# Bat species surveys in pine and oak forests; a comparison with conservation implications

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#### Abstract

Populations of Chiropterans have experienced massive regional declines in recent years. The northern long-eared bat (Myotis septentrionalis) has recently been classified as federally threatened and may be faced with local extinction on Long Island. While many factors have contributed to these declines, such as the introduction of white-nose syndrome (WNS), caused by the fungus (*Pseudogymnoascus destructans*), and habitat loss, it is believed that the conservation of bats through habitat protection is most optimally implemented at the local level by local governments and wildlife organizations. In order for this type of conservation to occur, it first must be determined what types of habitats the bats are most likely to be found in, to allow for more focused conservation efforts. Acoustic surveys, shown to be a viable method of characterizing bat populations on a local scale, were conducted in a variety of habitats at Brookhaven National Laboratory (BNL). Calls were sorted by species using two programs: Sonobat<sup>®</sup>, which automatically identified the calls using full spectrum acoustic analysis of bat echolocations, and SPECT'R<sup>®</sup>, which required us to manually identify the species by looking at a graph of each call. We found that from the mobile surveys there is a significant relationship between big brown bats (*Eptesicus fuscus*) and the planted white pine (*Pinus strobus*) forests as well as red bats (Lasiurus borealis) and pitch pine (Pinus rigida)/mixed oak- heath forest. For other species and the static surveys, there was insufficient data to make a conclusion. This knowledge can be used to protect the areas that will be most beneficial to bat conservation, and the data can be used to continue the long term studies conducted at BNL to determine the effects WNS has on local bat populations.

#### Introduction

Many bat species are experiencing severe population declines. A number of factors have contributed to these trends, including urbanization, deforestation, and more recently, white nose syndrome (WNS), a disease caused by the fungus *Pseudogymnoascus destructans*. In particular, the northern long-eared bat (*Myotis septentrionalis*), endemic to the northeastern United States and the Long Island area, has recently been listed as federally threatened. With so many issues forcing bat populations to critical levels, implementing appropriate conservation techniques is becoming increasingly paramount. There is a general consensus that bat conservation is best applied at the local level through local governments and wildlife organizations (Berkes 2007). Therefore, it is important to conduct research at the local scale to monitor populations and

species' preferred habitats so organizations can implement strategies to preserve and/or increase population sizes. When the preference of habitat by bats on Long Island is better understood, implementing local conservation strategies to protect those areas will be an easier endeavor.

With the recent outbreak of WNS in North America, there has been a significant amount of literature published about bat ecology, especially factors that have led to their decline. Many different factors have been documented including exposure to pesticides, loss or change in habitat, and interruption of hibernation (Menzel *et al.*, 2002). There has also been work done to document the urban factors that may influence bat populations like ultrasonic acoustic clutter and how it influences bat foraging behavior (Arlettaz *et al.*, 2001). A study conducted in West Virginia showed that northern long-eared bats prefer black locust (*Robinia pseudoacacia*) trees for their maternity roosts in Allegheny hardwood–northern hardwood forests (Menzel *et al.*, 2002). However, more research is needed on the types of habitats bats frequent on Long Island in order to better conserve all bat species.

The purpose of this study was to conduct bat acoustic surveys in locations where the vegetation, forest structure, and location of water sources have been well characterized to identify possible preferences in habitat. Bats, in general, can be hard to survey because of their small size and nocturnal activity pattern. Conveniently, acoustic survey techniques have proven to be valuable in determining the presence or absence of bat species in different areas as opposed to capture techniques, such as mist-netting (O'Farrell et al., 1999). Furthermore, acoustic techniques require much less labor to achieve the same level of accuracy as capture techniques. Acoustic surveys allow for a more complete sample of the species present in an area but cannot gauge population size reliably (Lacki *et al*, 2007). However, when surveys are taken at multiple locations, the results can be used comparatively to determine bat habitat preference.

In 2001, the forest composition and location of groundwater was surveyed at Brookhaven National Laboratory (BNL), where this study was conducted. The overall lack of urbanization, variety of forest types on the site and the effort placed into the vegetation surveys make BNL an ideal location for acoustic surveys. In this study, we used acoustic surveys as indicators of bat presence in an attempt to define areas which should be targeted for more focused management, with an emphasis on the recently threatened northern long-eared bat.

#### **Materials and Methods**

#### Study Site

This study was conducted in the forests of BNL. The sites surveyed were categorized as one of the following forest compositions: pitch pine (*Pinus rigida*)/ white oak (*Quercus alba*) forest, pitch pine/mixed oak/heath forest, planted white pine (*Pinus strobus*) forest, scarlet oak (*Quercus coccinea*)/heath forest, successional forest, and red maple (*Acer rubrum*)/scarlet oak/ mesic heath forest. The planted white pine forest present at BNL was planted by the Civilian Conservation Corps in the 1930s, and is not a natural occurrence. Data was collected between June 15th and July 23 during nights of low precipitation and wind,

Bat calls were recorded by two methods. To focus on smaller areas for a longer period of time, acoustic equipment was deployed in a number of microhabitats around the BNL property. *Static Surveys* 

Static Surveys were completed using a Song Meter SM2BAT+<sup>®</sup> detector made by Wildlife Acoustics<sup>®</sup>. Two Wildlife Acoustics<sup>®</sup> SMX-US ultrasonic microphones were attached to each detector and raised to approximately 3.66 meters above ground in order to maximize the odds of detecting a call. The detectors were set to stereo, allowing for two microphones at each site. Microphones were placed facing in opposite directions, increasing the effective range of the

detector. The height of the microphones placed them closer to the natural flight height of bats (Figure 6). When possible, microphones were placed at least 1.5 meters from any leafy vegetation to avoid noise due to leaf movement. Detectors were placed at predetermined locations for a minimum of four nights with little precipitation and low wind speeds (recording nights were postponed in the event of inclement weather in order to protect equipment). The detectors were programmed to begin recording approximately half an hour after sunset in 10 minute on/off intervals until sunrise. Survey locations were chosen in such a way as to adequately represent the habitat diversity and spatial area of undeveloped areas of BNL property. Within relatively unfragmented tracts of forest, chosen microhabitats were biased to preferred bat feeding areas, such as near bodies of water, in an attempt to maximize the number of calls. It has been shown that bat activity is significantly higher over rivers and lakes than other land types (Vaughan et al., 1997). Microphones were calibrated weekly for sensitivity. GPS locations at each site were recorded using a Garmin eTrex<sup>®</sup> handheld device.

#### Mobile Surveys

Mobile surveys were recorded using an AR125 <sup>®</sup> Ultrasonic Receiver made by Binary Acoustic Technology<sup>®</sup> attached to the top of a vehicle and Spectral Tuning and Recording Software (SPECTR III<sup>®</sup>) on a laptop computer. Surveys occurred along 4 predetermined routes approximately 3.2 kilometers long (See Figures 2-5). Routes were chosen to represent as much of the forest habitat diversity of BNL as possible. Routes were conducted between 2100 and 2230 each night, when bat feeding activity has been shown to be high (Anthony et al., 1981). Unlike the static surveys, recordings were taken on a continual basis for the duration of the survey. The vehicle speed was maintained between 5 and 10 miles per hour in order to detect as many bats as possible along the route. Temperature, wind direction and speed, relative humidity, cloud cover, and dew point were recorded at the start and end of each survey. Survey nights were postponed during high precipitation and winds to protect equipment and because it has been shown that bats are less active during heavy precipitation, theoretically because of thermoregulation problems caused by wet fur (Fenton et al., 1977).

#### Species Determination

Once the echolocation files were gathered from the static and mobile acoustic surveys the species that made each call was determined using two different programs. SonoBat<sup>®</sup> is a program that automatically determines the species that made the call using full spectrum acoustic analysis and comparing calls to a reference library. The results from this program were entered into a Microsoft<sup>®</sup> Excel<sup>®</sup> spreadsheet. The species were then determined again using Snapshot Characterization and Analysis Routine (SCANR<sup>®</sup>) software, which requires the user to determine the species manually by looking at the minimum frequency and characteristic slope in octaves per second (Sc). A combination of both programs reduced the number of calls that were indeterminate. Each call was linked to a GPS coordinate and data entered in a spreadsheet for later import into a geographic information system (GIS). ESRI<sup>®</sup> ArcGIS<sup>®</sup> shapefiles were then created for the mobile routes and static surveys to document the geographic locations of the calls. *Forest Composition Determination* 

Using the most recent map of forest composition for BNL and the GPS coordinates of each bat call from the mobile surveys; we associated the location of each call with a forest type. In  $\operatorname{ArcMap}^{\mathsf{TM}}$ , points were generated to represent each call. A 30 meter radius buffer was created around each point to represent the effective range of the detector (See figure 1). The vegetation type with the greatest coverage within this buffer was designated the vegetation type for that call. This vegetation data was similarly used to analyze the data from the static surveys. Our protocol

suggests that one detector active for 4 nights will obtain an accurate sample size for the immediate 100 acres (USFWS 2015). Because of this, each static survey location was represented by a point with a 100–acre buffer which allowed us to analyze the forest composition of each location relative to the number of calls that occurred over the duration of the survey.

#### Results

#### Mobile Surveys

The mobile surveys yielded a total of 129 bat calls, 96.1% of which were positively identified to species. Of all 129, 35.7% came from within pitch pine/white oak forest, 12.4% within pitch pine/mixed oak/heath forest, 33.3% within planted white pine forest, 13.2% within scarlet oak heath forest, 4.7% in successional forest, and 0.8% in red maple/scarlet oak/ mesic heath forest (See Table 1). None of the data was found to be normally distributed so a non-parametric Kruskal-Wallis test was performed on this data for each species using Minitab<sup>®</sup> to determine if forest type is a significant factor in the distribution of bats. When adjusted for ties, the p-values of the tests for big brown bat (*Eptesicus fuscus*) and red bat (*Lasiurus borealis*) were less than .05 and therefore statistically significant. Big brown bats were significantly more likely to be found in planted white pine forest (p-value = .006) and red bats were significantly more likely to be found in pitch pine/ mixed oak-heath forest (p-value = .027) than the other forest types (See Appendix A).

#### Static Surveys

The static surveys yielded a total of 407 bat calls, 66.8% of which were positively identified to species. Of the 407, 28.0% came from within pitch pine/white oak forest, 12.5% from pitch pine/mixed oak/heath forest, 0.0% within planted white pine forest, 57.7% from a scarlet oak/heath forest, and 1.7% from disturbed areas (See Table 2). For the sake of

consistency, a Kruskal-Wallis test, similar to that conducted for the mobile surveys, was performed using Minitab<sup>®</sup> on the static survey data. No p-values were less than .05 indicating that there was no significant relationship between species presence and forest type. See Appendix B for the resulting p-values from all of the Kruskal-Wallis tests.

#### Discussion

Our study was the first to utilize the Wildlife Acoustics detectors on BNL property for forest surveys, and will be contributing to a long term study of the area using similar methods. For this reason, there was a fair amount of trial and error in our methods, particularly in survey location choice. Typically with acoustic surveys, site locations are biased towards highly populated areas. Because these surveys have not been conducted previously, we instead surveyed as much of the BNL property as possible, resulting in our sampling areas with both high and low population potential.

Based on our mobile surveys and Kruskal-Wallis test, the bats whose foraging preference is affected by forest composition are the big brown bat and the red bat. The frequency of calls of big brown bat was particularly high in the white pine forest, while those of red bat was highest in pitch pine/mixed oak-heath forests. However, these results contradict those of the static surveys, which suggest that the locations where bat species are likely to be found are not necessarily influenced by forest composition. Some possible explanations for this are:

1. Certain forest types were not adequately represented in the static surveys. While we attempted to survey as much of the BNL property as possible, not all locations we surveyed with the same precision. In particular, the white pine forests only had one survey location. This site yielded no calls after 4 nights, and our protocol suggested that

we abandon the surrounding area, as this low number indicates that the area is not ideal for further study in the immediate 100 acres (USFWS 2015).

2. While our methods can indicate the potential importance of forest composition (or lack thereof) on bat populations, it does not indicate how features within these forests affect bat populations. Many features of forests can influence bat populations, such as wetlands and flyways, and their presence is independent of the forest composition. On the western boundary, there was a very high concentration of calls from the big brown bat in the white pine forest during mobile surveys. This area is located near recharge basins and a natural wetland off site, which may explain the bias towards that area. The Kruskal-Wallis test shows significant preference for the white pine forest by the big brown bat, but this significance may be coincidental due to the other factors present. Human activities can also encourage bat foraging, which our study does not consider. Insects are attracted to light sources (which are also present near the western boundary in the form of highway lighting) which, in turn, attract bats. Also, insects are drawn to wetlands, regardless of whether they are natural or artificial. The recharge basins on the western boundary may encourage bat activity just as if they were a natural wetland.

We believe that immediate conservation priority should be given to the microhabitats that other studies have shown to have higher bat populations rather than a specific forest composition. In particular, natural and artificial wetlands, as we see on the western boundary, have been determined to be important foraging locations of bats (Sirami 2013). This is not to say, however, that the forest composition is not important in bat conservation. We can see in our data that oak and pine forests are still utilized as foraging areas even when a wetland microhabitat is not present, which suggests that they may be utilized as roosting sites, which are also sites of conservation interest (Fenton 1997). In particular, late stage forests and dead trees, which are also present at BNL, have been shown to be suitable roosting sites for bats (Lacki *et al* 2007). In 2012, a wildfire burned through 200 acres of BNL property (See Figure 1). This area has still been used by bats for foraging, and the wildfire has provided a significant amount of roosting areas for bats on site.

These ideas suggest that conservation of bats should focus on maintaining diversity of habitats, rather than focusing on one area in particular (Fenton 1997). Habitat and foraging preference of bats is determined primarily by a species' specific ecology (Sirami 2013). Generalizations of habitat preference, and, by extension, conservation, become very difficult when focusing on many species. We believe that conservation efforts at BNL should encourage the maintenance of its diversity of habitats. Multiple habitats will increase the number of food sources available to the species on site (Fenton 1997). Because we know there are northern long-eared bats on site, more research should be done to learn more about their ecology specifically in order to ensure BNL doesn't have any negative impacts on the species.

Throughout the process of conducting the mobile and static surveys we experienced issues that should be addressed in the future. For the mobile surveys, the relatively short range of the microphone (30m) and the vehicle holding it being restricted to roads limits the area where bats can be detected. Also, at this time there is no sure way to distinguish manually between the calls of the silver-haired bat (*Lasionycteris noctivagans*) and the big brown bat; we had to rely on Sonobat<sup>®</sup> to differentiate between them. Further research into their calls may be beneficial so they can be manually distinguished easily. For the static surveys, there was a lot of background static in the calls from the surrounding foliage, cars, and machinery which influenced Sonobat's<sup>®</sup>

ability to identify the calls. We think the high amount of static also caused the complications we experienced with the program SCANR<sup>®</sup> where some of the calls would not appear, but they had appeared in Sonobat<sup>®</sup>. When placing the static detectors in the future, more effort should be made to ensure they are placed to minimize background noise. An experiment could be done in the future to compare the static and mobile detectors to see if one is better at filtering out the background noise or if it is just detector placement that influences the amount of background noise. Since this was the first time the static detectors have been deployed in forested habitats on the BNL property, the detectors were scattered around the entire property and might not have been placed in the best locations to adequately represent each forest type. Our data, along with more targeted detector placement, should be used to select high population areas for further studies at BNL.

#### Acknowledgements

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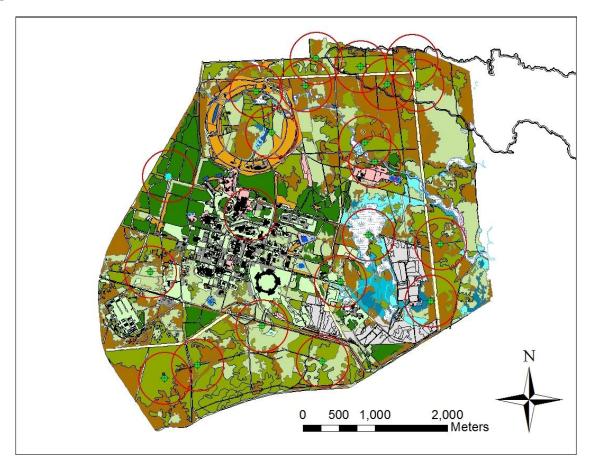
## Table 1: Mobile Survey Calls by Species

Mobile	Epfu	Labo	Laci	Lano	Unknown	Total	Percent of Total
Pitch Pine/ White Oak Forest	29	11	4	1	1	46	35.70%
Pitch Pine/ Mixed Oak/ Heath Forest	13	0	0	2	1	16	12.40%
Planted White Pine Forest	38	1	1	1	2	43	33.30%
Scarlet Oak Heath Forest	14	2	0	0	1	17	13.20%
Red Maple/ Scarlet Oak/ Mesic Heath Forest	1	0	0	0	0	1	0.80%
Successional	5	1	0	0	0	6	4.70%
						129	

## Table 2: Static Survey Calls by Species

Static	Epfu	Labo	Laci	Myse	Lano	Unknown	Total	Percent of Total
Pitch Pine/ White Oak Forest	53	12	5	3	9	32	114	28.00%
Pitch Pine/ Mixed Oak/ Heath Forest	9	0	4	1	3	34	51	12.50%
Planted White Pine Forest	0	0	0	0	0	0	0	0.00%
Scarlet Oak Heath Forest	113	19	6	23	8	66	235	57.70%
Disturbed	1	1	1	0	1	. 3	7	1.70%
							407	

## Figure 1: Static Detector Placement, Burned Area Outlined in Black



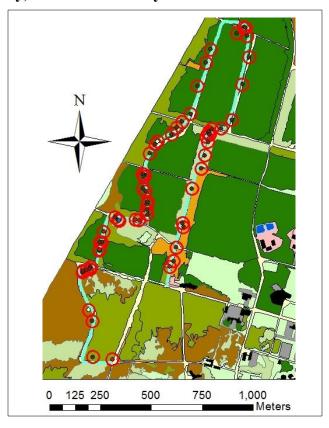


Figure 2: Mobile Survey, Western Boundary Path

Figure 3: Mobile Survey, Middle Path

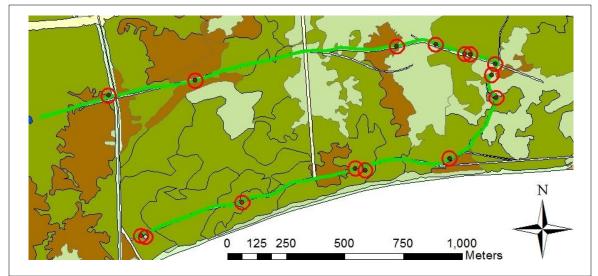


Figure 4: Mobile Survey, 860 Path

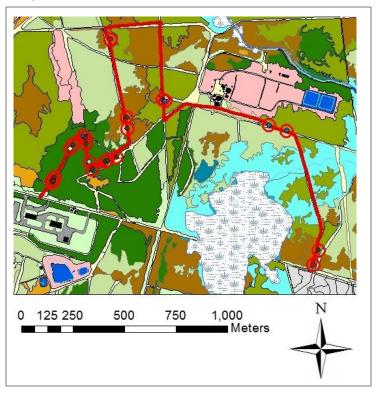
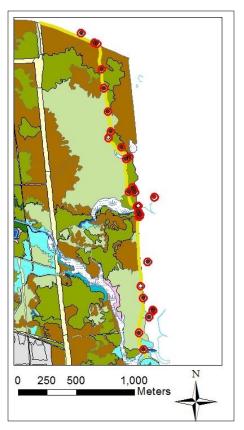
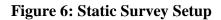
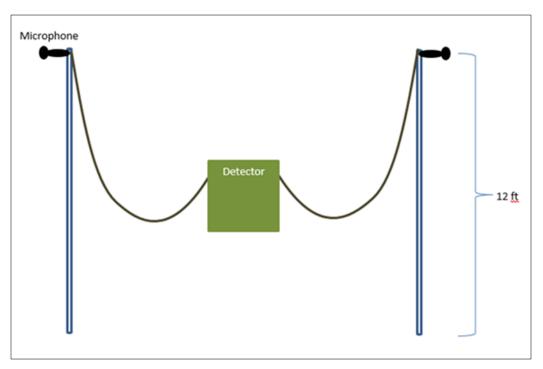


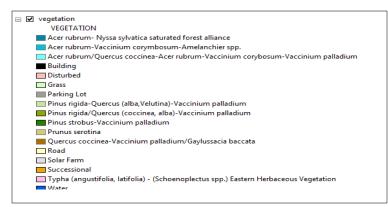
Figure 5: Mobile Survey, Z-Path







## **Figure 7: Forest Type Key**



**Figure 8: Species Key** 



# Appendix A: Kruskal-Wallis Tests – Mobile

Kruskal-W	/allis Test: E	Epfu versu	s Forest T	уре
Kruskal-W	/allis Test o	n Epfu		
Forest Typ	be N Me	edian Ave	Rank Z	
A 8	0.00000000	0 12.3 -	2.88	
B 10	2.0000000	0 34.1	1.78	
C 10	1.5000000	00 25.9	-0.14	
D 7	3.00000000	0 39.5	2.44	
E 10	1.00000000	0 26.6	0.01	
F 7	0.00000000	0 19.6 -	1.30	
Overall	52	26.5		
H = 16.19	DF = 5P = 0	0.006		

Kruskal-Wallis Test: Labo versus Forest Type
Kruskal-Wallis Test on Labo
Forest Type N Median Ave Rank Z
A 8 0.00000000 22.5 -0.81
B 10 0.50000000 35.6 2.12
C 10 0.00000000 22.5 -0.93
D 7 0.00000000 26.0 -0.09
E 10 0.00000000 25.3 -0.29
F 7 0.00000000 26.0 -0.09
Overall 52 26.5
H = 4.98 DF = 5 P = 0.418
H = 12.67 DF = 5 P = 0.027 (adjusted for ties)

Kruskal-W	/allis Test: Unknown versus Forest Type
Kruskal-W	/allis Test on Unknown
Forest lyp	oe N Median Ave Rank Z
A 8	0.00000000 24.5 -0.41
B 10	0.00000000 27.1 0.14
C 10	0.00000000 27.1 0.14
D 7	0.00000000 28.2 0.32
E 10	0.00000000 27.1 0.14
F 7	0.00000000 24.5 -0.38
Overall	52 26.5
H=0.40 D	PF = 5 P = 0.995
H = 1.87 D	PF = 5 P = 0.867 (adjusted for ties)

Kruskal-W	/allis Test: I	Lano versu	us Forest	Туре
Kruskal-W	/allis Test o	on Lano		
Forest Typ	e N Me	edian Ave	Rank Z	Ζ
A 8	0.00000000	0 24.5 -	0.41	
B 10	0.0000000	00 27.1	0.14	
C 10	0.0000000	00 29.7	0.74	
D 7	0.00000000	0 28.2	0.32	
E 10	0.0000000	24.5	-0.46	
F 7	0.0000000	0 24.5 -	0.38	
Overall	52	26.5		
H=0.99 D	F = 5 P = 0.	964		

Kruskal-Wallis Test: Laci versus Forest Type
Kruskal-Wallis Test on Laci
Forest Type N Median Ave Rank Z
A 8 0.00000000 25.0 -0.30
B 10 0.00000000 30.3 0.87
C 10 0.00000000 25.0 -0.35
D 7 0.00000000 28.6 0.40
E 10 0.00000000 25.0 -0.35
F 7 0.00000000 25.0 -0.28
Overall 52 26.5
H = 1.10 DF = 5 P = 0.955
H = 6.71 DF = 5 P = 0.243 (adjusted for ties)

# Appendix B: Kruskal-Wallis Tests – Static

Kruskal	-W	/allis 1	Test:	Epfu	versu	is Fore	st Ty	pe	
Kruskal	-W	/allis 1	Fest o	on Ep	ofu				
Forest 1	Гур	be N	Μ	edia	n Ave	Rank	Z		
F1	1	1.000	0000	00	7.5 -	0.58			
F2	7	6.000	0000	00	11.8	0.41			
F3	1	0.000	0000	00	3.5 -	1.24			
F4	4	2.000	0000	00	8.6 -	0.85			
F5	8	9.000	0000	00	12.9	1.09			
Overall		21		11.	0				
H = 3.21	. C	)F = 4	P = 0	.524					
H = 3.29	) [	)F = 4	P = 0	.510	(adju	sted fo	or tie	s)	

Kruskal	-W	/allis Te	est: L	abo	versi	us Fore	st Ty	pe	
Kruskal	-W	/allis Te	estoi	n La	bo				
Forest	Тур	be N	Me	dia	n Ave	e Rank	Z		
F1	1	1.0000	0000	0	17.0	0.99			
F2	7	0.0000	0000	0	12.0	0.52			
F3	1	0.0000	0000	0	7.5 -	0.58			
F4	4	0.0000	0000	0	7.5 -	1.25			
F5	8	0.0000	0000	0	11.6	0.33			
Overall		21		11.	0				
H = 2.77	7 0	)F=4 P	= 0.5	596					
H = 4.01		)F=4 P	= 0.4	405	(adju	sted fo	ortie	s)	

Kruskal	-W	allis T	Test:	Mys	e vers	us For	est T	ype	
Kruskal	-W	/allis <sup>-</sup>	Fest c	on M	yse				
Forest 7	Гур	e N	Μ	edia	n Ave	e Rank	Ζ		
F1	1	0.000	0000	00	8.0 -	0.50			
F2	7	0.000	0000	00	9.6 -	0.71			
F3	1	1.000	0000	00	17.0	0.99			
F4	4	0.000	0000	00	10.3	-0.27			
F5	8	0.000	0000	00	12.2	0.69			
Overall		21		11.	.0				
H = 1.86	5 D	F = 4	P = 0	762					
H = 2.93	D	F = 4	P = 0.	.570	(adju	sted fo	ortie	s)	

Kruskal	-Wallis Test: Lano versus Forest Type
Kruskal	-Wallis Test on Lano
Forest T	ype N Median Ave Rank Z
F1	1 1.00000000 13.5 0.41
F2	7 1.00000000 12.3 0.67
F3	1 0.00000000 6.0 -0.83
F4	4 0.50000000 10.6 -0.13
F5	8 0.00000000 10.4 -0.36
Overall	21 11.0
H = 1.21	DF = 4 P = 0.877
H = 1.43	DF = 4 P = 0.840 (adjusted for ties)

Kruskal	-Wallis T	est: Laci	versu	s Fores	t Typ	е
Kruskal	-Wallis T	est on La	aci			
Forest 1	Type N	Media	an Ave	e Rank	Z	
F1	1 1.0000	00000	16.0	0.83		
F2	7 0.0000	00000	11.6	0.30		
F3	1 0.0000	000000	7.5 ·	-0.58		
F4	4 0.0000	00000	10.8	-0.09		
F5	8 0.000	00000	10.4	-0.33		
Overall	21	11	.0			
H = 1.10	DF=4 F	e = 0.894				
H = 1.57	DF=4 F	P = 0.815	(adju	isted fo	rties	s)

Kruskal	-W	allis Te	st: I	Unkr	nown	versus	Fore	est Type	
Kruskal-Wallis Test on Unknown									
Forest 1	Тур	e N	M	edia	n Ave	e Rank	Z		-
F1	1	3.00000	0000	00	10.5	-0.08			
F2	7	4.00000	0000	00	11.7	0.37			
F3	1	0.0000	0000	00	3.0 ·	-1.32			
F4	4	6.50000	0000	00	11.0	0.00			
F5	8	3.00000	0000	00	11.4	0.25			
Overall		21		11.	0				
H = 1.80	ם (	F=4 P	= 0	772					
H = 1.83 DF = 4 P = 0.766 (adjusted for ties)									

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