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Impact of Intrinsic Physiological Factors in the Population Recovery of Myotis Lucifugus (Little Brown Myotis) From White-Nose Syndrome

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Impact of Intrinsic Physiological Factors in the Population Recovery of *Myotis lucifugus* (Little Brown Myotis) From White-Nose Syndrome.

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Abstract

White-nose Syndrome (WNS) has decimated populations of hibernating bats in the US. In particular, Myotis lucifugus (little brown myotis) has been one of the most affected. We investigated the energetic cost of innate immune response and Pseudogymnoascus destructans (Pd) fungal activity on post-hibernating little brown myotis and the link with the recovery of local populations in New England from WNS. We captured bats at one of each of 4 colonies in Eastern Massachusetts and Southern New Hampshire, consecutively, every 14 days to minimize disturbance to the colony. Blood samples were collected from each bat. Respirometry trials were performed on each bat the next day after capture. We examined basal metabolic rate (BMR), an important measure of energy expenditure, and bacterial killing ability of blood (BKA) and white blood cell (WBC) counts, an important measure of innate immune ability. Analysis of data is still in progress. We are focusing in particular on the analysis of data gathered in May and early June when fungal levels tend to be high. We will report on whether the relationship between fungal activity and energy use changes during female pregnancy. This will improve understanding on whether population recovery in some bat colonies is affected by cost of immune response and recovery to WNS. Our analysis will result in estimates of energy use rates and immune responses and fungal load of bats at different colonies with different demographic conditions. These results will be used in models to predict likelihood of colony recovery from WNS.

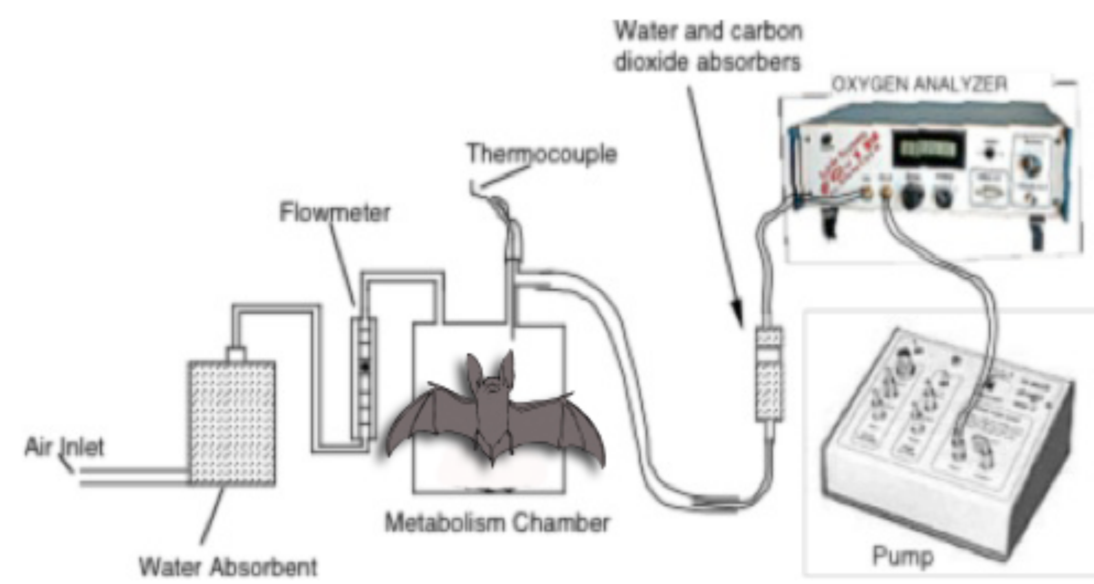
Introduction

White-nose syndrome (WNS) (caused by *Pseudogymnoascus destructans* (Pd) fungus) has been documented at the majority of known hibernacula throughout the Northeast including Massachusetts, since 2007, causing precipitous downward trends in wintering little brown myotis, *Myotis lucifugus.* Accordingly, most summer colonies in the area also declined dramatically in size, although some have started to recover. After external barriers are breached by an invading pathogen, a number of nonspecific, innate responses can occur. One is the activation of complement proteins in the blood. Nutritional and energetic costs are associated with maintaining normal immune function, and even a mild increase in immune system activity can lead to an increase in metabolic rate. As soon as they emerge from hibernation, some bats still fight the fungus, which causes WNS, and spend up to two months responding to and recovering from the disease, which should cost energy. Insectivorous bats emerging from hibernation are particularly vulnerable to energy costs because their fat reserves are limited and insect populations are low in spring. After hibernation, pregnancy begins in female bats which costs additional energy. Thus, if available energy is limited, female bats may be forced to make a trade off: spend less energy either on reproduction, threatening their ability to successfully reproduce, or on immune function, weakening their ability to fight the fungus. We hypothesized that fungal activity will cause an increased immune response, costing energy that would otherwise be used towards pregnancy.

Methods

Experimental Design: *Myotis lucifugus* (little brown myotis) were captured at return from first feeding from four maternity colonies in Massachusetts and New Hampshire in spring to summer. All bats were captured with harp traps, hand nets, or by hand. We assessed each bat for damage to wing using the Wing Damage Index (WDI). Bats with wing score (WS) 2 have a great number of black necrotic lesions, sometimes along with tears, in wing membrane with some associated white lesions (indicative of initial healing) on their wings. Fungus is being actively excluded during WS 2. Those with WS 1 have great numbers of white spots on their wings, which indicate recovery from fungus and healing is at its peak as damaged tissue flakes away. WS 0 indicates minimal to no damage remains in the wing membrane. All bats were swabbed for *Pd* fungal load as a measure of fungal activity.

Respirometry: Basal metabolic rate (BMR) is the rate of metabolism in resting, post absorptive individuals at thermoneutrality and is a widely used measure of rate of energy use in mammals. Bats are considered thermoneutral from 12pm-6pm at 30°C. During respirometry, bats were kept in a positive pressure, open circuit system, with dried CO_2 free excurrent air then subsampled by an Amtek electrochemical oxygen analyzer. Using respirometry (indirect calorimetry), dried incurrent airflow rate and incurrent and excurrent oxygen concentrations were recorded as part of measuring oxygen consumption rate in order to estimate BMR. The lowest oxygen consumption rate obtained in the 4-6 hour testing period was defined as BMR. After respirometry, bats were immediately returned to the colony site (where they were captured) and released.



Bacterial Killing Assay (BKA): *E. coli* was exposed to bat plasma for one hour and then plated. Colony forming units were then counted on experimental and control plates to estimate percent bacteria killed by plasma in one hour. BKA is a measure of complement protein activity.

Results

Myotis lucifugus

Basal Metabolic Rate (BMR)

In general, bats from the Ernst colony have a higher BMR than bats from the Singh colony (Table). Bats from the Howland colony have the lowest average BMR in not to early pregnancy, but have the highest BMR average in mid to late pregnancy. For WS 2 bats, the relationship between BMR and fungal load shifts from a positive relationship in not to early pregnant bats (Figure 1) to no relationship in mid to late pregnant bats from Singh colony.

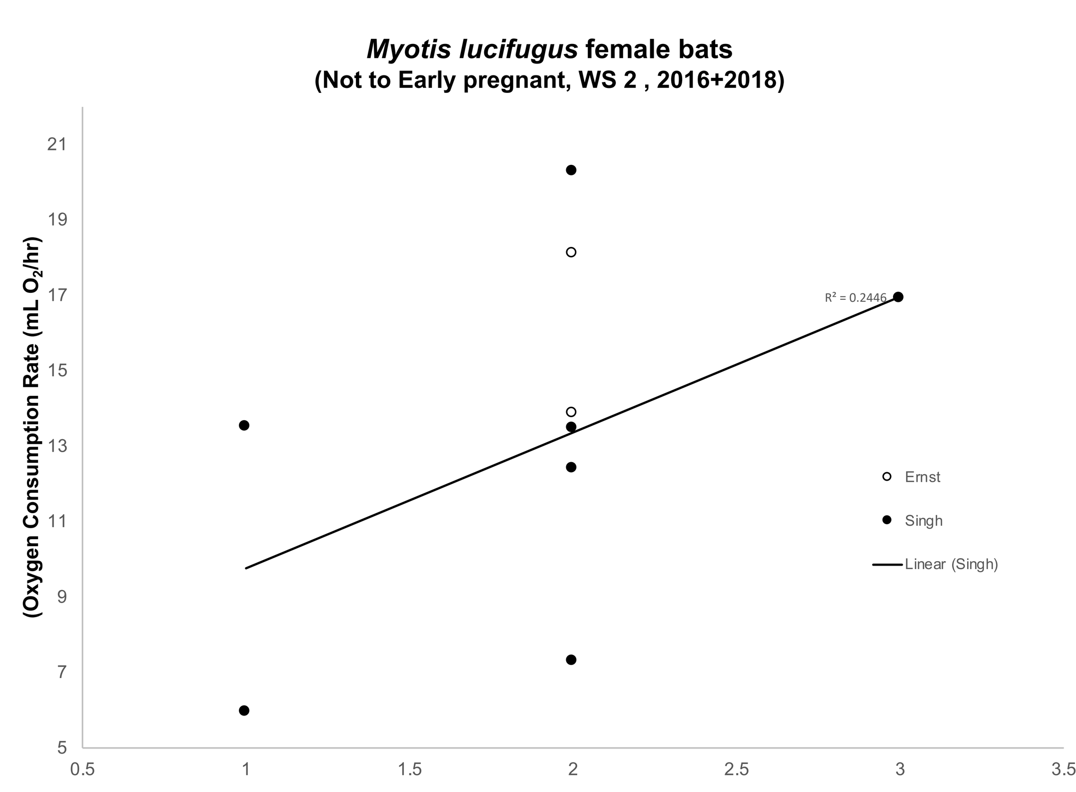


Figure 1. The two lines represent the relationship between fungal load and BMR for different colonies of bats of not to early pregnant *Myotis lucifugus* (2016+2018).

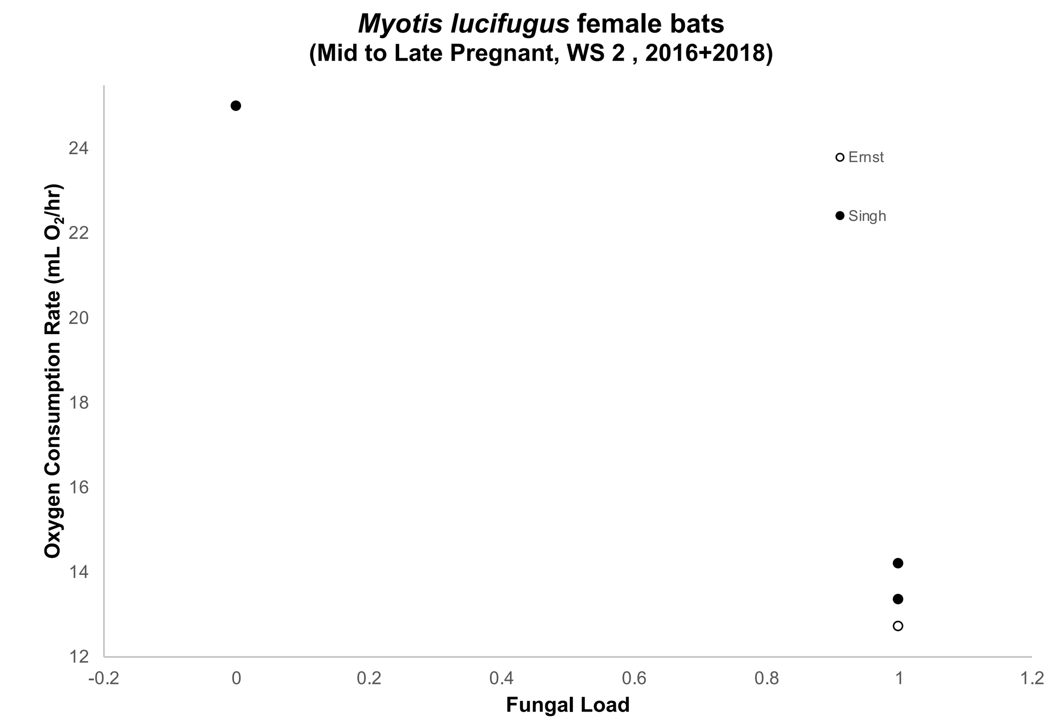


Figure 2. The two lines represent the relationship between fungal load and BMR for different colonies of bats of mid to late pregnant *Myotis lucifugus* (2016+2018).

Bacterial Killing Ability

In general, bats from the Howland colony have higher BKA than bats from Singh or Ernst colony. The relationship between BKA and fungal load shifts from no relationship in not to early pregnancy (Figure 3) to a slightly negative relationship in mid to late pregnancy (Figure 4) for the Singh colony. The Ernst colony shifts from a negative relationship in not to early pregnancy (Figure 3) to a positive relationship in mid to late pregnancy (Figure 4). Howland had only a positive relationship in mid to late pregnancy.

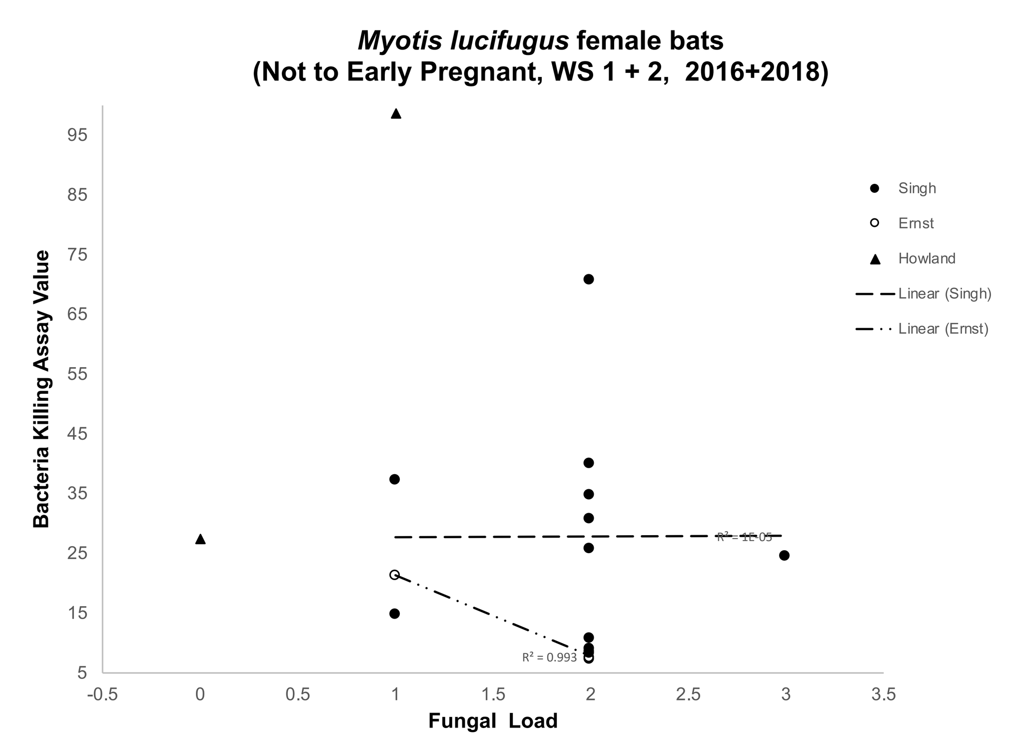


Figure 3. The two lines represent the relationship between fungal load and % of bacteria killed by bat plasma for different colonies of bats of not to early pregnant *Myotis lucifugus* (2016+2018).

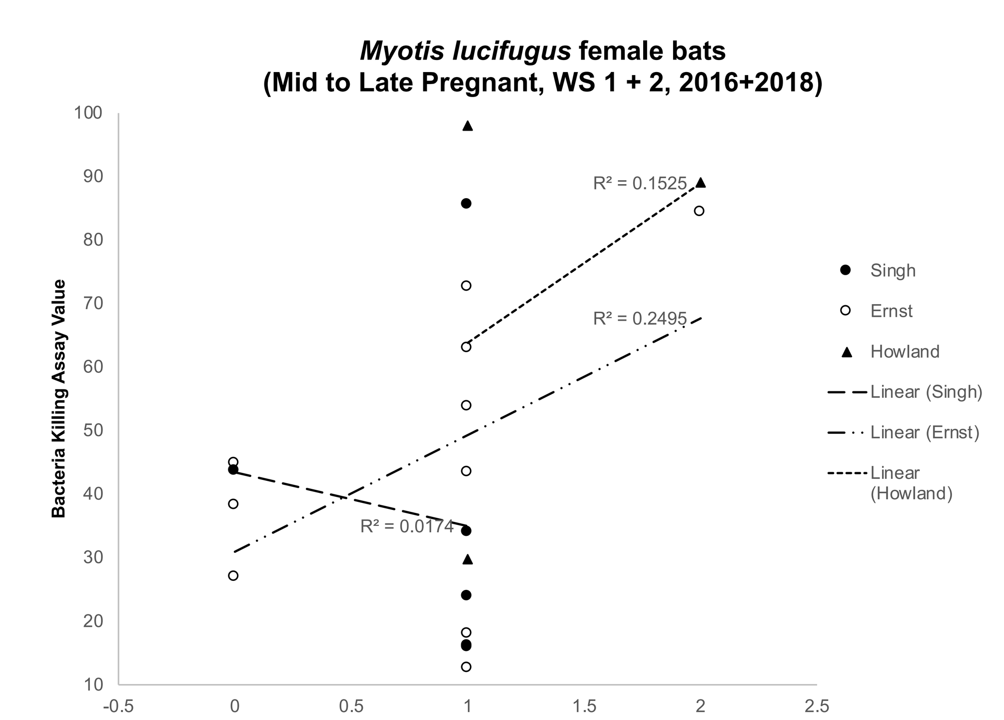


Figure 4. The two lines represent the relationship between fungal load and % of bacteria killed by bat plasma for different colonies of bats of mid to late pregnant *Myotis lucifugus* (2016+2018).

Table: Averages of BMR and BKA for Little Brown Myotis (WS 1 and WS 2; 2016 and 2018)

Colony Preg Stage	BMR	BKA
Singh 0+1	13.87 ± 1.83	27.83 ± 5.51
Singh 2+3	12.09 ± 2.40	36.39 ± 10.72
Ernst 0+1	14.57 ± 1.88	11.20 ± 3.40
Ernst 2+3	15.87 ± 1.72	45.64 ± 7.35
Howland 0+1	8.01 ± 0.01	63.07 ± 35.57
Howland 2+3	19.25 ± 3.22	72.21 ± 21.43

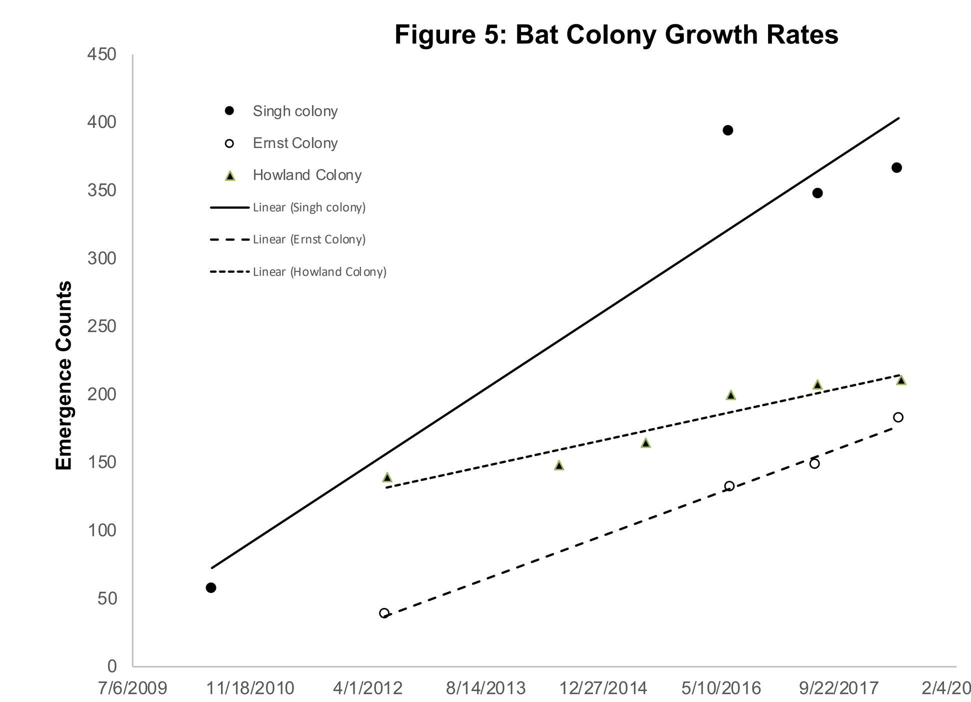


Figure 5. The relationship between years and emergence counts at *Myotis Lucifugus* maternity colonies.

Conclusion

Most studies of WNS have examined its impact during hibernation. We investigated immune function and energy cost of WNS infection in bats in order to understand more about the role of energy use and immune function in response to and recovery from WNS in bats after hibernation. Singh colony has the highest growth rate but the lowest energy expenditure (or BMR) on average throughout pregnancy and a modest immune (or BKA) response (see Table). Singh bats could expend less energy on immune response, allowing for more energy later in reproduction such as during lactation. Singh bats in early pregnancy spend more energy to fight the fungus. Later in pregnancy, Singh bats spend less energy to fight the fungus with more to spend on pregnancy. However, both Ernst and Singh bats increase BKA immune response later in pregnancy.

Our previous research found the impact of WNS as indicated by wing damage is greater in little brown myotis than in big brown bats. Both BMR and BKA were greater in little browns experiencing mild wing damage as compared to no wing damage than in big browns. This could mean more energy is expended during the response and recovery to WNS in little browns than in big browns.

The unique feature about our research is creating energy use and immune function indicators of colony health (see Table). This helps address the problem of finding strategies to help bats survive the disease and reproduce. WNS has impacted the local bat populations greatly within the past ten years, but now some of the local populations are starting to recover. Understanding the intrinsic factors that are influencing colony recovery of local bat populations can help in promoting further bat population recovery. After gathering data from about 20 bats from three different colonies in various stages of demographic recovery (see Figure 5), we have created numerical estimates (see Table) of energy use (i.e., BMR) and immune function (i.e., BKA) which can be used to predict how bats are recovering from the disease and reproducing, including growing in population size. These colony averages provide a starting point to understand how and why bat colonies differ in their recovery.

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