

Protocols for Passive Acoustic Sampling of Bats in the Plumas National Forest

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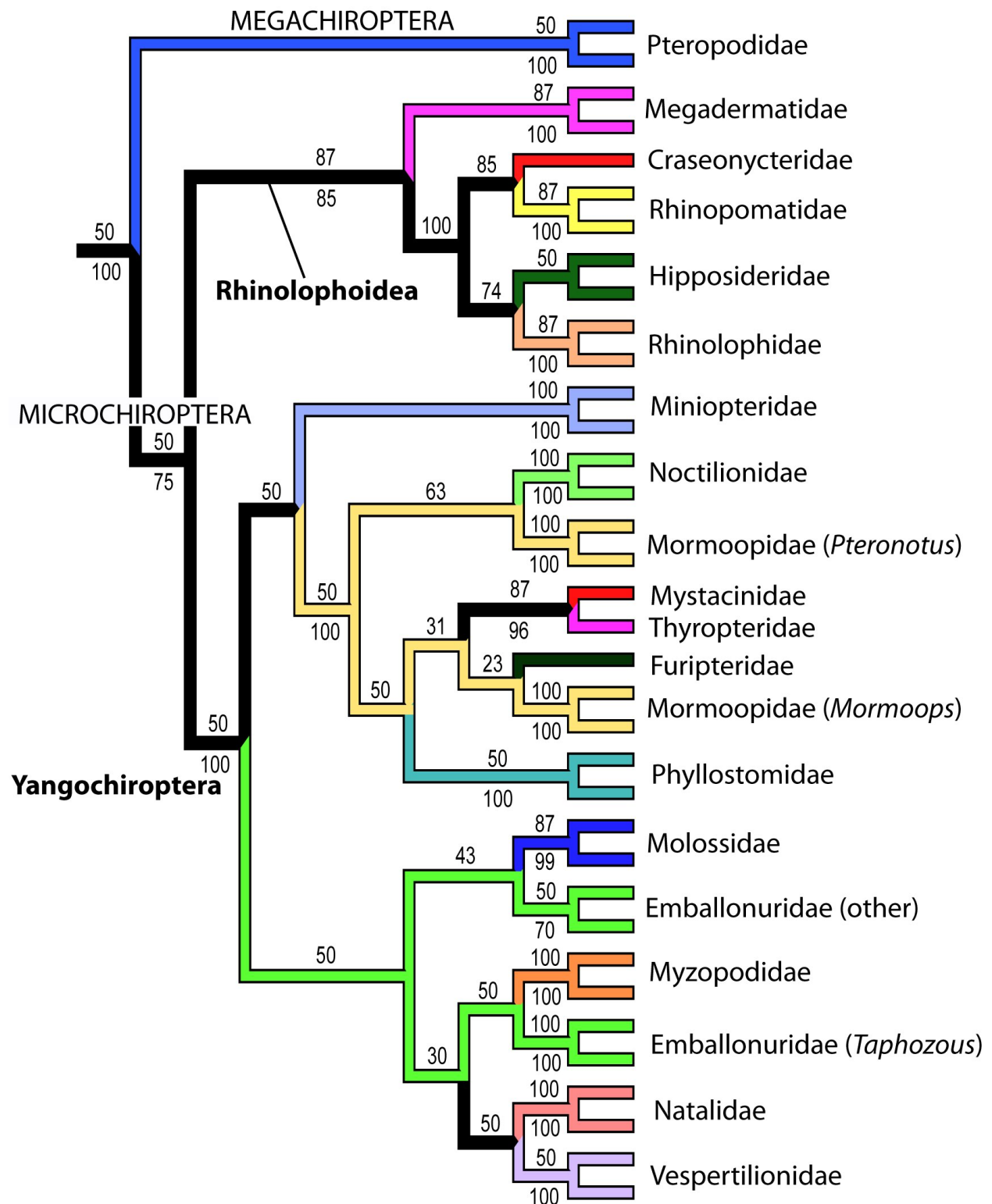
1 Background

1.1 Bats

1.1.1 General information

Bats are one of the most diverse group of mammals with around 1240 species (Tudge 2000; Schipper et al. 2008), second only to rodents. The distinctive characteristic of this group is their ability to fly.

There are two main group of bats (see dendrogram below, extracted from Agnarsson et al. (2011)), the Megachiropterans, or flying foxes, which are large frugivorous bats that inhabit exclusively in the tropics. There are around 170 species of megachiropterans, they search their food using the sense of sight and smell and are mostly diurnal. The large flying fox (*Pteropus vampyrus*) is the largest species of bats and belongs to this group, which can have a wingspan of 1.7 meters (5 feet 7 inches). Within this group only the bats of the genus *Rosettus* use a very primitive form of echolocation, and are nocturnal.

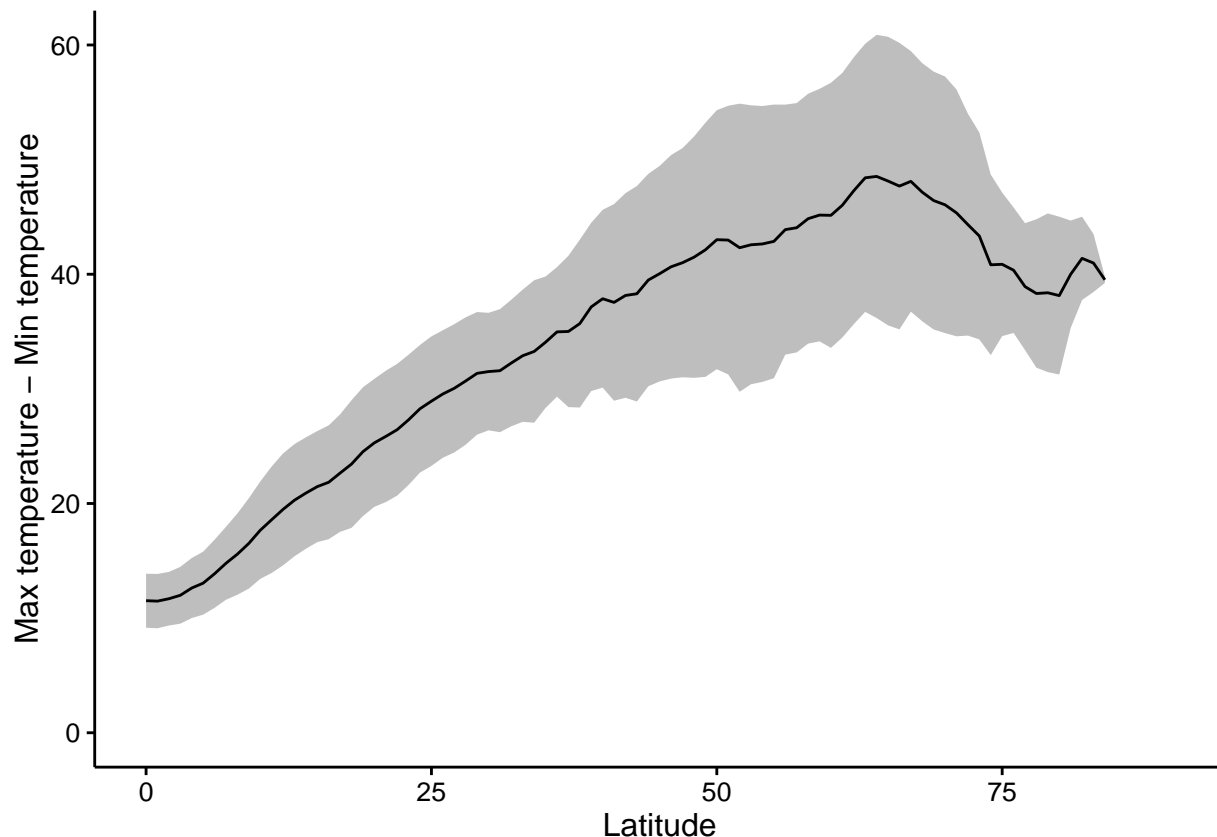


Microchiropterans are the most diverse and specialized group of bats, and they live in every continent but Antarctica. Most of them are insectivores, but some of them are pollinators. Usually these bats are a lot smaller than megachiropterans, they are nocturnal, and rely on echolocation to detect their preys and navigate through space

1.1.1.1 Adaptations for flight Chiropterans are the only mammals to achieve true flight, bats have several adaptations for flight (Norberg 1998). It is supposed that ancient bats started flying around 50 million years ago (Cooper, Cretkos, and Sears 2012), developing an enlarged hand with a thin membrane between them which is very different from the wings of birds. Little is known of the evolution of flight in bats, since the earliest fossils found of these groups were already winged (Gunnell and Simmons 2005; Jepsen 1966). However wings are not the only adaptation that bats and birds share, they both have a higher metabolism than other flightless vertebrates of the same size, their bones are lighter, and have enlarged pectoral muscles that allow them to fly (Norberg 1998).

1.1.1.2 reproduction Chiropterans as most of mammals breed live kits, most bats have a litter size of one or two. Several bats have maternity colonies,

1.1.1.3 Hibernation or migration Most north-american bats hibernate during the winter, but this is not true for all chiropterans. Some species migrate regionally (140 to 350 miles), in order to get to central hibernacula. Some other bats however can migrate upto 1180 miles to get to warmer latitudes (T. H. Fleming et al. 2003, McGuire et al. (2012)) and avoid hibernating all together. Some species such as the silver-haired bats (*Lasionycteris noctivagans*) may migrate over 155 miles a day (McGuire et al. 2012). In general bats that live in higher latitudes tend to be migratory whereas bats that live in lower latitudes usually don't migrate, this is partly due to colder temperatures in higher latitudes, but also there is a higher Temperature Annual Range in higher latitudes as seen in the graph below (data extracted from Hijmans et al. (2005)).



Bats in higher latitudes migrate to escape lower temperatures, which bring scarcity of invertebrates (T. H. Fleming et al. 2003). It has also been established that there are important difference between sexes in terms of migration patterns, where in most species, females migrate more often and further away than males. There are several studies that show that there are different metabolic needs between males and females, since males spend more energy in autumn due to spermatogenesis, and females spend more energy in spring due to

pregnancy and lactation, this would result in the differences in migration between them (Cryan and Wolf 2003).

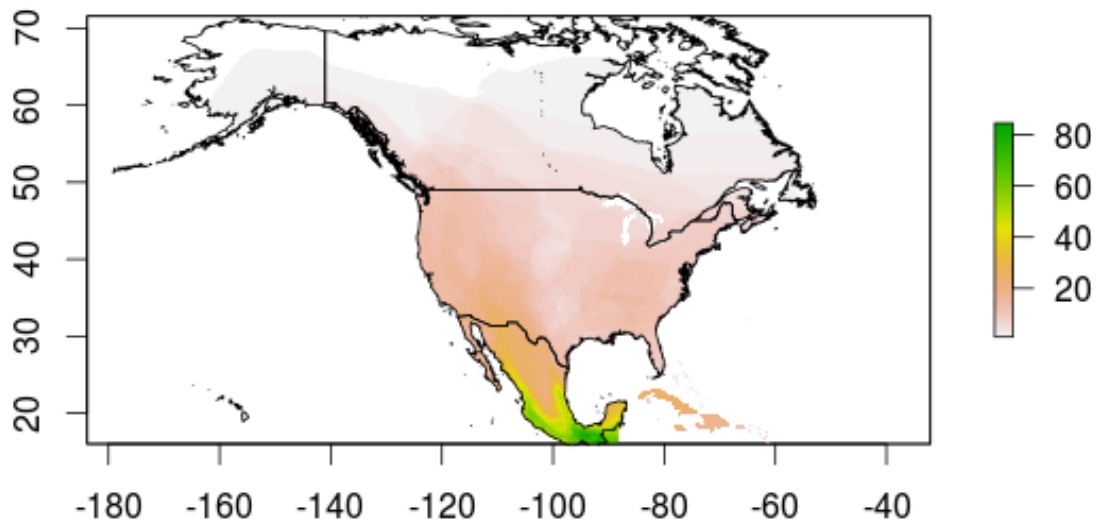
Most bats (around 70% of them) feed on invertebrates, other species are frugivores, or pollinators. Some very specialized groups of bats feed exclusively on blood.

periods of activity

echolocation

1.1.2 Bats in northamerica

- General information about bats biology, taxonomy, and natural history. main groups no species by region adaptations like hibernation in different areas There are 51 species of bats in North-america, those bats belong to four distinct families (R. D. Bradley et al. 2014). The patterns of diversity are shown in the map below, where we see that bats are more abundant in the southwestern portion of the United States (Jenkins, Pimm, and Joppa 2013)



1.2 Why Bats Matter

1.2.1 Ecosystem services delivered by bats

Bats are very important economically in the world. Their most important benefit without a doubt is that they feed on invertebrates and thus they are one of the major natural pest controls for crops, since over two thirds of all bats are obligated insectivorous (T. H. Kunz et al. 2011). Due to their insect control, only in agriculture, it has been calculated that bats save farmers in the United States 72 dollars/acre (Boyles et al. 2011), which projects to an economic value of \$22.9 billion dollars a year in the United States for the agricultural industry.

At the same time there are bats that are pollinators of flowers, and there are other frugivorous bats that help spreading seeds (T. H. Kunz et al. 2011). Bat pollination occurs in about 528 species of angiosperms world-wide. Even though most of north american bats are insectivorous, in arid habitats two families of succulent plants, Agavaceae and Cactaceae, rely on bats to be pollinated. Several of those species are very importante economically in northern and central america and suply food, fiber, tools, soaps, and medicine to the community as well as being the base of the multimillion dollar industry of tequila (Forster, Fleming, and Valiente-Banuet 2003).

1.3 Threats to bats

1.3.1 White nose syndrom

The White Nose Syndrome (WNS) is a fatal bat disease produced by a fungus, its name is based in the white color left on the infected skin of the muzzle, ears, and wings of bats. The syndrome is characterized by the presence of abundant and delicate hyphae and conidia on bat muzzles, wing membranes, and/or pinnae (D. S. Blehert et al. 2009). This desease usually causes aberrant behavior of bats during hibernation, including bats prematurely staging at hibernacula entrances, failure of bats to arouse normally in response to disturbance, and diurnal and mid-winter emergence (Langwig et al. 2012). The fungus that produces the disease its call *Pseudogymnoascus destructans*, formerly known as *Geomyces destructans*, because initial analysis of small subunit (SSU) and internal transcribed spacer (ITS) rRNA gene sequences placed this fungus into this genus (Gargas et al. 2009). The fungus has very slow growth on artificial media and produces arthroconidia on verticillately branched conidiophores and on prostrate hyphae, typical of genus *Geomyces*, but the asymmetrically curved conidia are unlike any species previusly described (Gargas et al. 2009).

Dermatologically speaking, until 2009 the WNS was poorly understood, so it was necessary detail information to compare the disease with other dermatology issues (C. U. Meteyer et al. 2009). The same year, it was published the necessity of histological examination by microscope to confirm the fungus. In this way, infected bats showed that fungal hyphae pervade the bat tissue filling hair follicles and sebaceous glands, yet the fungus does not typically lead to inflammation or immune response in the tissue of bats (C. U. Meteyer et al. 2009). Even more, it was demonstrated that this wasn't a typical disease where the fungus is an opportunistic pathogen, the exposure of healthy little brown bats (*Myotis lucifugus*) to pure cultures of *G. destructans* causes WNS (Lorch et al. 2011) *P. destructans* is capable of living at relatively low temperatures. Thermal performance curves generated for each isolate indicated thermal optima for growth between 12.5 and 15.8°C (54.5 to 60.44 °F) and an upper critical temperature for growth between 19.0 and 19.8°C (66.2 to 67.6°F) (Verant et al. 2012), no growth at 24°C (75.2°F) or above (Gargas et al. 2009). This makes this fungus to grow optimally at the temperatures found in winter bat hibernacula. Bats are thought to have lowered immune responses during hibernation torpor (Carey, Andrews, and Martin 2003), which explains the situation may predispose bats to infection by *P. destructans* (Gargas et al. 2009).

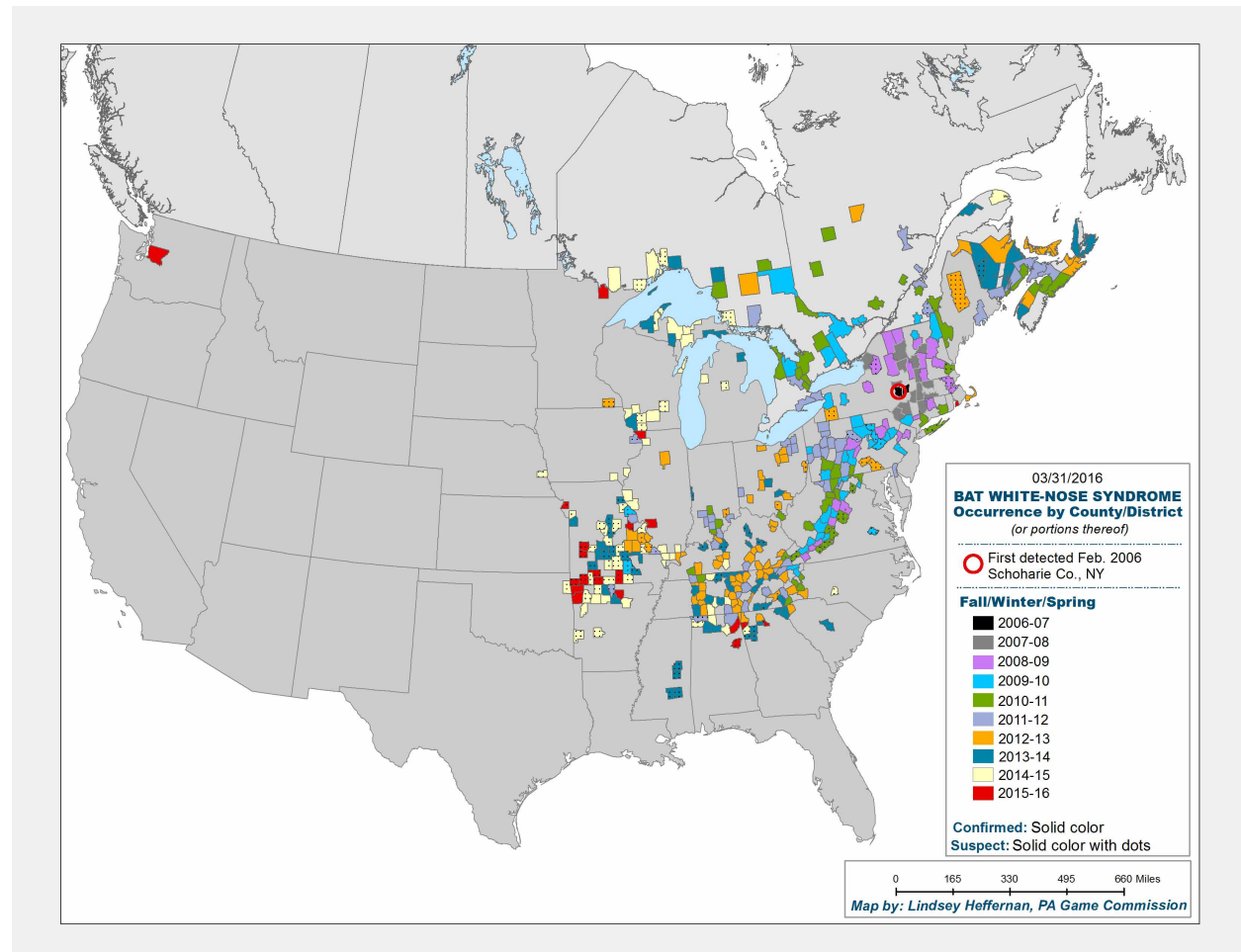
In general terms, novel pathogens introduced to new host communities can have devastating effects on wildlife populations, drive species to extinction and thereby decrease biodiversity (Daszak, Cunningham, and Hyatt 2000; Smith, Sax, and Lafferty 2006). To this date WNS has been estimated to have killed over five million North American bats (Verant et al. 2012).

It has seen that differences in temperature at locations within underground sites occupied by hibernating bats may influence both progression and severity of WNS among infected bats and environmental persistence and transmission of the fungus (Verant et al. 2012), even some modeling suggests that localized thermal refugia of 28°C (82.4°F) could improve survival by up to 75%, depending on how WNS acts to disrupt energy balance (Boyles and Willis 2009). It seems to be that in hibernating bats infected with *P. destructans* the impacts of disease on solitary species were lower in smaller populations, whereas in socially gregarious species declines were equally severe in populations spanning four orders of magnitude. However, as these gregarious species declined, we observed decreases in social group size that reduced the likelihood of extinction (Langwig et al. 2012)

WNS is dispersing notoriously trough North America. The first evidence of WNS in bats was on February 2006 in New York, and it was documented by a photograph taken at Howes Cave, 52 km west of Albany (D.

S. Blehert et al. 2009). Until 2009, the disease was spread in the northeastern United States and it was confirmed by gross and histologic examinations of bats at 33 sites in Connecticut, Massachusetts, New York, and Vermont (D. S. Blehert et al. 2009).

The USGS has information about WNS was confirmed in Michigan and Wisconsin at 2014, and for 2016 in State of Washington (see the map below (USGS 2016)). However, a new study indicates that six *Pseudomonas* isolates can inhibit the growth of *P. destructans* in vitro and should be studied further as a possible probiotic to protect bats from white-nose syndrome (Hoyt et al. 2015).

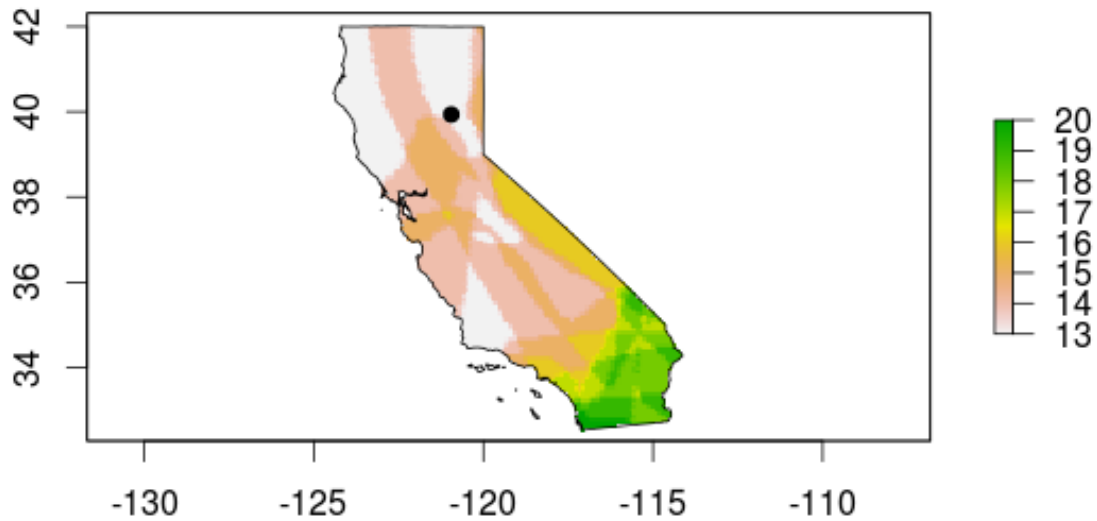


1.3.2 Eolic energy and bats

Besides WNS, the biggest threat to bats in northamerica is wind turbines. Every autumn high mortalities occur when migrating bats crash into this turbines (Cryan 2011). In a review of all multiple mortality events, defined as events where more than 10 bats died at a specific location on the same date, it was estimated that wind turbines have been the cause of more cumulative multiple mortality events than any other reason, followed closely by WNS (O'Shea et al. 2016). From 2003 to 2013 at least 5,626 bats of 27 species in 18 countries where registered to have died in wind turbines (L. Rodrigues et al. 2015), and this should be only a fraction of the likely mortality, with estimations of 888,000 bat deaths only in northamerica for the year 2012 (Smallwood 2013). It is also important to note that mortality in not equally distributed among bat species most deaths that happen in wind turbines correspond to migratory species that roost in trees (Arnett et al. 2008).

1.4 Bats in the Plumas National Forest

1.4.1 Species present in the Plumas National Forest



Small description on each of the species

- *Myotis yumanensis* (Myyu)
- *Myotis californicus* (Myca)
- *Myotis ciliolabrum* (Myci)
- *Myotis volans* (Myvo)
- *Myotis lucifugus* (Mylu)
- *Parastrellus hesperus* (Pahe)
- *Lasiurus blossevillei* (Labo)
- *Myotis evotis* (Myev)
- *Antrozous pallidus* (**Anpa**)
- *Eptesicus fuscus* (Epfu)
- *Lasionycteris noctivagans* (Lano)
- *Myotis thysanodes* (**Myth**)
- *Tadarida brasiliensis* (Tabr)
- *Lasiurus cinereus* (Laci)
- *Corynorhinus townsendii* (**Coto**)
- *Euderma maculatum* (Euma)
- *Eumops perotis* (Eupe)

1.4.1.1 Product Occupancy map for species studied in the Plumas National Forest

1.4.2 Bat species of Concern in the Plumas national Forest

Longer description of this species and reasons of why is a species of Concern

- *Antrozous pallidus* (Anpa)
- *Myotis thysanodes* (Myth)
- *Corynorhinus townsendii* (Coto)

2 Site Selection

2.1 The importance in selecting heterogeneous environments

This often improves the representativeness of the sample by reducing sampling error. It can produce a weighted mean that has less variability than the arithmetic mean of a simple random sample of the population.

In computational statistics, stratified sampling is a method of variance reduction when Monte Carlo methods are used to estimate population statistics from a known population.

The reasons to use stratified sampling rather than simple random sampling include[1]

If the population density varies greatly within a region, stratified sampling will ensure that estimates can be made with equal accuracy in different parts of the region, and that comparisons of sub-regions can be made with equal statistical power. For example, in Ontario a survey taken throughout the province might use a larger sampling fraction in the less populated north, since the disparity in population between north and south is so great that a sampling fraction based on the provincial sample as a whole might result in the collection of only a handful of data from the north.

Randomized stratification can also be used to improve population representativeness in a study.

2.2 Classifying Plumas National Forest into different environments

2.2.1 Layers used to classify the Plumas National Forest

- Elevation (m.a.s.l)
- Burn intensity basal
- Burn intensity canopy
- Burn intensity soil
- Distance to fire edge
- Distance to roads
- Distance to water bodies
- Fire interval
- Vegetation type

2.2.2 Methods used to classify the Plumas National Forest

- K-means

2.2.3 Product GIS layer of the Plumas National Forest Classified into 5 different environments

- Raster, and shapefile of the classification of the Plumas National forest

2.2.4 General Characteristics of the five types of environment

- Graphic output as a classification tree
- Table output as means and standard deviation of each of the variables for each environment type

2.3 Stratified random site-selection

2.3.1 Product 2000 stratified random points

- 400 points per habitat delivered in KML and shp formats
- Contemplates 200 sampling points per year for the next ten years, 40 points per habitat per year

3 Acoustic monitoring

3.1 Advantages and disadvantages of passive acoustic monitoring

- Acoustic bat detectors can be set anywhere
- Acoustic bat detectors can sample for days
- Acoustic bat detectors are not as accurate as mist nets
- Species detection are never 100% accurate

3.2 Setting of the Pettersson D500x bat detector

- Importance of setting the detection time to 3 seconds
- parameter settings
- Set all programs of the detector to the same parameters to avoid field mistakes

3.3 Installing a bat detector in the field

- Explanation of how to deploy the detector in the field

3.4 Field measurements to be taken in the field

- Description of each of the measurements to be taken in the field
- Basal Area
- Canopy cover
- Ground cover

3.5 Using sonobat to automatically classify bat calls into species

3.5.1 Filter low quality calls

- How to automatically erase bad quality calls in order to diminish sonobat running time

3.5.2 Classify bat calls

- How to batch-classify the calls for one site

3.5.3 Interpret the results made by sonobat

- Reading sonobat’s output files
- How to see which species are present according to sonobat

3.5.4 Get sonobat’s help to manually vet inconclusive calls

- How to get sonobat’s help to manually vet species that are uncertain to be present

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