Introduction

Bats play vital ecological roles throughout the world. In North America, where a majority is insectivorous, bats act as major predators on insect populations. Many of these insects are agricultural pests. Without bats, the increase in pest populations would lead to an estimated $3.7 billion in agricultural losses [3]. The value of bats to North American agricultural industry is estimated at $22.9 billion/year [3]. Currently several threats are impacting the bat populations in the eastern United States, including habitat loss, wind turbines, and White-nose syndrome. The objective of this study was to determine activity patterns of bat species in two forested habitats in Middle Western Georgia.

Georgia has a temperate climate with moderate winters and very warm summers, which are the ideal temperatures for insectivorous bats and insects. Insect activity has been known to increase as the temperature increases which causes a variety of chain reactions such as enhanced herbivory and increased reproduction. This increased insect activity can be detrimental to forests and agriculture, but may be beneficial for insect-eating bats. Likewise, lower temperatures are associated with decreased insect activity. Bat activities are strongly correlated with insect abundance. Bats are opportunistic feeders with the ability to change hunting techniques to catch the prey available. This adaptation allows bats to take advantage of prey whenever available [6, 15]. When insect abundance is very low, bats hibernate to reduce energy expenditure until prey are available.

The amount of food intake is extremely important for hibernating bats. The more food a bat intakes, the more fat reservoirs it has to utilize during the winter months in hibernation. Fat reservoirs are used in a multiple ways during hibernation, mainly for the maintenance of body temperature. Bats exhibit a variety of ways to maintain the proper body temperature for its current environment. During winter, there is a decline in both temperature and food supply. Bats will hibernate with the onset of these factors. Hibernation for bats is different than other mammals because they participate in the Torpor-Arousal Cycle. Torpor is a state that the body temperature drops to a few degrees above the ambient temperature and most, if not all, physiological functions of the bat...
such as metabolic rates and heart rate is slowed. Torpor, on average, lasts for 12-15 days depending on the bat’s physical condition (amount of fat reserves) and the hibernacula conditions (temperature) as well. Arousal occurs for various reasons: thirst, feeding, sex, and to warm back to a normal body temperature [12]. This process allows bats to survive long periods of low abundance or absence of insects.

White-nose syndrome

During the winter of 2006, a new disease began impacting bat populations in the Eastern United State. Bats were found dead in the areas directly around the hibernacula. The muzzle of the dead bats were covered with a white growth. This disease was named white-nose syndrome.

White-nose syndrome (WNS) is a fungal disease caused by Pseudogymnoascus destructans (Pd) [11]. Pd is a cold-adapted fungus that invades the epidermis and dermis, including the invasion of sebaceous and apocrine glands and hair follicles of bats during hibernation. The mechanism Pd uses to infiltrate the tissues is fungal hyphae—each of the branching filaments that make up the mycelium of a fungus—displacing the cutaneous tissues [9-11]. The invasion occurs because the immune system of hibernating bats is suppressed with all the other physiological functions as well as the temperature and humidity of the hibernacula. Pd only grows at cold temperatures <20 °C (<68 °F) [11]. The growth rate of Pd peaks at temperatures between 12.5 and 15.8 °C (54.5 and 60.4 °F) and declines at warmer or colder temperatures [11].

WNS manifests itself in the affected bats through different physical symptoms. The cutaneous fungal infection manifesting itself as white, filamentous growth on muzzle, and powdery growth on the surfaces of ears, wings, and tail membranes (the most notable for WNS) [8, 10, 13]. The infection also causes abnormal or increased frequency in arousal from torpor affecting metabolic rates, loss of fat reserves and water balance [2, 5, 7, 9, 11, 13]. This results in premature exhaustion of fat reserves prior to the availability of food [2, 5, 9-10, 13]. The starving bats are often seen flying outside of hibernacula in mid-winter searching for food during midwinter when food is least abundant [9, 13]. This results in the bats starving prior to warming temperatures and increases in the amount of insects present. During other times of the year severe wing damage affecting flight [8, 13]; cupping erosions, lesions, and ulcerations of epidermal and dermal tissues and underlying connective tissues can be seen[9, 13].

It is speculated that Pd is originally from Europe and later brought to the United States since the fungus has been seen on hibernating bats in Europe but there has been no indication of WNS or mass mortality in bat species there [8]. The disease was first noticed in New York State during the winter of 2006-2007. Since its first emergence in 2006, there has been an estimated 5.7-6.7 million deaths [8, 10, 11, 14]. WNS has spread rapidly throughout the United States affecting over 190 bat hibernacula in 20 states mainly in the Appalachian region of eastern North America [5, 8, 10] (Fig1).
WNS has affected at least 8 species of hibernating bats that are located in the Appalachian region. These species that are already infected or suspected to be include: Eptesicus fuscus, Myotis leibii, M. lucifugus, M. septentrionalis, M. sodalis (Indiana bats), M. grisescens (Gray bat), Lasiusus cinereus (may correlate with wind-energy development), and Perimyotis subflavus [2, 8-10]. The M. grisescens and M. sodalis species have already experienced drastic population declines before the onset of WNS and are already classified as Vulnerable and Near Threatened [2]. The continued spread of WNS may result in dramatic declines in all affected bat species. The decline of this taxonomic group will not only have tremendous ecological implications but also economic implications for the timber and agricultural industries of the United States. At present there is means to control the spread of the Pd fungus or prevent exposed bats from becoming infected. It is hoped warmer regions of each of the affected species geographic ranges will may act as refuges where survival rates may be higher than cooler more portions of the country.

This study was designed to measure the acoustic activity of bats to better understand how WNS would impact these local bats in Pine Mountain, GA. I predicted that I would observe activity throughout the fall and winter months because of the region’s slow gradual decrease in temperatures. To test this prediction, I selected two different types of sites, a managed area and unmanaged area, where acoustic Anabat II bat detectors were placed.

MATERIALS AND METHODS
Selection of Sites
Sites were selected along the Pine Mountain ridge region of western Georgia (elevation of 280m). Two forest sites were selected; 3 sample points were
placed in each forest site. One of the forest sites was under active forest management (managed site). Management treatments included forest thinning and prescribed burning. The sample points in the managed forest sites were designated CAM This resulted in an open forest. No forest management treatments were performed on the other forest site (unmanaged site). This resulted in a much more dense and cluttered forest habitat. Each sample point was marked with a PVC pipe hammered into the ground (Table 1). The two sites (managed and unmanaged) were only about 5-8 miles apart from one another so the weather patterns in each area should be relatively similar.

<table>
<thead>
<tr>
<th>Sampling Point</th>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample point 1</td>
<td>Managed</td>
<td>32° 45' 8.2&quot;</td>
<td>84° 54' 52.4&quot;</td>
</tr>
<tr>
<td>Sample point 2</td>
<td>Managed</td>
<td>32° 45' 17.3&quot;</td>
<td>84° 54' 56&quot;</td>
</tr>
<tr>
<td>Sample point 3</td>
<td>Managed</td>
<td>32° 45' 32.5&quot;</td>
<td>84° 54' 57.1&quot;</td>
</tr>
<tr>
<td>Sample point 4</td>
<td>Unmanaged</td>
<td>32° 45' 9.3&quot;</td>
<td>84° 54' 8&quot;</td>
</tr>
<tr>
<td>Sample point 5</td>
<td>Unmanaged</td>
<td>32° 45' 20.1&quot;</td>
<td>84° 54' 5.1&quot;</td>
</tr>
<tr>
<td>Sample point 6</td>
<td>Unmanaged</td>
<td>32° 45' 28.7&quot;</td>
<td>84° 54' 8.4&quot;</td>
</tr>
</tbody>
</table>

Table 1. This table is a description of each sampling point including latitude, longitude, and a brief description of the point. Sample points 1-3 are in the managed site and sample points 4-6 are in the unmanaged site.

Acoustic detection

To determine activity levels, Anabat II bat detectors were used to collect echolocation calls of bats using the surrounding area. Anabat II is an acoustic detection device for recording bat calls consisting of a high frequency microphone and a recording device. It uses frequency division to lower the echolocation calls of bats to an audible frequency. The detector has a microphone that is able to pick up calls and noises. Echolocation calls detected by the microphone were recorded and stored on a CF memory card. Detectors were set to record data from 19:00 that evening until 7:00 the next morning. The Anabat II detector was housed in a plastic weather proof box with a detachable lid. Each detector was oriented with the microphone facing the area with the least dense vegetation. To position the detector in the most desired position (usually toward an open clearing) and to prevent swaying, two ropes are attached to the box and staked to the ground with metal stakes. A weather data collector, HOBO Pro 2, was placed out at one of the three sites during monitoring period to record temperature and humidity fluctuations.

Each sample point was monitored for two consecutive nights once a month from October to December. Each week there would be three detector boxes placed out at random in the sites, at least one detector had to be in each site. This allows for a more variable data collection that week than if all the detectors were at the same site. The HOBO® weather collection unit was placed out randomly with one of the detectors to collect.
Analyzing collected data

The average temperature and RH for each night were calculated and highest and lowest recorded temperature (\(^\circ\text{F}\)) and RH (%) were recorded.

The software package AnalookW was used to convert the recorded echolocation calls into a sonogram (Fig 2). Each sonogram consisted of a series of individual pulses representing an individual ultrasonic vocalization. At least 5 pulses were required for a call to be considered for analysis. A sonogram of each recorded bat echolocation call was viewed and identified based on characteristic frequency shifts and patterns.

![Sonogram of PESU](image)

**Figure 2. Sonogram of PESU** is an example of what AnalookW does to recorded echolocation calls.

Call sonograms were compared to known call patterns from an eastern bat call library provided by Chris Corben. The number of call files recorded for each species was counted and used to determine activity patterns and species diversity among sample points and sites. The Shannon index was used to determine the diversity of species. Compared activity levels between managed and unmanaged sites, trends in activity patterns between months, and diversity between managed and unmanaged. Calculation of the standard deviation was performed. The Shannon indexes were used to perform T-Tests on different sets of data to confirm if there is any significant difference between sites and between months.

**RESULTS**

**Activity Levels**

From October 2014 to December 2014, a total of 283 calls were identified as bat calls, 270 of which were identifiable to certain species. These 270
calls represent ten different species of bat inhabiting the Pine Mountain area. Over all the activity in the managed area was 40% greater than the activity in the unmanaged area (Fig 3). The monthly activity (Fig 4) shows fluctuations in activity from month to month. There was a 27% decrease in activity from October to November, while there was a 97.5% decrease in activity from October to December. The activity of bats within the managed sites and unmanaged sites vary between locations. The sites in the unmanaged area had larger fluctuations during all three months of activity than the managed area. There was an average of 5% difference between sampling points of unmanaged, while there was only a 1.5% difference between sites of managed (Fig 5).

Species Specific Activity
Species richness of this sampled region is 10 different species of bats. Some calls did not have enough pulses to be identified as a single species or the pulses looked like it could be identified as two species. Calls unable to be identi-
fied to species were categorized into an appropriate frequency category. LACI and LANO were only recorded in the managed sites, while MYSE and MYLE were only recorded in the unmanaged sites (Table 2). TABR was located in both areas but during different months.

<table>
<thead>
<tr>
<th>4 letter code</th>
<th>Binomial Species Name</th>
<th>Common Name</th>
<th>Managed Sites</th>
<th>Unmanaged Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPFU</td>
<td><em>Eptesicus fuscus</em></td>
<td>Big Brown Bat</td>
<td>37</td>
<td>4</td>
</tr>
<tr>
<td>LABO</td>
<td><em>Lasiurus borealis</em></td>
<td>Eastern Red Bat</td>
<td>31</td>
<td>2</td>
</tr>
<tr>
<td>LACI</td>
<td><em>Lasiurus cinereus</em></td>
<td>Hoary Bat</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>LANO</td>
<td><em>Lasionycteris noctivagans</em></td>
<td>Silver-haired Bat</td>
<td>18</td>
<td>0</td>
</tr>
<tr>
<td>MYLE</td>
<td><em>Myotis leibii</em></td>
<td>Eastern Small-footed Bat</td>
<td>0</td>
<td>19</td>
</tr>
<tr>
<td>MYLU</td>
<td><em>Myotis lucifugus</em></td>
<td>Little Brown Bat</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>MYSE</td>
<td><em>Myotis septentrionalis</em></td>
<td>Northern Long-eared Bat</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>NYHU</td>
<td><em>Nycticeius humeralis</em></td>
<td>Evening Bat</td>
<td>39</td>
<td>3</td>
</tr>
<tr>
<td>PESU</td>
<td><em>Pipistrellus subflavus</em></td>
<td>Tri-colored Bat</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td>TABR</td>
<td><em>Tadarida brasiliensis</em></td>
<td>Brazilian Freetailed Bat</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 2. *Species specific activity: managed v. unmanaged* shows the number of calls for a specific species in both the managed and unmanaged areas. This table excludes the unidentified calls that were categorized in ranges.

Shannon Index (H’)

The Shannon index is a commonly used means of comparing diversity among plots. A high Shannon index value denotes greater species diversity, while a low Shannon index value denotes a community with fewer species of a community dominated by a small number of species. The overall diversity for the 2 sites over the 3 months was H’=2.27. The diversity for the managed sites over the 3 months was H’=1.88. The diversity of the unmanaged sites over the 3 months was H’= 2.0 (Fig. 6). There was no significant difference in diversity between the two forest sites (p=0.8536).

When diversity was measured for each month, there was no significant difference between bat diversity in October (H’= 2.05) and November (H’=2.13; p=0.9034). There was a significant decrease diversity from October to December (H’=1.04; p=0.007). There was a significant decrease in diversity from November to December (p=0.0161) (Fig 7).
Weather Data

The weather data collection came from the HOBO Pro 2 unit. Every time the Anabat II detector went out so did the weather data. The relative activity of bats greatly depends on the ambient temperature [12]. The average temperature, the temperature highs and lows, average RH values, and the RH highs and lows were calculated for each sampling period (Fig. 8).

October had the warmest temperatures with a high of 75.8 °F and an average of 58.1 °F for the dates sampled during the study. There is a gradual decrease in temperature during the three months the study was conducted. The lowest average temperature 34 °F occurred in December. The relative humidity stayed reasonably around the same percentage throughout the three-month duration of this study. The lowest average value of RH was 76.9% and the highest average value was 96.7%.

DISCUSSION

At the beginning of this study, I predicted that because of the slow gradual de-
crease in temperatures in Pine Mountain, GA, I would observe acoustic activity through the fall and winter months. From the collected data, my prediction was confirmed. Although there was a decrease in activity from October to November to December, I observed calls from multiple species of bats from all three months. LACI and LANO were only recorded in the managed sites, while MYSE and MYLE were only recorded in the unmanaged sites despite the temperature (ref table 2). LACI and LANO are open habitat foragers, which is congruent with the type of sampling points that were studied in the managed site. MYSE and MYLE are cluttered habitat specialists corresponding to the unmanaged site's sampling points. Bat activity and temperature are positively correlated. When the temperature increases, bat activity increases and when the temperature decreases, bat activity decreases [15]. This relationship of activity and temperature is observed in the data collected from this study (Fig. 5 and Fig. 8).

From the species richness data, it was assumed that the December Shannon index value would be lower than the total Shannon index value (ref figure 9). It was surprising to see that the managed site had a lower diversity ($H' = 1.88$) than the unmanaged sites ($H' = 2.00$). This may be due to the inclusion of both northern long-eared bats and small-footed bats in the unmanaged area not found in the managed area. The northern long-eared bat is known to specialize on very dense forest areas. This describes the habitat found in the unmanaged area.

From my findings during this study, I conclude that White-nose syndrome (WNS) will not have as large an impact on bats in western Georgia as more northern regions of the Eastern United States. Since there is a gradual decrease in the temperature, this allows for insect activity to be more active positively correlating to the activity of bats [12]. Assuming the activity of bats is associated with insect availability, the bats have a greater opportunity to gain body mass. Increased body mass has been seen to help bats resist the symptoms of WNS compared to bats with smaller body masses [10].

Georgia has very mild winters while many northern states start to see declines in temperatures much sooner than December. The bats in those northern states will go into hibernation around late October – early November. By cross referencing the weather data collected and the activity from the three months of study, I can assume that these southern bats go into hibernation around mid to late November. Since the temperatures gradually decrease through the fall and winter months, hibernating bats have more time to feed, which can result in bigger fat reserves and a larger body masses for the winter hibernation period. A bat with a larger body mass, compared to a bat of that same species with a smaller body mass, is more likely to survive the onset of WNS during hibernation because its fat stores will not exhaust as quickly.
Bibliography