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NSF Grant Proposal

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**Title:** A Survey on The Small Brown Bats of the Surry Mountain Dam Area, New Hampshire

**Question:** Can *Rhodococcus rhodochrous* aid in recovery from White Nose Syndrome in local bat species?

**Background:**

* This paragraph explains what White Nose Fungus Syndrome is.

White Nose Fungus is caused by a fungus called *Pseudogymnoascus desctructans*, which takes advantage of the hibernation patterns of small bats. It grows over the muzzles of the bats, and all over the skin, especially the wings. Infection usually causes death (1).

* This paragraph talks about the significance of my observations at Surry Mountain Dam, and how there could still be a population of bats in Keene.

I have noticed the bat population in Keene, especially at Surry Dam, seems to still be present in some way, since a quick visual count shows a prevalence of at least one species. The last time a study was done on the bat population was in 2013 (4). I would like to see which local species, if any, have developed a tolerance to WNFS (“White Nose Fungus Syndrome,” which is caused by *Pseudogymnoascus destructans*), or if there still hasn’t been much recuperation (2)(1).

* This paragraph is about the bacterium that was recently discovered to have inhibiting effects on the fungus *P. destructans*.

There is a type of bacteria, *Rhodococcus rhodochrous*, that was discovered in Missouri that is able to inhibit or completely stop the growth of the fungus (2). I want to know if that bacterium is found in any part of their cave environment, especially in bats that have survived the latest devastation caused by White Nose Fungus. This would require test to be done on microflora of bats in different hibernacula to see if any of those bacterial species are present, where bats have survived.

**Significance:**

In New Hampshire, bats provide an important part of the ecosystem in that they eat plant parasites, and act as vectors for seed dispersal for local plant species. There has been a decline in bats in the area due to White Nose Fungus Syndrome (4), and this could affect local agriculture. Many crops and other vegetation will be damaged by the 2.4 million pounds of insects that will go un-eaten, and this will affect the agriculture and economy of New England (3). One main goal of this project to assess the current state of the bat populations in the western part of New Hampshire as far as density and evenness are concerned.

There are many other species around the world that are affected by invasive fungus like chitrid fungus (6), and specific remedies are hard to find. *R. rhodochrous* is a bacterium that could have certain qualities genetically that inhibit fungal growth. It is important to know the role that *R. rhodochrous* could play in the rehabilitation of bat species that have been decimated by fungus, to inform initiatives in the future.

If our bacterium becomes incorporated in the microbiome of these bats, that will show evidence of human-facilitated symbiosis, and could help scientists in the future to find bacterium that can destroy the fungus.

It is important to keep working to understand the relationship between certain genera of bats and the species of fungus that has devastated bat populations around the whole country (1). This grant would allow research to be done to help understand the relationship between the bats of our ecosystem and *R. rhodochrous*, and will ensure a more definitive link between inhibition of *P. destructans* in bats and the bacterium.

**General Hypothesis:**

When *R. rhodochrous* is introduced to the bat community in Keene, NH, the levels of *P. destructans* within the community will decrease.

**Proposed Methods:**

Hypothesis: Bats can spread *R. rhodochrous* to one another after inoculation if the two species can directly benefit from one another.

It is important to know if the bats would be able to transmit the bacterium, *R. rhodochrous* to one another. Some in vitro experiments concerning the microflora of the noses of bats (*Myotis lucifugus*) will be conducted, to see if bats will incorporate the bacterium into their microbiome.

*Rhodococcus rhodochrous* is normally found in soil, so the best way to give this bacterium to bats would be to directly inoculate their nose with broth culture of the bacteria.

In one group, 5 individuals of *M. lucifugus* bats will be inoculated with the bacterium, and put back into the enclosure with 15 other bats who did not receive treatment with the bacterium.

In another separate enclosure, 20 individuals of *M. lucifugus* will be kept as a control, and won’t be treated with any of the bacterium.

These trials will be run for a week at a time, after which point, all of the individuals in the study will be tested for the presence of *R. rhodochrous*, and the two results will be compared and replicated once a month for three months. Each group will be tested for the presence of the bacterium using qrt-PCR from a swab once a day for one week.

This could be tested in populations that are in captivity, in case inoculation from *R. rhodochrous* actually has physiological implications in the bats in vivo. We will collect pre-weight and post-weight measurements for bats before and after exposure to *R. rhodochrous*. This part of the study could potentially be done at the Stone Zoo in Stoneham, MA, which is currently trying to update their program on genomic medicine of zoological species.

At Surry Dam, if the trials at Stone Zoo go well, bats that are captured will be given the bacterium. Fungal infection will be measured by unit of cubic cm of infected tissue before exposure to the bacterium, and after exposure to the bacterium. In subsequent trips to the dam, the same measurement will be taken from bats that were not initially inoculated with the bacterium, too see if the bacterium will be passed along in a natural system.

For each bat in the wild, a nose swab will be done, and bacteria will be grown on benzene agar. The bats that are swabbed that have no evidence of fungus will be compared with those swabs that do have fungus to see if there is any difference in the amount of *R. rhodochrous* cultured. *Rhodococcus* species can grow on benzene agar for an exclusion assay, since they can use benzene as their primary source of carbon, at levels that are toxic to most other bacterium (5).

We will introduce the bacterium to wild populations. If the hypothesis is correct, this incorporation of this beneficial bacteria should help the bats that remain to fight off the fungus later on, and could help the different species of bats survive infection in the future.

Hypothesis: *Eptesicus fuscus* populations in Keene have increased since the last study that was done in 2013 at Surry Dam.

In our area, *Myotis leibii*, the Eastern Small-footed Bat; *M. septentrionalis*, the Northern Long-eared Bat; *M. lucifugus*, the Little Brown Bat; and *Eptesicus fuscus*, the Big Brown Bat were captured in 2013 as a part of the study about how bat populations in Keene have been ravaged by White Nose Fungus (4). Many of the species of bats were caught at a rate of less than 1 bat per night for each species, with the only exception being the Big Brown Bat, which normally doesn’t contract the fungus at as high of a rate as the other bat species (4).

In Surry, NH, there are different areas in the public parks system where the nets would be put up. The study that was last done near Surry Mountain Dam used 40 different netting locations, to avoid the tendency of bats to become net-shy (4).

The animals that are captured, though they might be scarce, will be inspected for signs of infection, and tagged if they are from one of the three myotis species. They will also be assessed for general health and weighed to keep data that could be useful in tracking the efficacy of this treatment. Fungal infection will be measured as cubic cm of infected tissue. Over the two years, there will be a recapture every two months during the summer time and fall, since capture rates are abysmally low during winter months, due to hibernation (4).

**Predictions:**

If the overall health of the bats improve with the introduction of the bacterium and become a part of their native flora, there should be an increase in the *Myotis* species after some time. The populations of smaller arthropods that are eaten by the bats will have increased with the decrease of the overall bat population, and the population of bats could rise dramatically with the overabundance of food resources.

Another thing is that there could be a high population of the Big Brown bat compared to 2013 for this reason. Hopefully some individuals from the smaller bat species will still be present so that the human-facilitated symbiosis of *R. rhodochrous* can be tested in a natural system.

If there are smaller bat species still around, the *R. rhodochrous* will allow more fecundity in the population, so after the first few recaptures, there will be more *Myotis* species present in the Keene bat community.

In the Stone Zoo study, there will be more *R. rhodochrous* in all of the individuals in the treated population compared to the control, with a much higher population being maintained on individuals who were inoculated. After the first two days, the population of *R. rhodochrous* will decrease, and as the two species begin to coexist, the levels of *R. rhodochrous* will stabilize.

This example figure shows the predicted results of the Stone Zoo trials.

**Literature Cited:**

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Pathogen?" (PDF). *Science*. 323 (5911): 227. Accessed 23 October 2018.

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