles to southern West Virginia, and 150 miles to the Kentucky populations);
- the limited number of caves that support VBEB in the three smaller sub-populations (The States of Virginia, Kentucky, and North Carolina each have only one cave that supports groups of more than 100 hibernating individuals);
- the geographic isolation and genetic distinction of the four sub-populations, making it unlikely that bats could naturally re-colonize areas historically occupied by another sub-population once the effects of WNS are ameliorated.

Given these factors, a portion of the VBEB working group convened to develop appropriate "triggers" for initiating VBEB captive propagation efforts. Those conclusions are fully detailed in Appendix B-1 and are summarized here.

In that document, Dr. Phil Miller, of the Conservation Breeding Specialist Group, considered a range of population viability analysis techniques that are commonly used to help identify a maximum acceptable level of annual mortality from a threat. These analyses evaluate the likelihood that a population or taxon will become extinct within a given timeframe. In some cases, the wildlife demographic processes that are affected by threats, and the risks associated with them, operate together in a continuous manner such that a certain level of annual mortality from a potential threat is deemed to be non-threatening at low levels of intensity, but will become unacceptable at higher levels. Other types of threats operate in a binary fashion, meaning that either the threat is present and the impact is severe, or the threat is absent. An example cited for this latter type of threat is the chytridiomycosis fungal infection in amphibians. Once the fungus is introduced into an area, it spreads rapidly through the amphibian population and causes rapid and precipitous declines in population abundance. As a result, once the pathogen has entered a given ecosystem, all amphibian populations in the area are at a very high risk of extinction in the very near future. The threat of WNS appears to provide a parallel example in that it spreads very rapidly through hibernating populations upon infection, and leads to severe declines in total population size in a very short period of time. In these types of binary effects scenarios, it is difficult to conceive of a useful management trigger that can be defined in terms of a population impact exceeding a demographic threshold. Rather, a high risk of exposure carries with it a correspondingly high risk of significant impact, perhaps even extinction. Using this scenario, an appropriate trigger

for initiating VBEB conservation management action would be defined in terms of the proximity of an unaffected cave to the epidemic front and the predicted length of time before the front reached the cave.

Using this type of model as a trigger, the working group considered factors such as the previous rapid spread of WNS (over 100 miles within one year of documentation and 500 miles within two years), the proximity of WNS to VBEB caves, the high and rapid level of associated mortality (up to 97% within two years) once WNS enters a site. The group determined that large-scale reductions in the overall population of VBEB are likely imminent. They concluded that "aggressive and immediate management actions to prevent drastic population decline appear warranted," particularly within the northeastern West Virginia/northwestern Virginia population. The three remaining distinct VBEB populations to the southwest should be also be targeted for subsequent action once the epidemic front reaches a critical proximity. A critical assumption behind this determination is that VBEB are susceptible to WNS mortality in a manner similar to those documented for six other hibernating bat species in the northeastern United States. The possible need for research to validate the assumption that VBEB are suceptable to WNS is described below.

Other Alternatives Considered

The following alternatives to immediately initiating captive holding and propagation were considered. None of the available alternatives provide a reasonable means of reducing the risk of VBEB extinction or local extirpation within the immediate future as effectively as captive propagation.

Wait until Significant VBEB Mortality is Documented

WNS was first documented within the range of the VBEB in February 2009. Visible signs of infection were limited to a small number of individual bats of two species, the little brown bat and tricolored bat. Although some dead bats were found, large scale mortality has not yet been observed at sites within the range of VBEB. If the effects of WNS in West Virginia follow a similar pattern as experienced elsewhere, then WNS will become much more prevalent on bats within the area during the second hibernation season after it is detected (winter 2009/2010) with significant bat morality occurring late in that season.

It is possible that VBEB may be resistant or less susceptible to WNS than other species that have already been exposed. Therefore, we considered conducting laboratory tests to determine VBEB susceptibility prior to initiating captive holding and propagation efforts. However, because these tests would have to be conducted during the hibernation period, when signs of WNS and associated mortality typically occur, the earliest they could be conducted would be during fall and winter 2009/2010. Results from the tests would likely not be available until late that winter season. By that time, if VBEB were susceptible to WNS, it is likely that signs of susceptibility would already be evident in the field. Therefore, conducting these tests is not a timely or prudent alternative, since similar results will likely be available within the same time period by monitoring caves in

this area using already established and planned methods that involve less expense and labor.

A related alternative would be to wait to initiate captive holding efforts until VBEB mortality from WNS is documented in the field, thus confirming VBEB susceptibility. If VBEB are in fact susceptible to WNS, by the time that susceptibility is documented, WNS may have spread throughout a good proportion of VBEB caves and their associated populations. The likelihood of a quick spread through VBEB populations is high due to the limited number of caves that support VBEB; the close proximity of these caves to each other; and the fact that during the fall swarming season, VBEB that use many different hibernation sites mix together as they travel from cave to cave to mate and that many summer colonies converge into a few hibernacula in the winter. A large number of other migratory hibernating bat species are also known to use VBEB caves.

As a result, if we were to wait until WNS is documented within VBEB populations before initiating captive holding efforts, it would increase the likelihood that any bats gathered for captive efforts would have already been exposed to the syndrome. Bats that have been exposed to WNS may already have health complications and would require a higher level of medical care. It would be much more difficult to rescue, rehabilitate, quarantine, and care for affected bats than it would be to work with healthy animals gathered before they were affected. To date, efforts to rehabilitate WNS-affected bats of other species have had limited success. Attempting to rehabilitate WNS-affected VBEB would be complicated by the fact that so few VBEB have ever been held in captivity, and techniques for their holding and care have not been fully developed. Taking action to initiate captive holding prior to the 2009/2010 winter season will allow more time and provide more appropriate conditions for VBEB captive husbandry techniques to be perfected using healthy bats, which would increase the likelihood of project success and reduce associated labor and medical expenses.

Finally, although it does appear that species such as the big brown bat may be less susceptible to the effects of WNS, to date there are no cave-dwelling bat species that have been found to be immune. Given this available information, the high rate and rapid development of WNS mortality experienced by most bat species that have already been exposed, and the distributional factors that increase the possibility of rapid extinction of VBEB, we determined that the potential benefits of delaying captive holding efforts in order to prove susceptibility were significantly outweighed by the potential risks and consequences of not initiating those efforts in a timely manner. If monitoring in subsequent years demonstrates that VBEB are not affected by WNS, captive holding could be discontinued. Any information obtained by holding VBEB in captivity may provide useful information that could be applied to holding other insectivorous bat species that are affected by WNS (such as Indiana bats) or could provide data that would help determine why VBEB are not affected, which could be used to help discern potential treatment options for other bats.

Summer censuses of VBEB maternity caves in West Virginia conducted in June 2009 documented the largest number of bats observed since the censuses began in 1983. At

this time, this subpopulation is doing well and there are sufficient bats that removal of individuals for a captive colony will not negatively affect the subpopulation's viability. Therefore, it is prudent to act now before WNS results in mortality in the wild population.

Treat Affected Caves

As a newly emergent syndrome, little is known about the etiology of WNS. The causes of WNS and mechanisms of transmission are still being investigated and although work to identify and test potential treatment options is underway, feasible approaches have yet to be identified. Options such as treating affected caves with fungicide or biocontrol agents are being investigated and considered. However, these agents have not yet been tested or, in some cases, have not been identified. There are also secondary concerns that need to be addressed such as minimizing impacts to non-target organisms such as cave invertebrates and bats, ensuring the beneficial microbiotic communities of caves are not altered, and assessing how to effectively treat large or complex caves/mines. Any initial attempts at using these types of methods will be experimental and measures such as these will likely only be tested on selected sites where potential adverse effects could be minimized. As a result, it is not known when effective measures to control the effects and spread of WNS will be developed. Even when more information becomes available, or when potentially feasible treatment options have been identified, it may take a few years to fully implement and test these measures to the point that they could be safely used in the most important VBEB caves. By the time that these measures are available for use, it is likely that WNS will have spread throughout the range of the VBEB and significantly reduced VBEB population numbers, or potentially resulted in species extinction or local extirpation of genetically distinct subpopulations.

However, over the long term, measures such as treating caves may provide a solution that would allow for containment and control of WNS, and provide habitat that would allow captive-held VBEB to be released back into the wild. Therefore, while this alternative does not eliminate the need for captive holding, treating caves should still be pursued in concert with captive holding and propagation.

Attempting to Treat Affected VBEB Individually

Another alternative would be to treat affected VBEB individually by applying fungicide or other treatments to bats while they were hibernating. However, as was described under treating caves, potential options for these types of treatments have not yet been tested, or in some cases even identified. It may take a few years to fully identify and test these types of treatments to the point that they could be widely used. By the time that these measures are available for use, WNS could have spread throughout the range of the VBEB and have significantly reduced the VBEB population numbers, or potentially resulted in species extinction or local extirpation of genetically distinct subpopulations.

Additionally, experimental treatments on individual VBEB would be labor intensive, logistically difficult, and stressful to individual bats. Many VBEB roost on high ceilings or other inaccessible portions of caves which could make it difficult to treat all or even most of the bats in a particular cave. Unless treatment options are developed that provide

long-term immunity and all bats (including non-VBEB) that roost within a particular hibernacula are treated, untreated bats could re-infect treated bats with WNS, rendering the treatments ineffective. Treating bats during the hibernation season would increase the amount of human disturbances occurring at hibernation sites, which is known to cause adverse effects to the bats. Finally, if WNS remains within affected cave environments such as sediments and cave walls, then every year caves and individual bats roosting within those caves would have to be treated every year, requiring a large annual outlay of labor and expense.

Summary

There are no alternatives that provide as effective a means of reducing the risk of VBEB extinction as captive holding and propagation. Waiting to initiate captive holding until VBEB mortality was documented was considered. However, this was deemed to be undesirable because it would increase the likelihood that significant population level mortality could occur before captive holding efforts could be initiated and that these efforts would then need to start with bats already compromised by exposure to WNS. It would be much more difficult to rescue, rehabilitate, quarantine, and care for affected bats, than it would be to work with healthy animals gathered before they were affected. Treating affected caves or individual bats was also considered. However, effective treatment measures have yet to be identified and once identified, they will need to be tested. By the time effective treatment options are tested and available for use, WNS could have spread throughout the range of the VBEB and have significantly reduced the VBEB population numbers, or potentially resulted in species extinction or local extirpation of genetically distinct subpopulations. Captive holding and propagation is currently the only potentially feasible option available for ensuring that healthy populations of VBEB are maintained until the threat of WNS has abated.

Relationship to the Recovery Plan

The VBEB Recovery Plan was finalized in 1984 (Service 1984). At the time the plan was finalized, the primary threats and factors limiting species recovery were the limited number of suitable winter and summer roosting sites, and human disturbances to those sites. As a result, most of the recovery actions identified in the plan focused on identifying caves that VBEB use and protecting those sites from human disturbance through direct purchase and/or by gating.

WNS was initially discovered in 2006, and bat mortality associated with the syndrome was not documented until 2007. The most recent 5-year review for the VBEB was finalized in summer 2008. At that time, WNS was not known to occur within the range of the VBEB, but the review stated that if WNS spread to this area it could have "devastating population-level effects on the species" (Service 2008a). The 5-year review also identified the need to revise the recovery plan to incorporate updated information on species biology and threats. WNS was first documented to occur in the range of VBEB in West Virginia in February 2009. Because of the age of the recovery plan in relation to this newly emerging threat, recovery actions designed to address WNS were not included in the plan, and captive propagation is not listed as a potential recovery strategy for the

VBEB. Due to the potential for large-scale and imminent VBEB mortality within the next hibernation period (winter 2009-2010), we have prioritized measures to plan and implement specific recovery actions such as captive propagation, rather then delay initiation of the actions in order to formally revise the plan.

Coordination with States and other Partners

Initial plans for this effort were developed by members of an informally established VBEB working group. Participants included representatives from the Smithsonian National Zoological Park, Bat Conservation International, the Conservation Breeding Specialist Group (SSC/IUCN), the Association of Zoos and Aquariums Bat Taxonomic Advisory Group, Mesker Park Zoo and Botanic Garden, U.S. Geological Survey (USGS) Fort Collins Science Center, WVDNR, Virginia Department of Game and Inland Fisheries, North Carolina Wildlife Resources Commission, Kentucky Department of Fish and Wildlife Resources, U.S. Department of Agriculture National Wildlife Research Center, and the USGS National Wildlife Health Center. A list of participants is included in Appendix A. We anticipate continuing to coordinate with members of this group, as well as other species and topical experts, throughout the captive holding and propagation effort. All efforts to collect, hold, and release VBEB will be coordinated with representatives from the affected State wildlife resource agencies.

Purpose and Goals

The primary purposes of the VBEB captive holding, propagation, and reintroduction effort are to:

- Reduce or alleviate the risk of extinction, regional extirpation, and/or the loss of existing genetic diversity
- Maintain population(s) in confinement until treatment and control measures for WNS are developed or natural mortality subsides to low levels
- Provide a source of individuals to use to restore wild populations within the historic range of the species once the threat of WNS has been reduced or subsides.

A secondary purpose is to gain additional information on VBEB biology and maintenance requirements (e.g. nutritional requirements, physiology) that will assist managers in developing appropriate responses to WNS for this species and other insectivorous cave-dwelling bats.

Phased Approach

It is anticipated that this project will be implemented using a phased approach. Because VBEB have never before been held for any duration in captivity and protocols for holding, propagating, and releasing other insectivorous bats are not well established, this effort will use an adaptive management type of approach. Throughout the three project phases, husbandry techniques, facility set-ups, and other management approaches may be modified based on the responses of the captive VBEB and the most recent scientific developments. Techniques are expected to improve as we refine methods and identify more effective or efficient means to accomplish the specified goals. Updates and

supplements to this Captive Propagation Plan (Plan) will be made as the project progresses. The goals and success criteria below, and the protocols described throughout this document represent our initial plans.

Phase One-Captive Holding
The initial phase of the project will focus on captive holding. The goal of Phase One will be to develop and implement effective husbandry techniques to maintain healthy VBEB in captivity. This will involve initially bringing a limited number of individuals into captivity so that husbandry protocols can be tested. Success criteria for this phase will be that:

- adult VBEB exhibit feeding and roosting behaviors sufficient to maintain appropriate weights and overall fitness, and
- adult VBEB exhibit no signs of WNS or other diseases, and
- minimal mortality is experienced.

Phase Two – Captive Propagation
Once the goals of Phase One are met, the project will progress towards captive propagation. Goals of Phase Two will be to maintain secure, genetically diverse populations of VBEB in captivity and develop a breeding protocol so that productivity sufficient to maintain a viable captive population is achieved. This could include bringing in sufficient additional individuals to support a population with long-term viability, establishing populations in multiple facilities, or maintaining populations from all four separate sub-populations. In addition to the criteria listed for Phase One above, success criteria for Phase Two will be that:

- adult VBEB are successfully bred, and
- young survive to reach sexual maturity, and
- productivity equals or exceeds mortality, and
- appropriate procedures to maintain and track genetic diversity are implemented.

The duration of Phase Two will depend on how quickly progress is made towards addressing the threat of WNS in the wild.

Phase Three – Population Augmentation and Release
Once sufficient measures to address WNS are in place so that it could reasonably be expected that VBEB could survive in the wild, the project will move into the final phase, population augmentation and release. Prior to implementing this phase, specific protocols for selecting release sites, releasing bats, and monitoring released bats will need to be developed. The goal of this phase will be to release captive-bred or held VBEB so that self-sustaining wild populations can be established or retained. Ideally this would include the re-establishment or retention of all four genetically distinct sub-populations that currently exist. In addition to the criteria listed for the two phases above, success criteria for Phase Three will be that after being released into the wild, captive bred or held VBEB:

- survive and are capable of sustaining themselves, and
- appear to adopt natural behavioral patterns, and
- successfully breed and raise young, and
- that monitoring over multiple years documents that populations in the wild are increasing or remaining stable through natural reproduction.

Project Specific Planning

Partners, including the U.S. Fish and Wildlife Service (Service), wishing to conduct specific VBEB captive holding/propagation actions must obtain all necessary state and federal permits and produce a Project Specific Plan (Project Plan) before conducting any activities. Project Plans for potential captive holding/propagation activities will be developed in cooperation with and approved by the appropriate Service Field Office(s) (FO) before activities begin. The Service's West Virginia Field Office (WVFO), the lead office for the VBEB, shall approve each site plan before its implementation. For activities involving collection or release of VBEB outside of West Virginia, coordination with the local Ecological Services FO shall also occur. All Project Plans will also be coordinated with relevant state wildlife agencies. Collection of bats, successful production of progeny, number of progeny produced, etc., are never certain, but Project Plans should include as much information as possible. At a minimum, they must include the following:

- an outline of the qualifications of project coordinator and implementation team, and a list of any cooperating parties and their responsibilities;
- project-specific goals and objectives relevant to the appropriate project phase as described above;
- a description of facilities to be used and proposed methodology;
- detailed explanations of how the project will address issues regarding source populations, quarantine, husbandry, genetics, and plans for release or disposition of individuals as described below;
- a detailed budget, including partner contributions and other likely sources of funding; and
- a description and copy of all required permits.

Reporting

Due to the uncertainty and experimental nature of VBEB captive holding/propagation efforts and the continually developing nature of information regarding WNS, routine communication and open sharing of information between all partners will be critical to long-term project success. Any partner conducting VBEB captive holding/propagation must routinely coordinate with the Service and appropriate state wildlife agencies to provide updates on project status and significant accomplishments or problems. Any protocols developed, lessons learned, and other information gained during the course of a project should be routinely shared with other parties working on WNS research and response activities, including other parties engaged in similar captive holding/propagation efforts for the VBEB and other bat species.

Any partner conducting VBEB captive holding/propagation studies, releases, or release monitoring studies will at a minimum provide an annual report of activities to the Service, the appropriate state wildlife agency, and other involved partners. This report will include:

- a brief description of their program, including objectives and status;
- list of cooperators, if any;
- activities conducted and the results including a description of significant successes or obstacles encountered;
- a brief description of the status of captive or reintroduced populations;
- a summary of any recommended changes or updates to project protocols and the rationale; and
- a description of any additional efforts made to disseminate results of their work, including papers published, presentations made, or meetings attended.

In addition, partners should maintain detailed records of all activities conducted, including life history observations, fecundity, survival, mortality, results of any veterinary or other testing conducted, and any other conditions/observations important to successful propagation of the species.

Source Populations

The northeastern West Virginia/northwestern Virginia subpopulation is the largest and closest to the current range of WNS and should be targeted first for source population collection efforts. Other subpopulations can be targeted after captive holding methods become established.

Capture site selection should take into consideration the ease of collecting, minimizing stress to the bats, approval of the land owner, and the size of the population using the cave. Caves with relatively small colonies should be avoided if there are other options so that the integrity of the colony is not impacted by the removal of the collected bats. Sites known to have WNS should be avoided. The actual method of capture will vary from site to site, but harp trapping and hand collection are probably the two methods that will result minimize stress to the animals.

It is probably best to collect bats in the fall. At this time the females are not pregnant and potential resorption of embryos or miscarriage of fetuses is not an issue, the young bats are developed and on their own, and the bats should be putting on fat reserves for winter which may help them survive until they become accustomed to feeding in captivity. This will also minimize the potential that collected bats will already be affected by WNS. In addition, during the swarming and hibernation season, bats from various summer sites mix together and the genetic diversity at any particular site will likely be greater than during the summer.

Collection during other times of year would increase the potential for mortality and should be avoided unless absolutely necessary. During the spring, bats will have already

lost significant fat reserves during hibernation, and would therefore have reduced fitness and ability to survive the stress of adapting to living and feeding in captivity. This would also increase the chances that the bats were already compromised by exposure to WNS. Collecting bats during hibernation is also discouraged. This would increase the amount of disturbance caused to the remaining hibernating bats, and increase the chances that the collected bats may have already been exposed to WNS. In addition, once bats have entered full hibernation they may not adapt well to captivity. Collection efforts for other species indicate that hibernating bats will continue to attempt to return to the hibernation state even when kept at warm temperatures (G. Tuner, PA Game Commission, personal communication). Bats brought into captivity during hibernation would have to be repeatedly checked for WNS, and potentially given veterinary care resulting in increased disturbances and loss of fat stores. Thus there is increased potential for mortality.

To the extent possible, the animals in the captive population should reflect the genetic diversity of the wild population. For the VBEB, there is very little genetic diversity within each subpopulation. In addition, banding data have shown that bats in the various summer colonies mix together during the fall swarming and hibernation periods. Therefore, collecting bats from a number of fall swarming or hibernation sites may not be necessary to capture a representation of the natural genetic diversity. Initially in Phase One an equal number of males and females should be taken into captivity to develop protocols for maintaining the bats and to determine if there are sexual differences in the bats' response to captive confinement. An initial collection should not exceed 40 to 50 bats. Once it is demonstrated that these bats can be maintained in good health in captivity, additional bats should be added to the captive colony. The total number to be maintained and the appropriate sex ratio will be determined during Phase Two and is described in more detail in the Genetics, Breeding, and Numbers section of this document. The chance of reestablishing a wild population using captive bats will probably be greater if the bats released were captured from the wild and not captive-reared, because wild bats may retain familiarity with cave and foraging sites. Therefore, the bats taken into captivity should include both older bats and juveniles to increase the likelihood that some of the wild-caught bats will be alive when it is time to reintroduce bats into the wild.

The bats should be transported in a manner that minimizes stress to the animals. Soft-sided cages should be used and the bats should be kept in a dark, quiet environment. Bats should be transported to the holding facility as soon after capture as possible. When capturing bats, basic biological data should be collected for each bat handled (body mass, forearm length, wing score index, etc.). Individual bats should be marked so they can be identified.

Each Project Plan should describe the collection site(s) selected, rationale for selecting the site(s), number of bats of each sex to be collected, methods to be used (including method of bat identification), and personnel that will be handling the bats. The Project Plan must be approved prior to proceeding with the collection.

Quarantine

This effort involves three different types of activities/periods that will require separate quarantine protocols:

1. collecting source populations and transporting them to the holding facility;
2. holding the bats; and
3. bringing in additional specimens.

When dealing with a disease such as WNS that appears to be highly transmissible, establishing and implementing appropriate quarantine procedures are critical. Because the etiology of WNS is not yet fully understood, quarantine procedures will need to be continually reviewed and updated to reflect the most recent developments in WNS research. Other potential diseases associated with bats, such as rabies, will also need to be addressed. A summary of current suggestions regarding quarantine concerns and procedures developed by the VBEB working group is included in Appendix B-4. The information contained below is not intended to be a complete list of quarantine requirements; rather it outlines the types of considerations that will need to be taken into account before initiating any of these activities.

Collecting and Transporting Source Populations

When possible, collections should be made from sites that do not show evidence of WNS. Unless necessary for the retention of genetic diversity or species survival, bats collected for source populations should appear healthy and free of WNS and other health complications.

Collectors should use the most up-to-date WNS decontamination guidelines. These guidelines can be found at: http://www.fws.gov/northeast/white_nose.html. In order to minimize the potential to introduce WNS to new areas, the movement of bats collected from areas potentially affected by WNS into areas that are unaffected by WNS should be restricted. Therefore, until modes of WNS transmission are better understood, the initial facilities selected for holding the bats would ideally be located in close proximity to the areas where the bats are collected. Once collected, bats should be transported as soon as possible to the holding facility. Transferring collected bats between different vehicles, holding locations, or containers should be minimized. Bats should only be handled by individuals with appropriate rabies vaccinations and using personal protective gear that can be decontaminated or disposed of after use.

Holding Bats

Any organization or entity that receives VBEB will be required to develop site-specific quarantine procedures prior to accepting any specimens. Quarantine and decontamination protocols should be established for anyone entering the holding facility or handling the bats, as well as for quarantining VBEB from contact with other animals. Facilities to hold captive VBEB should be constructed in such a manner that captive bats will not be in contact with wild bats or with other species being held in the facility that could be a vector for potential disease or infection. Once in the holding facility, VBEB should be initially maintained in conditions to minimize the potential for WNS

development (e.g. at temperatures that would discourage growth of *G. destructans – see Appendix B-4*), and be tested for the presence of the fungus. VBEB caretakers should not be handling other bat species that may have been recently exposed to WNS. Bats should only be handled by individuals with appropriate rabies vaccinations and when using personal protective gear that can be decontaminated or disposed of after use.

Bringing in Additional Specimens

Because this effort will be conducted using a phased approach, and because there is potential to be working with four genetically-distinct subpopulations, it is likely that a facility may bring in separate groups of individuals over a period of time. Prior to introducing animals into an established captive population, or bringing additional VBEB into a facility already holding other VBEB, the new animals should be quarantined to ensure they will not be introducing WNS or other diseases into the established population. Quarantine locations should be sufficiently separated from established VBEB populations so that the possibility of bat-to-bat and bat-to-human-to-bat transmission of diseases or pathogens is minimized. Prior to introduction, animals should be tested for the presence of the WNS fungus and treated with fungicides or other control measures if they are available. Animals should also be screened and/or treated for the presence of ectoparasites such as strebilid flies and mites. Quarantine procedures for introductions will need to be modified and updated as information on WNS is developed. Project/facility specific procedures must be developed and approved as part of the Project Plan prior to collecting any additional individuals that may be brought into facilities holding established VBEB captive populations.

Husbandry

Since VBEB have not been held in captivity before, there are no established husbandry protocols for this species. The initial phase of this captive holding/propagation effort will focus on the development of effective husbandry techniques. Members of the VBEB working group with experience in maintaining other bats in captivity developed a set of preliminary husbandry recommendations that are attached in Appendix B-3. Initial attempts at holding VBEB in captivity should closely follow those recommendations until more species-specific protocols are tested and refined.

Because VBEB use caves/mines for roosting habitat, all captive VBEB should be provided with access to secure roosting areas that to the extent possible mimic these natural conditions. As noted in the Recovery Plan, VBEB are particularly sensitive to disturbances to both their maternity and hibernation roosting habitat. Excessive disturbance can cause the bats to abandon the roosting area and/or their young, and cause increased stress. Holding facilities should be constructed so that roosting areas are segregated from flight areas and will not be disturbed when staff access flight areas. Care should be taken to minimize disturbances to roosting VBEB and occupied roosting areas when cleaning, feeding, or performing other required care.

In the wild, VBEB primarily eat moths and other soft-bodied prey. However, moths are generally not commercially available, and bats will most likely also need to be

conditioned to eat other more commonly available food items such as mealworms. These food items may have harder bodies and may cause accelerated tooth wear compared to wild prey items. Efforts should be made to develop feeding regimens that allow bats to retain familiarity with natural foraging behaviors and prey items while still providing a nutritionally complete diet from readily available sources. Captive VBEB should be periodically evaluated for signs of excessive tooth wear and diets should be modified accordingly.

Each facility or partner proposing to hold captive VBEB should establish procedures for husbandry, monitoring health and fitness of captive bats, and for providing any required veterinary care. Monitoring should be sufficient to track the status of individual bats but should not result in undo stress to the animals. All facilities approved to hold VBEB should describe the type and frequency of examinations and treatment that they will provide and how they will access qualified veterinary support. Protocols for cleaning and disinfecting enclosures, feeding and watering materials, and all other items used by captive VBEB must be developed and implemented.

All facilities holding VBEB should employ a means to mark or otherwise definitively identify all bats brought into captivity and subsequently produced. Methods used should be designed to minimize incidental damage and stress to VBEB. Methods could include the use of passive integrated transponders (PIT) tags, banding, and/or tattoos. Detailed records on individual bats including source location, health and fitness, breeding status, progeny, and ultimate fate should be maintained.

Genetics, Breeding, and Numbers

Background

As described above, VBEB are distributed throughout four genetically-distinct regions roughly located in northeastern West Virginia/northwestern Virginia, southern West Virginia, eastern Kentucky, and southwestern Virginia/North Carolina (Piaggio 2009). The results of that study suggest complete loss of connectivity among regional populations. Because known colonies of VBEB are in such disparate regions and these regions are outside the known dispersal distances of these bats (Humphrey and Kunz 1976), these populations no longer maintain genetic connectivity. Therefore, each regional population is likely an isolated entity subject to genetic drift and inbreeding. That study also found little genetic diversity within regional populations. The population within western Virginia/North Carolina had the lowest overall diversity with haplotypes approaching fixation and there was evidence of a population bottleneck in all regions except Kentucky. This reduced genetic diversity means that genetic drift may be driving diversity within these populations and that biodiversity and evolutionary potential has been diminished.

Implications

Establishing founder populations with sufficient numbers and genetic diversity will be an important component of the long-term success of this project, particularly during Phase Two (captive propagation). Appendix B-2 contains a set of detailed recommendations

developed by members of the VBEB working group with expertise in genetics and conservation breeding to aid in addressing these issues. Although more detailed information and guidance is presented in the text of the appendix, a summary of the recommendations is as follows:

1. If feasible, maintain separate captive populations for each of the four identified distinct population segments. Start with northeastern West Virginia/northwestern Virginia population immediately, and use management triggers to initiate additional populations when necessary.

2. Use 100 individuals as effective founders for each distinct population to maximize the genetic variability in captivity. Because of early mortality and failure of some individuals to breed, 200 individuals from each population should be collected from the wild.

3. Use software-based genetic and demographic management tools to identify population sizes consistent with a long-term management goal of 90% heterozygosity retention for 100 years. Develop a multi-institutional cooperative breeding program to adequately house the requisite number of individuals. Adopt a group-level genetic management strategy.

4. When entering into propagation, individuals could be analyzed for genetic makeup and this information used to establish a propagation plan that will maximize genetic diversity in the offspring produced.

It is recognized that issues such as the availability of funding and facilities willing to work with VBEB, the limited number of bats in each population, the success of source population collection efforts, and the unanticipated effects and spread of WNS may limit the ability of managers to fully achieve the goals and objectives presented. However, every effort should be made to implement these recommendations to the extent practicable. Prior to implementing activities under Phase Two, facilities should review the genetics recommendations and, under consultation with the Service and appropriate state wildlife agencies, develop detailed plans to address these concerns. Methods to track breeding, kinship, and genetic make-up of the populations should be described and implemented. Progress towards meeting the stated goals should be reviewed annually.

Release/Reintroduction

Because no treatment or control measures for WNS are currently available, specific locations and methods for release of captive held/propagated VBEB cannot yet be identified. Once measures to address WNS are in place or when WNS mortality naturally subsides, so that it could reasonably be expected that VBEB will survive in the wild, more detailed planning for this phase can occur. Project-specific protocols for selecting release sites, releasing bats, and monitoring released bats will be developed and approved prior to implementing any release or reintroduction efforts. The following concerns and issues should be addressed in all project-specific release plans.

Sites should not be selected for reintroduction activities unless measures to address the threat of WNS have been developed for that site, the land owner approves of the proposed action, and there is some assurance that the habitat the bats are released into

will receive long-term protection (e.g. conservation easement, ownership by federal or state agencies committed to habitat protection, or formal land owner agreement). Sites should be surveyed prior to potential reintroductions to determine if the site is currently occupied by VBEB. If the site is already occupied by VBEB, an evaluation of the viability of the existing population should be made. Releases should not occur at already occupied sites unless they will not adversely affect the existing population. Priority will be given to release sites that are within the current range of the VBEB and that have documented historical use. If those types of sites are not available, consideration will be given to the creation of new sites within the current range (e.g. artificial construction of caves, restoration or stabilization of mines/caves) or to using other caves/mines within the current range that have similar characteristics to historical sites. Release into sites outside of the historical/current range will only be considered as a last resort and after compliance with all requirements of the National Environmental Policy Act, ESA, and other applicable federal, state, and local regulations is obtained.

As noted in other sections of this document, there are currently four genetically and geographically distinct sub-populations of VBEB. Priority will be given to projects that will retain these distinctions by correlating release locations with bats derived from appropriate source populations. However, we recognize that issues such as the availability of funding and facilities willing to work with VBEB, the success of source population collection efforts, and the unanticipated effects and spread of WNS may limit the ability of managers to develop and maintain sufficient numbers of bats to support viable captive populations of all four sub-populations. Release efforts that do not maintain current genetic and geographic distinctions will be considered when necessary for the survival and recovery of the species, and when other alternatives are not feasible.

VBEB summer/maternity and hibernation habitats are characterized by different environmental conditions. In order to fully complete their lifecycle and retain natural behavioral patterns, released bats will have to have access to, and knowledge of, the locations of both habitat types. Site-specific release plans will need to incorporate measures to ensure that there is a reasonable expectation that released bats will be able to find and access both types of habitat. Although there may be a number of options for successfully accomplishing this goal, examples of appropriate measures could include releasing bats at a site that has historically been used for both types of habitat or releasing groups of bats that include original founder animals that may retain memory of the location of nearby caves that they previously used.

All release efforts should incorporate at least semi-annual measures to monitor the survival and fate of released bats. This could include periodic surveys of release sites and expected maternity and hibernation roost areas, radio telemetry, mist nets surveys, and/or other means of marking and tracking released bats. Monitoring efforts should be designed to minimize disturbance and other adverse effects to released bats, while still providing sufficient data to evaluate the success of individual release efforts. Subsequent release efforts may be modified or approved/disapproved based on the success of previous efforts.

Disposition of Surplus Individuals

During the course of the captive holding/propagation projects, care will need to be taken not to produce more young than can be safely held within the capacity of approved facilities, while still producing enough young to maintain viable populations in captivity. All facilities engaged in holding and/or propagating VBEB should develop a plan to manage breeding so that captive population size does not routinely exceed the carrying capacity of existing approved facilities. However, because techniques for breeding VBEB are not well-established and survival and mortality rates are uncertain, it is possible that surplus individuals may occasionally be produced. This is most likely to occur during the captive propagation phase, before any control/treatment options to WNS have been identified. Once the population augmentation and release phase is reached, it is not expected that there will be "surplus" individuals. Wild populations of VBEB have recently been expanding and there are no signs that carrying capacity has been reached. It is therefore anticipated that any individuals produced during Phase Three will be able to be released.

Facilities planning to engage in VBEB captive propagation should develop an estimate of the number of VBEB that can safely be supported and coordinate with the Service and state wildlife agencies to develop a plan for the disposition of any surplus individuals prior to initiating propagation efforts. The Service must concur with the proposed disposition of animals prior to the need for such disposition occurring. Options for surplus bats may include transfer to other facilities that are initiating VBEB captive holding/propagation efforts, transfer to zoos or other similar facilities for educational purposes, or transfer to research organizations working on the treatment and causes of WNS. Transfer to other facilities for research or captive holding/propagation should only occur after a project-specific research or captive propagation plan has been approved, the proposed research or captive propagation project has been determined to be a high priority project by Service, and after compling with all requirements of the National Environmental Policy Act, ESA, and other applicable federal, state, and local regulations is obtained. VBEB may not be transferred to any facility until after the facility has obtained all required permits to hold and handle live VBEB, and/or dispose of VBEB mortalities. For research uses, Institutional Animal Care and Use Committee documentation should be in place and provided to the Service prior to receiving and VBEB. All acquisitions and dispositions for VBEB captive holding and propagation should be conducted in accordance with the Association of Zoos and Aquariums's Acquisition and Disposition Policy (http://www.aza.org/ad-policy/).

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APPENDIX A:

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APPENDIX B:

Documents prepared by the VBEB Working Group

PRELIMINARY VIRGINIA BIG-EARED BAT
CAPTIVE PROPAGATION DOCUMENTS

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GENETICS

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Page 14: Some general thoughts on demographic and genetic management of captive populations of endangered species, with references to the VBEB (P. Miller)

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QUARANTINE

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Some Thoughts on Identifying "Triggers" for Initiating Aggressive Conservation Action for the Virginia Big-Eared Bat

Phil Miller
IUCN/SSC Conservation Breeding Specialist Group

Risk assessment methodologies are very useful for evaluating and comparing the demographic impacts of different threats to endangered species populations, and for providing insight into the relative benefits of alternative management strategies designed to ameliorate those threats. In the wildlife management community, these methodologies usually fall under the collective term *population viability analysis*, or PVA (Beissinger and McCullough 2002; Morris and Doak 2002).

Wildlife managers often need to know the likelihood that a population or taxon will become extinct within a given timeframe, or how likely will be the decline below a pre-determined threshold population size (otherwise known as *quasi-extinction*). A range of PVA techniques – from relatively simple count-based risk assessments to full-blown spatially explicit, individual-based simulation models of demographic and genetic processes operating in small populations of threatened species – are available to address such a question. These techniques can help identify a maximum level of annual mortality (for example) that cannot be exceeded in order to keep the risk of population decline or extinction below an acceptable level. Excellent examples of such applications of PVA can be found in the world of fisheries management, where harvest quotas must be carefully evaluated and targeted to ensure sustained yields from populations that are to remain viable in the long-term.

The wildlife demographic processes that are affected by threats, and the risks associated with them, often operate together in a continuous manner. In other words, a given increase in the magnitude of a threat will frequently yield a corresponding increase in the magnitude of the risk, as shown schematically in Figure 1. Explicit in this scenario is the notion that a threatening activity is deemed to be non-threatening at low levels of intensity, but will become so at higher levels. Again, this is the basis of sustainable fisheries management and terrestrial game management. Consider hunting-based mortality as the process in question. When the threat intensity exceeds the threshold value **M***, annual mortality becomes too high and the risk threshold **R*** is exceeded – thereby triggering management actions that are designed to reduce hunting to more tolerable levels.

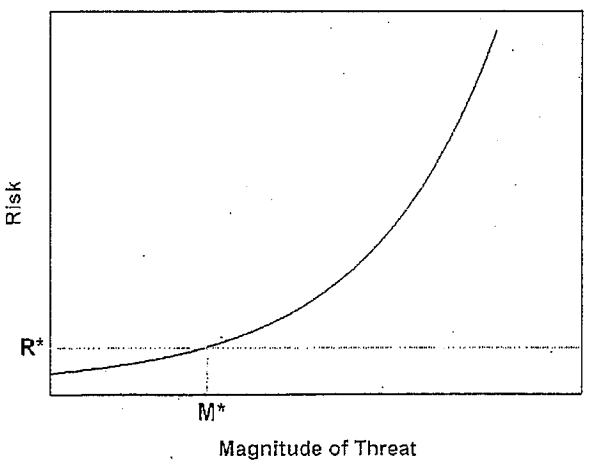

Figure 1. Generalized relationship showing risk of wildlife population decline under increasing magnitude of a given threat. Under this relationship, a specific risk tolerance threshold R* corresponds to a specific threat intensity M*, which can ultimately be translated into demographic characteristics of the population in question.

It is important to note that the value of the risk threshold R* shown in Figure 1 is determined not by biological theory or statistical logic, but by considerations of conservation policy and is derived from stated levels of risk tolerance among those setting the policy.

While many activities and processes that threaten wildlife populations today act in this type of continuous manner, there are others that do not follow such a pattern. Instead, we can think of their impact as operating essentially in a binary fashion: either the threat is present and the impact is severe, or the threat is absent. An excellent example of this phenomenon is chytridiomycosis in amphibians (Lips et al. 2006 and references therein). Chytridiomycosis results from infection by the fungus *Batrachochytrium dendrobatidis*. First seen in wild amphibian populations in 1999, at least 50% of species and more than 80% of individuals in a newly-infected area can be expected to disappear within a year, with the most severe impacts appearing to be in the Neotropics although the problem is global (Pounds et al. 2006; Lips et al. 2006). Although a very small number of amphibian species appear to demonstrate resistance to infection, introduction of the fungus into a naïve area typically leads to very rapid spread through water and direct animal contact, resulting in rapid and precipitous declines in population abundance. In other words, there is no evidence for a sort of dose-dependent response among amphibians to chytrid infection – once the pathogen has entered a given ecosystem, all amphibian populations in the area are at a very high risk of extinction in the very near future.

I see many parallels between chytridiomycosis in amphibians and white nose syndrome in bats of the northeastern US. WNS is only recently described, appears to spread very rapidly through a hibernating population upon infection, and leads to severe declines in total population size in a very short period of time – often just a single period of overwintering. Because of these parallels, I would argue that the "continuous effects" model of threat and its relationship to population risk shown in Figure 1 is also not valid for bats affected with WNS. A critical assumption behind that statement is that each bat species is equally and highly sensitive to infection with the pathogen and development of WNS upon infection. In species with low levels of sensitivity to the pathogen, it may be possible to describe the threat in a more continuous fashion. Additional research into susceptibility among different populations and taxonomic units will be required to address this issue.

If we assume (i) that the Virginia big-eared bat is highly susceptible to infection with the pathogen that causes WNS; and (ii) that the pathogen and resulting disease act in a manner similar to that of chytridiomycosis in amphibians, it is difficult to conceive of a useful management trigger that can be defined in terms of a population impact exceeding a demographic threshold. To consider an alternative to this approach, we can return to our Neotropical amphibian case study. Lips et al. (2006) documented the spatial trend in documented amphibian population declines and extinctions across Central America,

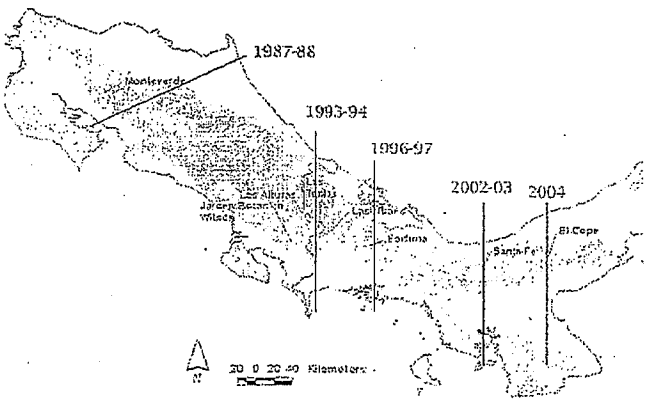

Figure 2. Locations in Central America with documented locations of amphibian population declines resulting from chytridiomycosis, and timelines associated with onset of infection. From Lips et al. (2006).

thereby allowing researchers to visualize an epidemic wave-front and to estimate the rate of spread of the fungus into new habitats (Figure 2), ranging from 28 to 100km/yr. Variance in the rate of spread is probably a function of the habitat conditions present in a region, as *Batrachochytrium dendrobatidis* strongly prefers moist and cool habitats. Using this information, Lips et al. (2006) were able to move ahead of the epidemic wave-front, identify a site at risk of chytrid infection, and document the onset and subsequent progression of the population-level impacts of chytridiomycosis.

In a similar fashion, historical data on the onset of WNS in caves of the northeastern US can be used to map the same type of epidemic wave-front and its movement southward from New England to West Virginia. This would allow broad estimates of the rate of spread of the syndrome which would, in turn, allow managers to identify those caves or cave complexes – currently disease-free – that that are in closest proximity to the epidemic front.

A trigger for initiating Virginia big-eared bat conservation management action would then be defined in terms of the proximity of a naïve cave to the epidemic front and, using the rate estimate, the predicted length of time before the front reached the cave. A tiered system could be put in place, where highest priority locations are those within a given distance of the epidemic front as they are expected to become exposed to the pathogen within a specified time interval. As a very general example, "Level 1" management action would be triggered for a cave that is within, say, 50 miles of the wave front as we may expect exposure to the pathogen in the next 1-2 years based on an estimate of epidemic front movement. "Level 2" management action may be assigned to a cave complex that is within, say, 100 miles of the wave front as we may expect exposure in the next 3-5 years. These target values are purely for purposes of demonstration only; if the general principle is seen as valid, intensive discussion is necessary to arrive at logical threshold values that can be used in practice.

Even if we discard the numerical thresholds held up above as simple examples, it is safe to say that the VBEB population in Pendleton County, WV is at extremely high risk of exposure as WNS has been found just a few miles away from their hibernacula. If we adopt the "binary effects" model described above, this high risk of exposure carries with it a correspondingly high risk of significant impact, perhaps even extinction. Aggressive and immediate management action to prevent drastic population decline in this complex appears warranted. The three remaining distinct VBEB populations to the southwest can be targeted for subsequent action once the epidemic front reaches a critical proximity.

Of course, success of this type of scheme depends critically on comprehensive monitoring of progression of the epidemic front. Careful consideration of the resources necessary to achieve the desired level of confidence in monitoring results will allow a more realistic assessment of the funds, equipment and personnel required.

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Barbara Douglas
March 9, 2009

Virginia Big-Eared Bat Captive Propagation Triggers (Part 2)

The range-wide population of Virginia big-eared bats (*Corynorhinus townsendii virginianus*) is currently estimated to be approximately 15, 000 bats (USFWS, 2008). This population is distributed throughout four genetically-distinct regions roughly located in northeastern West Virginia, southern West Virginia, eastern Kentucky, and western Virginia/North Carolina (Piaggio, 2009). The northeastern West Virginia region, which encompasses Tucker, Pendleton, and Grant Counties, supports the largest population segment. Over 60% of the range-wide population hibernates in seven caves in these counties. Caves in this region also support approximately 77% of the range-wide maternity population (USFWS, 2008). In addition, all five caves currently designated as critical habitat for the subspecies occur in this region (44 FR 61290-61292). The area encompassed by the hibernation and maternity caves in the northeastern region of West Virginia is approximately 30 miles long and 36 miles wide. Four caves within this area are already known to be affected by WNS, including one maternity cave designated as critical habitat, and one hibernation site that supports approximately 125 Virginia big-eared bats (C. Stihler, personal comm., 2009; J. Wallace, personal comm., 2009). As of 2007, there were only 3 caves that were located outside of West Virginia that supported more than 200 hibernating Virginia big-eared bats (USFWS, 2008). These caves are located in Tazewell County, VA; Avery County, NC; and Lee County, KY. They are all currently outside the known affected range for WNS, but are within approximately 40 miles, 115 miles, and 180 miles respectively of currently affected sites.

As shown in Figure 3, data on the presence of WNS in caves in the northeastern US shows the spread of WNS starting in an area near Albany, New York and emanating outward into other areas including New England and West Virginia. WNS was first documented in a cave frequently visted by the public near Albany in 2006. By 2008, it had spread over 130 miles to the north and 80 miles to the south and was known to occur in approximately 25-30 caves. Almost all of the known hibernacula within 80 miles of the original cave were affected. As of mid-winter 2009, WNS had spread 500 miles to the southwest in Virginia, 140 miles to the northeast in New Hampshire and 120 miles to the south in New Jersey (Butchkowski, 2009; Hicks et al. 2009). If current trends regarding the rate of WNS spread continue, WNS can be expected to be in all the major Virginia big-eared bat caves in the northeastern West Virginia region, including all caves designated as critical habitat, within the next year. Further, WNS would be expected to occur in range of the other genetically-distinct Virginia big-eared bats populations, including NC, VA, and KY, within the next 1-2 years.

Although no Virginia big-eared bats have been shown to be affected by WNS to date, WNS has had devastating effects on other species of bats in affected caves. Species that aggregate in dense clusters during hibernation have been particularly hard hit, and Virginia big-eared bats behave in this way during hibernation as well. Affected hibernacula in New York have been subject to colony declines of over 90% (Hicks, 2008). In the absence of data suggesting otherwise, it is prudent to assume that Virginia big-eared bats could experience similar mortality rates if they are susceptible.

Given the current proximity of WNS to the largest and most significant concentration of Virginia big-eared bats, the expected rate of spread of WNS to the remaining outlying populations, and the expected high and rapid mortality once WNS enters hibernation sites, large-scale reductions in the overall population of Virginia big-eared bats are likely imminent. Aggressive and immediate management action to prevent drastic population decline appears warranted.

In addition, zoos and other bat holding facilities have little if any experience working with Virginia big-eared bats, and the captive husbandry protocols that have been developed are experimental. Once a cave is affected by WNS, it will be much more difficult to rescue, rehabilitate, quarantine, and care for affected bats, than it would be to work with healthy animals gathered before the caves were affected. Taking immediate action now will allow more time and provide more appropriate conditions for captive husbandry techniques to be perfected, which would increase the likelihood of project success.

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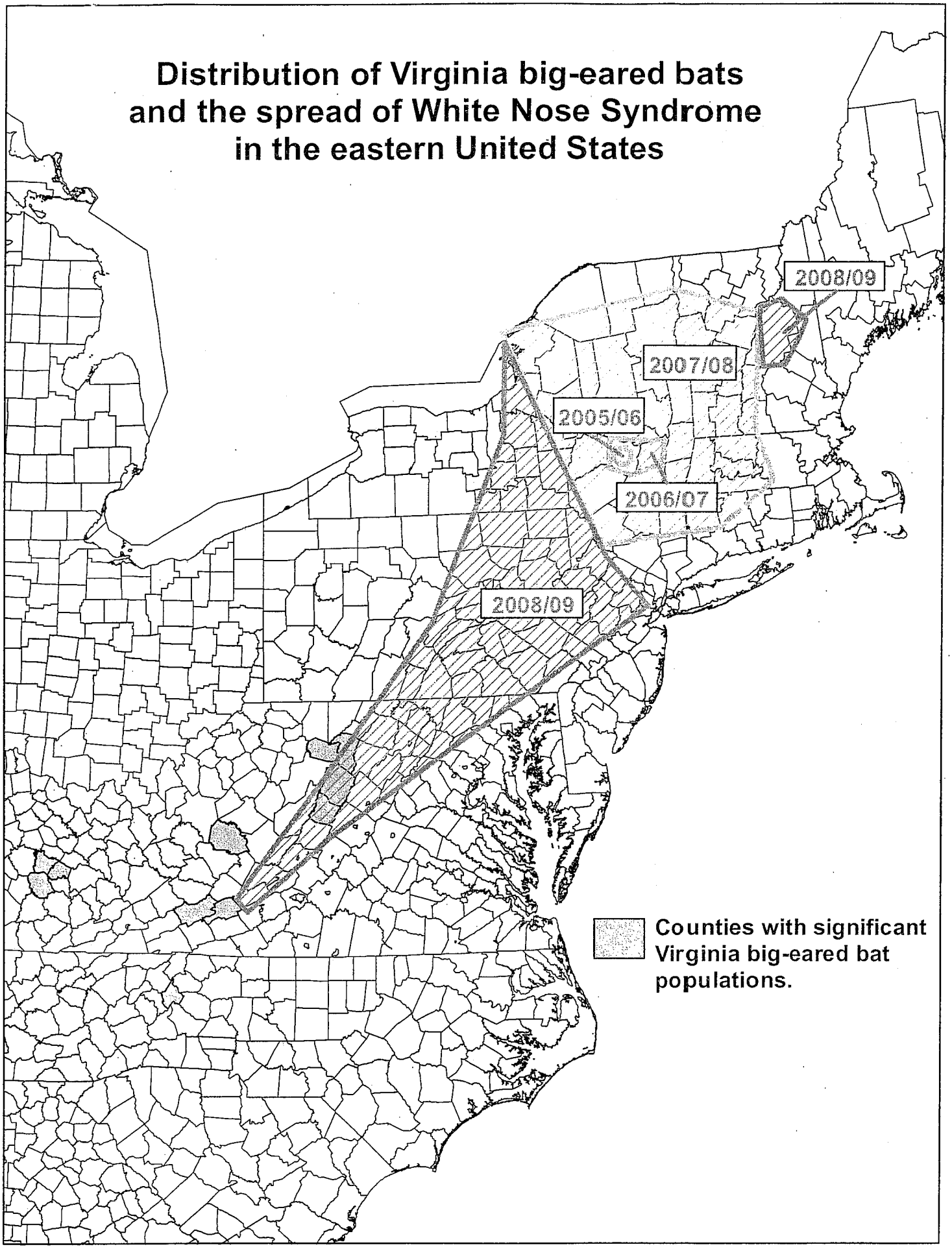

Distribution of Virginia big-eared bats
and the spread of White Nose Syndrome
in the eastern United States
2008/09
2007/08
2005/06
2006/07
2008/09
Counties with significant
Virginia big-eared bat
populations.

Virginia Big-Eared Bat
Captive Population Genetic Management Working Group
Summary of Discussions

26 February, 2009

Participants:
Toni Piaggio – USDA/ National Wildlife Research Center
Meredith Bartron – U.S. Fish & Wildife Service, Northeast Fishery Center
Phil Miller – IUCN/SSC Conservation Breeding Specialist Group

Outlined below are the tasks addressed by the Working Group in a conference call of 26 February, with recommendations to the appropriate management agencies for action.

Utility of captive population management

As a complement to the recommendations to be made by the Triggers Working Group, we believe that development of a captive management strategy is needed so that if and when the decision is made to initiate a captive population, the necessary plans are available. In order to minimize the risk of significant decline or extinction of the Virginia big-eared bat, with initial priority given to the population in and near Pendleton County, WV. If white nose syndrome (WNS) continues to spread southwestward, other distinct VBEB populations may become candidates for a similar captive management program.

Recent work by Toni Piaggio and her colleagues demonstrates marked levels of genetic differentiation between the four distinct population segments. Furthermore, this differentiation is not likely to be the result of recent anthropogenic activities on the landscape, but is probably a consequence of the geologic structure of the region. This means that these four population have probably been isolated for some extended period of time through natural processes acting on the landscape. Therefore, it seems logical that in an ideal conservation management strategy for this taxon, and when necessary, each of the four populations would require their own captive populations if faced with the imminent threat of WNS. We realize that this can greatly increase the resource burden on the overall program, but is considered defensible on the basis of sound conservation biology principles.

Numbers of founders for initiation of a captive management program

In one sense, the Pendleton Co. population presents us with an ideal situation for initiating a viable captive bat population, as large number of (presumably disease-free) individuals remain available for collection. This is certainly not always the case when critically endangered species are being considered for such a program. Consequently, we have the opportunity to sample nearly the entire spectrum of population genetic diversity from this population through collection of a relatively large number of bats from the Pendleton Co. population. Population genetics theory tells us that sample 100 individuals from this population will capture about 99.5% of the total diversity in the wild. Because there will inevitably be some level of post-capture mortality, and because some (many?) individuals will fail to breed in captivity, it is necessary to start with a larger number of founders so that the effective number is equal to 100. We therefore recommend the collection of 200 individuals, in an equal sex ratio, as founders for the Pendleton Co. captive population. Ideally, collections would be from multiple caves so as to obtain a more representative genetic sample.

Ideally, the set of founder individuals would all be unrelated to each other. While difficult to achieve in practice, it may be preferable to collect from hibernacula instead of maternity caves as the degree of relatedness within a given maternity cave may be higher. Additionally, it may be possible to use Toni Piaggio's set of genetic markers to evaluate statistically the degrees of relatedness between individuals that come in from the wild. In general, we recommend that efforts be directed toward creating the best founder population we have so as to improve the chances for success down the road.

Short- and long-term goals for the captive populations

Once the captive population is established through the successful introduction of founders into captivity, the two most important *short-term goals* for the program are:

- to create conditions under which bats will successfully reproduce in captivity; and
- to then grow the population as rapidly as possible to the target size established by the long-term goal (see below).

Husbandry will no doubt be a difficult factor to deal with in the early stages of a captive program for this species, but it is vital that nutrition and other issues be resolved as fast as possible so that the captive population does not decline in size and become genetically unstable.

The long-term goals of a captive management program are usually determined by the desired level of retention of genetic variability over a specified period of time. Nearly all programs managed by the international zoo community identify 90% heterozygosity retention over a 100-year timeframe as the "standard" long-term genetic goal. It is recognized that the program is not meant to last that long, but a long time horizon for genetic management guarantees a highly favorable outcome in the shorter-term. Assuming this is our long-term goal, general principles of population biology are used in computer software packages to assist population managers determine how many individuals are needed to establish a long-term target population size. Once this target size is known, one can determine the number of institutions that would be required to house this number of bats.

Unfortunately, since bats are colonial breeders, typical methods of managing genetic diversity in captive populations – creating detailed studbooks, making individual mating pair recommendations based on identifying genetically important individuals, etc. – do not usually apply. Population managers have developed simple techniques for managing species in groups, usually involving the creation of separate subgroups and wholesale movement of subsets of individuals (juveniles, males, etc.) between groups each generation. More detailed information on these techniques will be made available when the timing is appropriate.

Summary of Recommendations

1. If feasible, maintain separate captive populations for each of the four identified distinct population segments. Start with Pendleton County population immediately, and use management triggers to initiate additional populations when necessary.
2. Use 100 individuals as effective founders for each distinct population to maximize the genetic variability in captivity. Because of early mortality and failure of some individuals to breed, 200 individuals from each population should be collected from the wild.
3. Use software-based genetic and demographic management tools to identify population sizes consistent with long-term management goal of 90% heterozygosity retention for 100 years. Develop multi-institutional cooperative breeding program to adequately house the requisite number of individuals. Adopt a group-level genetic management strategy.

Virginia Big-Eared Bat
Captive Population Size Requirements

Prepared by:
Phil Miller
IUCN/SSC Conservation Breeding Specialist Group

Introduction

The tables and graphs below give estimated numbers of individuals that must be maintained in captivity in order to retain 90% of the original genetic variation (heterozygosity) captured from the founders over the specified timeframe. In deriving these calculations, we assume a generation length of 6 years.

Since various aspects of VBEB captive population dynamics are uncertain, we derive these size requirements under a set of alternative assumptions. First, we assume a range of effective population size ratios, N_e/N. The upper end of this range of ratios is set at 0.3, which corresponds to the maximum N_e/N ratio for a variety of wild mammal populations studied by Frankham et al. (1995). Many species show much smaller N_e/N ratios, so a minimum ratio of 0.1 here is not unreasonable. Secondly, we assume a range of potential maximum captive population growth rates. For reference, assuming exponential growth dynamics, an annual growth rate of 3.5% leads to a doubling of population size of 20 years, while a 7% growth rate doubles the population size in 10 years. This may be a reasonable range of possible growth rates given the complex husbandry issues that must be resolved for successful captive population management.

Values in the tables that are not in parentheses indicate that the genetic goals can be achieved within the specified timeframe. Parameter combinations that cannot achieve the specified genetic management goal are indicated by table values given in parentheses. In these cases, the values in parentheses are the number of years that 90% of the original levels of heterozygosity can be retained.

Example 1 (Table 1): 100 founders, 90% heterozygosity for 50 years
$N_e/N = 0.1$, $\lambda_{max} = 1.03$
Genetic goal cannot be achieved – 90% heterozygosity retained for only 13 years

Example 2 (Table 1): 100 founders, 90% heterozygosity for 50 years
$N_e/N = 0.25$, $\lambda_{max} = 1.03$
Genetic goal can be achieved for the length of the program with 217 individuals

It is important to remember that the analyses shown on the following pages are assumed to pertain to only a single distinct VBEB population, such as that occupying Pendleton County, WV and immediate surroundings. Additional captive populations of the recommended size would be necessary if other distinct populations are to be brought into captivity.

Table 1.
Number of founders: 100
Goal: 90% heterozygosity for 50 years

	N_e/N				
λ_{Max}	0.10	0.15	0.20	0.25	0.30
1.03	(13)	(23)	(39)	217	152
1.04	(14)	(28)	357	196	149
1.05	(15)	(38)	287	189	147
1.06	(18)	638	265	185	146
1.07	(21)	478	253	183	146

Figure 1.

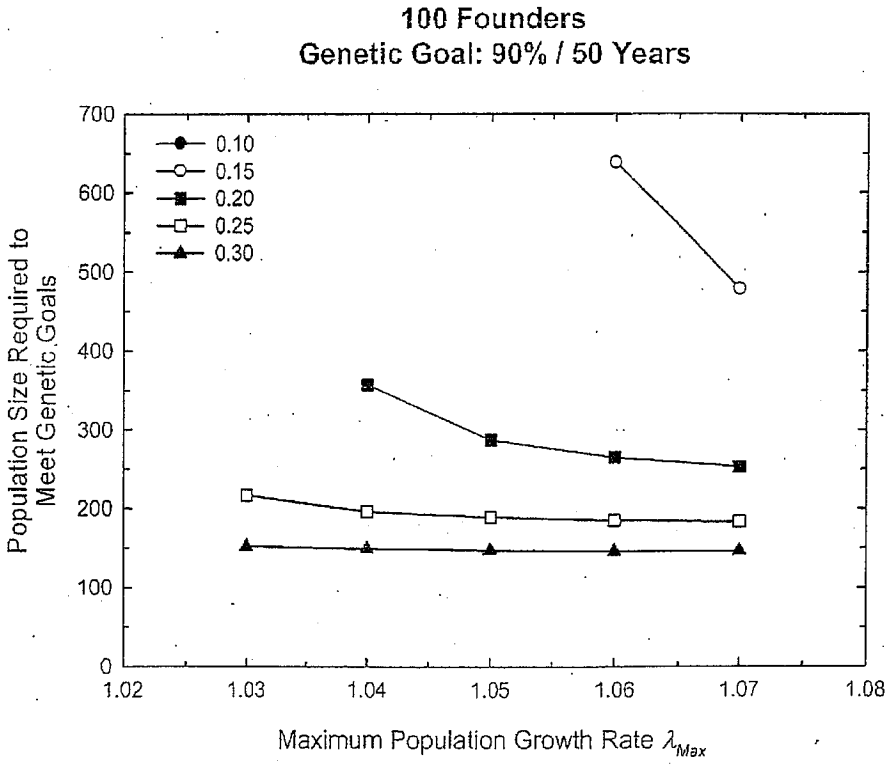


Table 2.
Number of founders: 100
Goal: 90% heterozygosity for 25 years

		N_e/N				
		0.10	0.15	0.20	0.25	0.30
λ_{Max}	1.03	(13)	(23)	110	86	72
	1.04	(14)	175	109	86	72
	1.05	(15)	164	109	86	72
	1.06	(18)	159	109	86	72
	1.07	(21)	157	109	86	72

Figure 2.

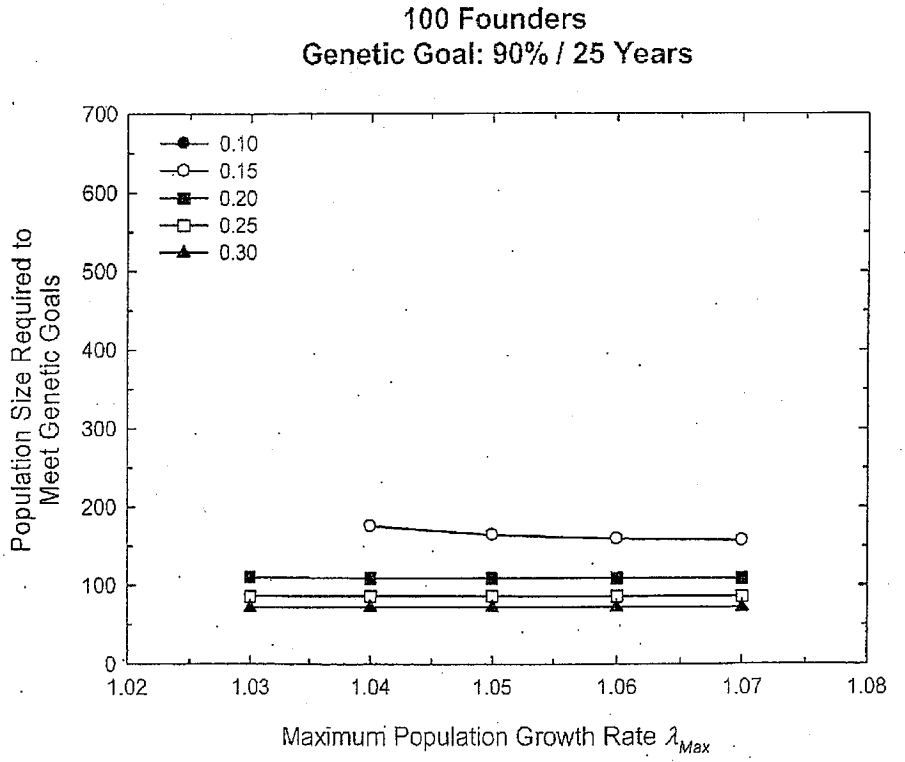


Table 3.
Number of founders: 50
Goal: 90% heterozygosity for 50 years

		N_e/N				
		0.10	0.15	0.20	0.25	0.30
λ_{Max}	1.03	(4)	(8)	(12)	(16)	(22)
	1.04	(5)	(8)	(13)	(18)	(25)
	1.05	(5)	(9)	(14)	(21)	(32)
	1.06	(5)	(9)	(16)	(26)	523
	1.07	(5)	(10)	(18)	(37)	286

Figure 3.

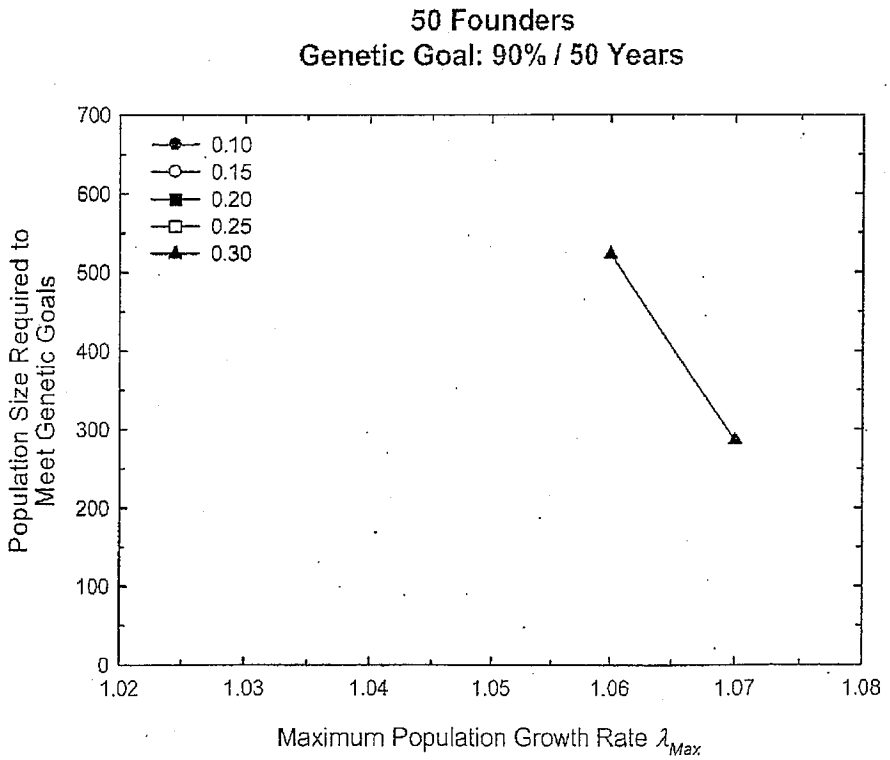
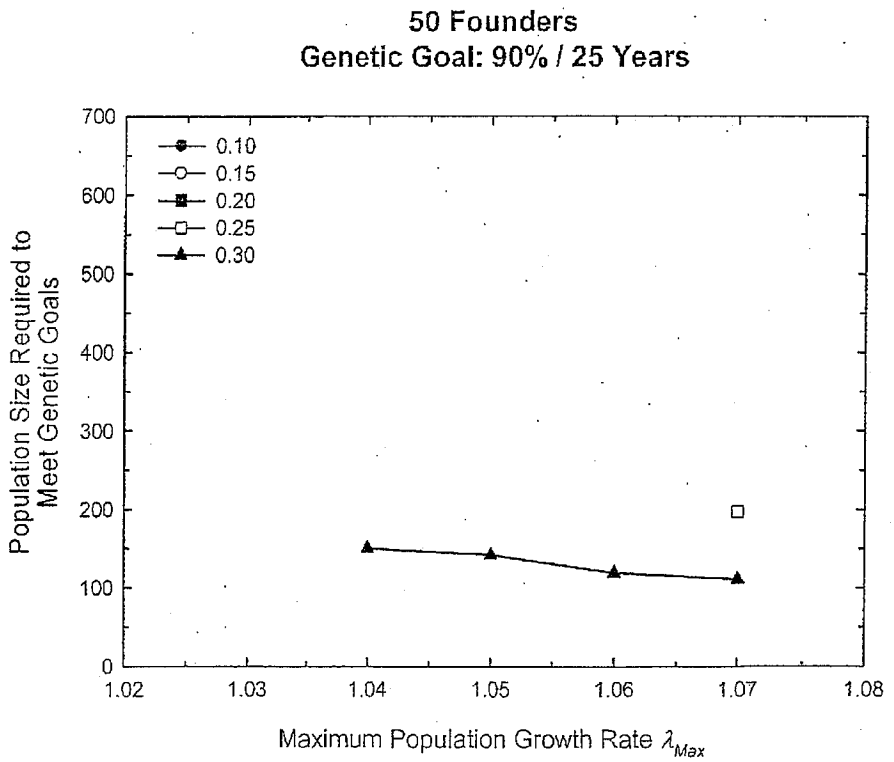


Table 4.
Number of founders: 50
Goal: 90% heterozygosity for 25 years

		N_e/N				
		0.10	0.15	0.20	0.25	0.30
λ_{Max}	1.03	(4)	(8)	(12)	(16)	(22)
	1.04	(5)	(8)	(13)	(18)	150
	1.05	(5)	(9)	(14)	(21)	142
	1.06	(5)	(9)	(16)	(25)	119
	1.07	(5)	(10)	(18)	197	111

Figure 4.



Some General Thoughts on Demographic and Genetic Management of Captive Populations of Endangered Species, with Reference to the Virginia Big-Eared Bat

Compiled by
Phil Miller, IUCN/SSC Conservation Breeding Specialist Group

Introduction

A comprehensive literature exists on the techniques for successfully managing captive populations of endangered species. In general, "success" in a captive program is largely defined by two criteria:

- Retention of high levels of genetic diversity – achieved through planned program initiation and implementation of specific breeding strategies; and
- Minimization of adaptation to captivity – achieved through proper genetic management and attention to the social / behavioral environment the species experiences in captivity.

This document will focus on the first of these criteria, with emphasis on considerations for setting genetic goals for a captive program, selecting founder animals to initiate a program, and developing breeding strategies.

Most of the information presented here will be quite general, with Frankham et al. (2002) used as a primary source of background material. In addition, more detailed complementary documentation is provided as an Appendix that focuses on amphibian captive population management. As will hopefully be demonstrated below, there are likely to be many parallels between the characteristics of amphibian captive programs and those of a species like the Virginia big-eared bat.

Stages of an endangered species captive program

Following Frankham et al. (2002), a captive management program for an endangered species is composed of six phases (Figure 1):

- Decline of the wild population, with resultant loss of demographic and genetic stability;
- Initiation of the captive population through obtaining founders from the wild;
- Growth of the captive population to the desired size;
- Maintenance of the captive population over generations at the target size;
- Reintroduction of captive individuals into native habitat;
- Management of reintroduced population in the wild.

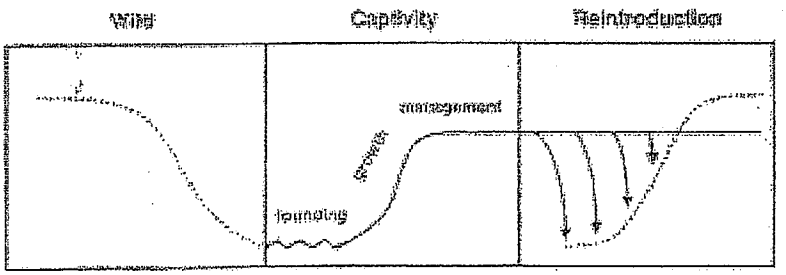


Figure 1. Phases of a captive breeding program. Adapted from Frankham et al. (2002).

Initiation of the captive population

The main consideration here is the number of individuals from the wild – hereafter labeled as *founders* – that should be brought into captivity. However, a complicating factor is potential genetic substructuring of the population through geographic isolation of the taxon in its current range. Decisions need to be made on whether distinct population segments (DPSs) or evolutionary significant units (ESUs) exist within the range of the species, and if those individual units should be treated separately with regards to *ex situ* and *in situ* management. Such treatment may preserve genetic differentiation among units, but the trade-off is potentially substantial increases in resources necessary to manage multiple captive populations.

The size of the founding population should be chosen so as to sample a large proportion of the wild population's genetic diversity – as much, in fact, as practical. We can use population genetics theory to help us predict how much of the total genetic diversity (heterozygosity) can be captured from the wild population by collecting a given number of founders. This relationship is shown in Figure 2.

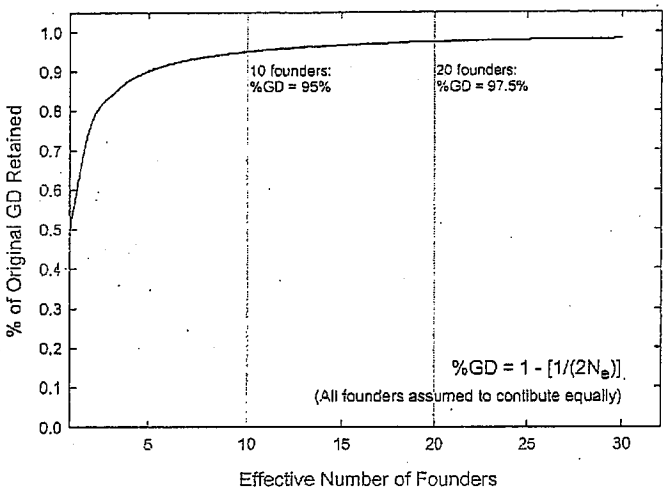


Figure 2. Genetic diversity captured from a wild population as a function of the number of founders used to initiate a captive breeding program. Note that this is the *effective* number of founders, defined as those that survive to contribute to the captive population.

The relationship in Figure 2 indicates that 10 effective founders (with equal sex ratio) will capture 95% of the wild population heterozygosity, while 20 will capture 97.5%. It is important to note that we are talking here about effective founders – in other words, only those individuals that survive the transfer to captivity and subsequently reproduce under captive conditions. Therefore, if it is anticipated that some potential founders brought into captivity will not survive to reproduce, the total number of individuals used to initiate the program must be increased accordingly. There is relatively little apparent return in heterozygosity sampled beyond 20-30 founders, but every little bit of genetic variability is important when establishing a solid genetic foundation upon which to build a long-term captive population management plan.

In addition to sampling heterozygosity, population managers are sometimes concerned with the preservation of rare alleles at generic loci that may confer unspecified fitness advantages or other benefits. The requirements for capturing allelic diversity are usually more stringent, especially when some alleles are quite rate. The relationship between allelic diversity captured and effective founder number is given in Figure 3, where we assume generic loci with two alleles and the starting frequency of one allele is either $p = 0.05$ or $p = 0.01$. Note that the number of effective founders required to capture rare alleles is substantially increased. In general, capturing and maintaining rare alleles in captive populations is not considered to be a primary focus of successful management.

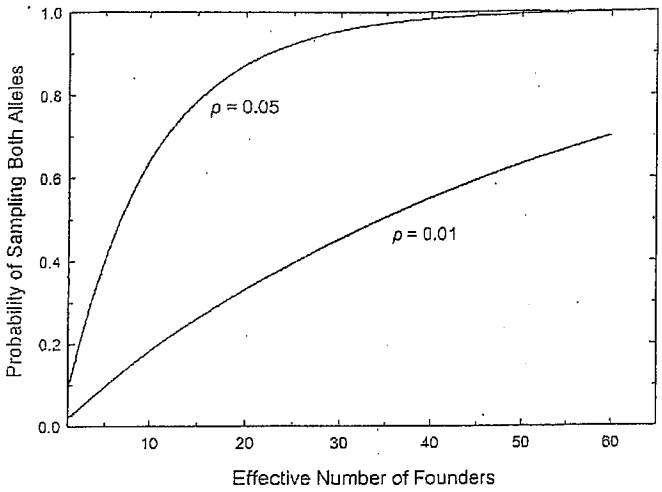


Figure 3. Probability of sampling at least one copy of each allele at a given genetic locus as a function of effective founder numbers, for two loci with allele frequency $p = 0.05$ and $p = 0.01$.

Ideally, founders should be unrelated to each other so that inbreeding can be minimized or avoided altogether in the early stages of a breeding program. In fact, this is an explicit assumption in all pedigree analysis conducted during later stages of the program. However, it may be difficult to achieve this goal, or at best, difficult to determine the degree of relatedness among individuals collected from the same geographic location. Molecular information, usually obtained through DNA minisatellite (fingerprinting) or microsatellite analysis, can be very helpful in determining the relationships among potential founders. This type of screening can help in identifying valuable animals for subsequent breeding priority, based on the identification of rare and/or desirable alleles at specific loci (e.g., MHC). Caution is advised, however, when using rare genotypes as a criterion for developing detailed breeding recommendations as overall levels of genetic diversity may be compromised in these allele-targeted breeding strategies (for examples, see Miller 1991; Miller and Hedrick 1995).

Growth of the captive population to the target size

Once the founding population has been established, the captive population must then grow in size as rapidly as possible to reach its target number. This target population size is determined by the long-term goals that are set for the captive program. For endangered species conservation breeding programs, the goal is almost always genetically based, and typically focuses on the retention of a high level of genetic diversity over a specified period of time. The most common genetic goal for a captive breeding program is to retain at least 90% of the original founder diversity over a period of 100 years in captivity. Achieving this goal will provide a population with the genetic material necessary to minimize risk of short-term damage from inbreeding and genetic drift, and will also provide the genetic variation required for long-term adaptation to environmental conditions upon reintroduction back to the wild.

There are many factors that determine the captive population size necessary to achieve a particular program goal, including the size of the founder population, generation length, captive population growth rate, and the ratio of effective population size to total size, or N_e/N. In general:

- A larger number of founders requires a smaller effective population size to achieve the program goal as "genetic bottleneck" effects are reduced;
- Populations with longer generation times require smaller target sizes as rate of loss of heterozygosity is slowed over time;

- Populations with higher growth rates require smaller target sizes as rate of loss of heterozygosity is slowed over time;
- Populations with higher N_e/N require smaller target sizes as inbreeding and drift are reduced in intensity over time.

Relationships between some of these variables are shown in Figure 4.

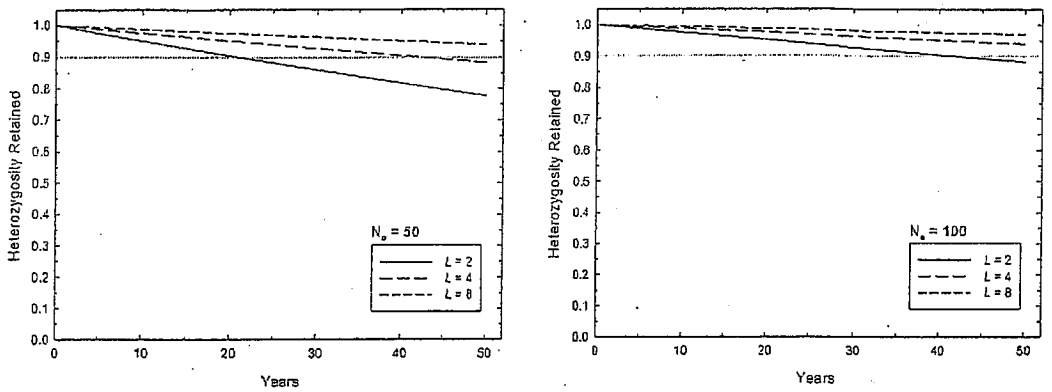


Figure 4. Retention of heterozygosity in a captive population over 50 years as a function of generation length, *L*. Shorter generation times lead to higher levels of heterozygosity lost. Red horizontal line references 90% retention of heterozygosity. Note that larger effective population size (right panel) leads to higher levels of retention compared to the smaller effective size (left panel).

In this early phase of captive program management, emphasis is given to growing the population as rapidly as possible in order to minimize the genetic impacts of small population size. All adults should produce offspring, with even low levels of inbreeding considered tolerable if the pair in question show strong mating preferences.

For some endangered species brought into captivity, it is a cold, hard reality that a desired genetic goal cannot be achieved, and the goal must therefore be scaled back to more realistic levels. This may be due to inadequate space in zoos. For example, with a small founder base of black-footed ferrets (*Mustela nigripes*) and a short generation time of just 2.5 years, almost 20,000 spaces were required to achieve the captive program goal of 90% retention of heterozygosity for 100 years. In light of this information, the program goal was readjusted to 90% retention of heterozygosity for 50 years.

Management of the captive population to achieve program goals

As stated above, genetic diversity in captive populations is eroded through reduced fitness of offspring from inbred matings (inbreeding depression), and the random loss of heterozygosity through genetic drift. Allowing individuals to breed randomly, according to their own designs, will lead to unacceptably high rates of loss of diversity. Random breeding will also lead to unequal genetic contributions among founders, as some individuals will breed frequently while others may not breed at all. This can have a considerable deleterious effect on effective population size and, by extension, retention of genetic diversity. To counter this process, population managers use analytical methods to identify genetically important individuals that should be given higher priority for breeding in a given year. These methods are designed to, over time, equalize founder contributions and produce a more genetically diverse captive population.

The metric most commonly used to identify genetic importance is mean kinship (Ballou and Lacy 1995). Mean kinship is defined as the average degree of relatedness of an individual to all other living individuals in the population, including itself. An individual with low mean kinship has few relatives in the population and, therefore, has genetic variability that is comparatively under-represented in the living captive population. Given our goals for genetic management, this individual is given high priority for breeding in order to reduce the risk that this genetic variability is lost if the animal dies without leaving an adequate number of descendants. Reducing the average mean kinship across all individuals in the population results in a population that has a correspondingly low level of inbreeding and a reduced rate of loss of heterozygosity.

Computer software (PM2000: Lacy et al. 2000; PopLink: Faust et al. 2008) is available to assist in the calculation of mean kinship lists and the requirements for captive populations to achieve identified program goals. Mean kinship calculations ideally require complete knowledge of the pedigree of each individual in the population, typically recorded in studbooks maintained by trained zoo professionals. With this information in hand, lists of individuals sorted by mean kinship are readily available, and pairings between high-priority individuals can be identified for the upcoming breeding season.

Remember that another important captive population management strategy involves maximizing the N_e/N ratio. Attention to this population characteristic can yield significant benefits to the long-term genetic viability of the captive population. This ratio can be increased by:

- Equalizing family sizes across breeding pairs. As variability in family size increases, genetic contributions among founders become more unequal, and retention of genetic variability declines. Amazingly, reducing this variance in family size to zero yields a population whose effective size is *twice* the census size: $N_e \approx 2N$.
- Maintaining roughly equal numbers of breeding males and females.
- Increasing generation length when possible. This can be done through various manipulations of the captive population, and can be difficult to achieve in practice.
- Equalizing population size over time. Fluctuations in total population size can lead to genetic bottlenecks which greatly reduce N_e.

Captive management for species maintained in groups

In order to optimally manage the genetic structure of a captive population using mean kinship, the complete population pedigree must be known. Unfortunately, meeting this condition may often not be possible for species that are maintained in groups or colonies, as parentage for individual offspring may be impossible to determine. Without this information, the effective size of the population and the degree of heterozygosity retained over time cannot be accurately calculated. This very well may be the situation for the Virginia big-eared bat when contemplating management of the species in captivity.

Genetic management of groups is a poorly understood aspect of conservation genetics, and is the subject of active research within the zoological community. Despite this uncertainty, there are some general protocols that can be employed to strengthen the genetic integrity of captive populations of group breeders. These protocols typically focus on minimizing the intensity of inbreeding as a means of reducing the rate of loss of heterozygosity.

Genetic management of groups usually involves setting up separate breeding colonies, and then moving groups of individuals – usually males, but this can be different depending on the specific technique employed – in specified "directions" between groups at regular intervals. This is demonstrated in more detail in Figure 5.

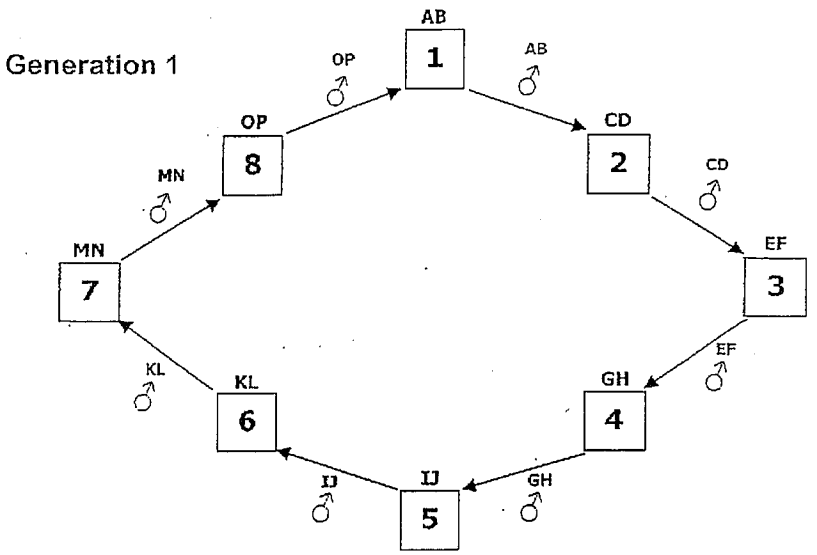


Figure 5. Schematic diagram of a group management protocol for inbreeding avoidance described by Princée (1995). Numbered boxes indicate separate breeding groups, with lineages identified by letters above the groups. In the first generation of the protocol, male offspring are moved clockwise to the next breeding group for use in the next generation. In generation 2, male offspring are moved two steps in a clockwise direction, and male offspring are moved in four steps in a clockwise direction in generation 3. Because of the relatively small number of groups, inbreeding is unavoidable after the third generation. This cycle of movement is repeated starting in generation 4.

If we assume that the Virginia big-eared bat must be managed as a group breeder, there are broad recommendations that can be made for the initiation and maintenance of a captive program. These recommendations depend largely on the duration of the species' reproductive lifespan, generation length, and N_e/N ratio. A type of decision tree for identifying optimal group management strategies can be found on page 12 of the Appendix. Given a basic knowledge of the life history of the Virginia big-eared bat, the most likely candidate group management protocol can be found on page 18 of the Appendix.

Reintroduction of captive individuals back to the wild

The ultimate goal of any conservation breeding program is the reintroduction of individuals bred or held in captivity back into their native wild habitats. The art and science of reintroducing endangered species includes a substantial literature – and an equally substantial level of controversy over its value. While successful reintroduction programs are relatively rare, the goal remains appropriate and captive population management strategies must keep it as a focus for the long-term viability of the species in question.

In the interest of brevity, the primary issues surrounding captive population management for reintroduction are only summarized here. A more detailed treatment may be found in Frankham et al. (2002).

- Processes that erode genetic variability – founder bottlenecks, inbreeding depression, and genetic drift – can seriously compromise the genetic viability of captive populations.
- Populations of endangered species can become genetically adapted to captive conditions; this process of adaptation is not restricted to birds and mammals (e.g., Araki et al. 2008). Minimizing the length of the captive program, as well as the intensity of selective pressures imposed on the captive individuals, are among many techniques identified to help reduce the magnitude of this serious problem.
- During the initial trial stages of a reintroduction program, genetically surplus individuals (i.e., those with many relatives in the population) are considered most appropriate for release candidates. Use of these individuals will not compromise the genetic integrity of the source captive population. Once the wild population appears to show stable growth dynamics, captive individuals with high levels of genetic diversity and desirable wild behaviors should be selected for release. As with the early stages of a captive program, high priority is given to growth of the newly reintroduced wild population. Once established, the reintroduced population can periodically receive captive animals through supplementation until levels of genetic variation in the wild mirror those in captivity.

Conclusion

The intent of this document is to focus more on the general principles of genetic and demographic management of captive populations – not on prescribing specific targets and techniques for such a program for the Virginia big-eared bat if deemed attractive or necessary. It is hoped that the guidelines described herein will be valuable for contemplating the details of a program for this species.

Additionally, it is recognized that other aspects of a captive population program – nutrition, husbandry, enrichment, institutional commitment, etc. – may potentially offer substantial obstacles to a program's success. These cannot be dealt with in this focused document, but must be considered with equivalent rigor and responsibility.

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Virginia Big-Eared Bat - Husbandry Recommendations & Considerations

Colony size - Target captive colony size is being determined elsewhere. However due to the time intensive process of teaching a Virginia big-eared bat to eat in captivity, it is recommended that they be brought into captivity in small groups (approximately 5 individuals per facility). This represents a reasonable number of bats for a caregiver to train to eat at one time. Bringing in large numbers of VBEB at one time could easily overwhelm the capabilities of most, if not all, current bat holding facilities.

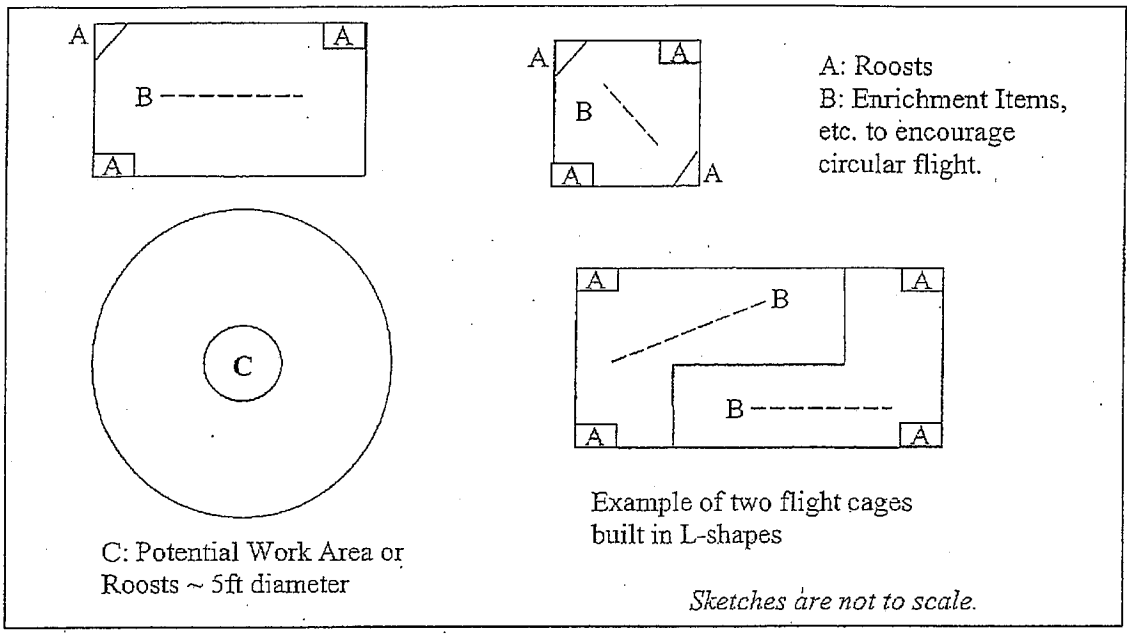
Colony composition - The specifics of the animals being collected will determine to some extent the long-term success of the program. Suggest collecting younger animals (can be judged via tooth length). If possible to determine current breeding males & females then would suggest collection of active breeders/known producers. If females are collected when they are pregnant, it is very possible they will abort or resorb their fetuses. In addition, a sex ratio skewed toward females is likely to reduce the agonistic interactions between animals by reducing male-male competition. We recommend a 1:3 (male:female) ratio.

Labor costs/considerations – Based on experience with this and other similar species, effectively caring for and monitoring a breeding colony of 20 -25 VBEB will take about 3 hours per day for an experienced caregiver. However, it could take twice that time in the initial period. The success of a colony of a challenging species like VBEB will depend in a large part on finding experienced caregivers who have worked with colonies of insectivorous bats. In addition to understanding the logistics of captive care of bats, caregivers will also need to closely monitor individuals to track aggressive interactions (particularly during breeding season). Ideally colonies would have a small team of caregivers with a single primary to reduce the stress of multiple people interacting with the colony.

Diet – Ratio of soft to hard insects in the wild is 50-75%:50-25%. In captivity suggest presenting *both* gut-loaded mealworms and wax worms. Animals should have access to both species of larvae over night. Smaller mealworms are more likely to aid VBEB in learning to eat mealworms in captivity (no larger than "large" size, "medium" preferred) and should definitely be available during training periods. Once the bats have learned to self-feed larger sizes might work. During the training period larger mealworms must also be on hand in case bats need to be maintained exclusively on viscera. Commercial insect suppliers have historically had problems with availability of larvae. The ideal captive situation would have a breeding colony of mealworms to ensure the availability of a high quality diet. Larvae should be gut loaded in accordance with standard captive insectivore diets (see references). See feeding tips appendix for additional information.

Flight Cages – If quarantine considerations permit it, larger flight cages can be built outside. A flight space that is 11 x 6 x 2 – 3.5 m (35 x 20 x 6.5-12.5 ft) would be ideal (length x width x height). Flight cages should be "furnished" with center obstacles to encourage a circular "endless" flight path. Indoor flight cages should be 24 x 24 ft. In addition, several roost boxes should be furnished on either end (3 or more), so that bats can escape from one another or shift roosts, etc. Roost boxes should be 1 x 0.5 x 1 m (3 x 2 x 3 ft) and be able to be secured to keep bats inside. Food should be provided in all the roost boxes. A narrower flight space may be used if it is constructed in an L shape. Circular flight cages have also been used with great success, but the width of the "doughnut" should be 12 – 15 ft. See sketches for illustrations of these options. If possible each captive colony would have at least two flight areas, so

that males could be separated from females prior to giving birth. Care should be taken to keep pregnant and nursing females in the flight cage and roosts that they are accustomed to, in other words, move the males.



Flight Cage & Roost Construction Materials – While the outer layer of flight cages needs to be sturdy enough to protect bats from natural predators, it is best not to have wire or metal roosting surfaces. Metal surfaces are associated with an increase in thumb and toe injuries. Polypropylene mesh (1/4 in or smaller) is preferred for surfaces with which bats will come in contact. Roost boxes constructed of wood lined with nylon window screen are preferred. However, if it must be a non-absorbable surface, hard plastic or plexiglass can be used but must be lined with plastic tarp or cloth and covered with nylon window screen. Hang a flexible barrier from the ceiling of each roosting cage (fabric cloths can be laundered and bleached).

Temperature/Humidity – Temperature should be between 20 – 32 C (68 – 90 F). A temperature gradient in roosting cages must be provided so pregnant females and young can access warmer temperatures and males can access cooler areas. This does not have to be fancy; you can use red light bulbs in screened clamp lights to provide heat. Humidity levels should be at a minimum 60 – 65%. Higher humidity levels are better for the bats, but may be conducive to fungal growth in cages.

Other Considerations – The majority of facilities holding insectivorous bats and people already in possession of the expertise needed to hand feed new bats are dedicated to bat rehabilitation. These facilities may suffer from two critical drawbacks: a rehab facility is only as good as the rehabber (there is a wide range in care standards) and any facility that is actively engaged in releasing recovered bats to the wild poses the most challenging quarantine issue. This does not eliminate high-quality, established rehabilitation facilities as potential colony sites, but it is a concern. Zoos have a great deal of experience with quarantine and do not hold

releasable bats, but do not in general have the requisite experience in the care of insectivorous bats – particularly bats that are new to captivity. If we are going to consider housing animals in zoos or other facilities that do not have a history of providing captive care to insectivorous bat colonies, then a program needs to be implemented as soon as possible to train primary caregivers.

References

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Appendix: Feeding Tips

- Mealworms (*Tenebrio molitor*) and wax worm larvae (*species*). Keep Add 5 grams of Vionate vitamin/mineral supplement and 5 grams of Missing Link to every 100 grams of insects.)
- Start by hand feed bats mealworms and/or wax worm larvae, whichever they will accept, or a mix of both if they will accept them. They are aerial feeders and will not instinctive take food from trays initially. If bat accepts food readily, allow it to eat as much as it will readily consume once a day. Amount each individual will eat will vary from bat to bat and season to season (2 to 6 grams daily); females increase their intake significantly during pregnancy. Weigh bats daily and increase feedings per day for any bat that loses weight for two consecutive days. Do NOT mistake a pregnant female for an overweight bat!
- Bats may not accept whole mealworms or wax worms initially. If this is the case, feed viscera\ of 10-20 large (30-35 mm) mealworms per bat **twice** a day or more often as needed to maintain body mass.
- Leave plastic containers filled with mealworms and wax worm larvae in each roosting cage throughout the night to encourage bats to self-feed. (Add 5 grams of Vionate vitamin/mineral supplement and 5 grams of Missing Link to every 100 grams of insects.)
- Insects should be removed from cages, dead worms and debris removed, and live worms put into medium (e.g. wheat bran, monkey chow) during the day. Medium should also include calcium, e.g. powdered calcium Carbonate (% by volume: 5% calcium carbonate to 95% medium).
- Water trays should be left on the bottom of the roosting cages in shallow containers at all times and the water changed twice a day. Containers should be so shallow bats can walk through them without drowning.
- Keep calcium available in roosting cages at all times.
    - Leave calcium blocks in cage or add calcium carbonate to a separate container of water (5% by volume).

Notes on quarantine guidelines
25 February 2009
David Blehert, FWS, Madison, WI
Tabitha Viner, National Zoo, Washington, DC

Characteristics of the Geomyces sp. associated with WNS

- Grows optimally at 4-10 degrees C
- Maximum growing temperature of 20 degrees C
- Requires growth for 10-14 days to get appreciable biomass

Hypotheses

- Spores may be viable in the environment for 1-2 weeks
- Prepatent period between exposure to fungal spores and visible manifestation of disease is at least 2 weeks
- Housing bats at warm temperatures (25-37 degrees C) in a controlled environment for longer than 2 weeks should significantly reduce if not clear the fungus (spores) from the bats and the environment. Hyphae may persist within the skin of bats, but may not be viable or infectious.

Quarantine-relevant items to be determined

- Standard antifungals (eg itraconazole) effective for Geomyces-infected bats
- Sensitivity and specificity of PCR for wing membrane or swab samples (in progress)
- Test interval for PCR tests in quarantine
- Necropsy protocol

Quarantine suggestions

- Due to the possibility of rabies infection, bats should be quarantined for a minimum of one year
- During quarantine, housing temperature should be maintained at the upper range of temperature as determined by husbandry requirements
- Bats should be allowed to acclimate to the captive environment before quarantine testing is begun
- Suggested testing protocol
    - PCR on wing membrane swab or punch biopsy for Geomyces. Testing frequency to be determined. Possibly at t = 2 weeks, 6 weeks, and 11 months??
    - Fecal culture for Salmonella and other pathogens
    - Fecal PCR for Histoplasma, Cryptococcus
    - Fecal parasite screen X3
    - Full necropsy per outlined protocol (to be determined; will include collection of wing and muzzle skin for analysis) on any deceased individuals