The effect of rain noise on roost emergence in Neotropical bats

Bachelor Thesis

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Preface

Frame of the assignment

The thesis and colloquium is the final part of the study Forest and Nature Management. It is one of the assessment requirements in order to obtain the bachelor diploma of this university. By implementing the thesis research, the candidate student demonstrates that he / she is able to function successfully in the professional domain for which he / she has been educated. He / she demonstrates to be able to formulate and analyze a problem, to formulate research questions, to identify suitable methods, collect information and data, analyze, order and interpret these, and – finally – to find a useful solution to the identified problem. The thesis assignment is implemented during a continuous period of half a year (gross). It is concluded with writing a report (the thesis), presenting this to the assessors (in a colloquium) and defending or explaining it in a criterion-based interview.

Experiences

During the process of doing this thesis I discovered how much I learned during my education and I that I am capable of working independently. I found out where my interests lie and where not. Although the data processing sometimes was mind numbing and the writing tedious, I enjoy the whole project very much.

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Abstract

This report is the result of a study done at the Smithsonian Tropical Research Institute, Panama. It is part of a bachelor thesis of the Forest- and Nature Management – Tropical Forestry Program at Van Hall Larenstein University of Applied Sciences, The Netherlands. This study is the first in a series of experiments on the effect of rain on tropical bats. Little is known about how bats are affected by rain. One fifth of all bat species are threatened. As increased global warming causes weather patterns to change, tropical regions will receive more precipitation. To establish adequate conservation programs the results of this series of studies could provide vital information. The aim of this preliminary study is to learn whether bats recognize rain noise, if this influences their roost emergence behavior, and whether it influences species differently. To test this, a speaker, capable of playing ultrasonic as well as sonic frequencies was placed in front of roost entrances of two different bat species. With this speaker the noise of rain and ambient noise, absent of rain, was played back on separate nights. The emergence behavior of the bats was recorded with a camera with a night-function. The videos were analyzed with event recording software. The results show that bats do delay their emergence when they are exposed to the noise of rain, while they do not delay their flight when exposed to ambient noise, absent of rain. The two study species did not show a difference in delay. This demonstrates that bats recognize the noise of rain and use this to evaluate their environment.

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1. Introduction

When rain hits the forest floor and the tree canopy, it creates acoustic noise that could distract foraging bats, mask their echolocation calls, or mask useful sounds of their prey. These perceptual challenges that rain introduces to bats could greatly influence daily tasks, such as hunting for food. However, as not much is known about how bats are affected by rain, it is difficult to predict how climate change will influence future bat populations. As increased global warming changes weather patterns, tropical regions will likely receive more precipitation [1-3]. This will likely have a big effect on tropical bat species, and understanding which conservation programs will be adequate to mitigate these effects is of the utmost importance.

This study on rain noise is the first, in a series of experiments, designed to understand how bats perceive rain, how it influences their hunting success, and how it affects species with different foraging strategies differently. The results of these studies could provide fundamental information for future conservation efforts. The aim of this preliminary study is to learn whether bats recognize rain noise, if this influences their roost emergence behavior, and whether it influences species differently.

1.1 Noise

"Noise (…) is anything influencing a receiver's receptors other than a signal of interest." [4]Virtually all animals, irrespective of their sensory system, experience noise. So do animals that rely on acoustic signals. Sound produced by other organisms (e.g., birds, frogs and insects), the abiotic environment (e.g., rain, wind, and creeks), or an anthropogenic source (e.g., traffic and compressor stations) can mask acoustic signals of interest and therefore be experienced as noise.

To avoid interference of noise, many species are able to adapt their behavior in these situations. Male nightingales (*Luscinia megarhynchos*), zebra finches (*Taeniopygia guttat*), blue-throated hummingbirds (*Lampornis clemenciae*) and cotton-Top Tamarins (*Saguinus Oedipus*) increase the amplitude of their acoustic communication signals when subjected to noise [5-8].

A major source of noise is rainfall. Rain is a frequently occurring phenomenon in almost all climates. Animals have adapted to rain noise in various ways. Males of the Chiloe Island ground frog (*Eupsophus calcaratus)* increase the rate and duration of their mating calls when exposed to rain noise [9], while tawny owls (*Strix aluco*) stop vocal activity during heavy rain [10].

1.2 Bats and Noise

An order of animals that relies heavily on sound are bats (*Chiroptera*). Bats are highly diverse and found in almost all terrestrial habitats [11]. They exploit many different food sources and have adapted their behaviour, sensory-, and motor systems in various ways [11, 12]. Bats do not use acoustic signals only for communication [13], but mainly use them for spatial orientation and hunting through echolocation [14]. Therefore, acoustic noise could form greater challenges for bats than for many other animals that primarily rely on other sensory systems for orientation and foraging.

Lab experiments have shown that gleaning bats avoid loud noise (traffic, vegetation and digitally generated broadband), and their hunting success decreases in these noisy environment [15]. Besides avoiding noise, bats can also reduce the masking effect of noise on their echolocation calls by altering their acoustic behavior. For example, the Mexican free-tailed bat (*Tadarida brasiliensis*) lengthens the duration of its echolocation calls when exposed to the anthropogenic noise of compressor stations in New Mexico, US. [16].

1.3 Bats and Rain

It has been shown that rain causes bats to delay their emergence, which otherwise is strongly correlated with the sunset [17, 18]. It has also been observed that bat activity is reduced on rainy nights [19, 20].

One apparent reason for bats to avoid rain is that bats with wet fur suffer increased metabolic costs [21]. Additional reasons might be that rain-generated noise masks echoes of prey sounds or their environment. For example, the frequency range of rain noise strongly overlaps with the frequency range of insect and frog mating calls. Thus, signals from potential prey might be masked by the rain noise and not perceivable to bats that use prey signals during foraging (passive hunters). The frequency range of rain noise also strongly overlaps with the frequency range of most bat echolocation calls and thus rain noise could mask echoes of the environment. This would reduce the bats' spatial orientation. Furthermore, raindrops might act as acoustic clutter, which produces echo noise. When bats echolocate, the raindrops create additional echoes that might overlap or mask the echoes from objects of interest, e. g. prey or other small objects. This would also hinder the bats' spatial orientation and the detection of small insects. Finally, insectivorous bats may avoid rain because prey might be less available when it rains. Dechmann [22] mentions that insect swarms are dispersed by rain and Koskimies [23] (in Potter, 2015)[24] says that rain belts contain fewer aerial insects. However, Chase [25]observed that rain showers are often accompanied or followed by brief blooms of insects.

1.4 Study Species

Bats with different hunting strategies might encounter different challenges when hunting during a heavy rainfall. The common big eared bat, *Micronycteris microtis*, is a small leaf-nosed bat (*Phyllostomidae*) that mainly feeds on large insects [26] and occasionally on small vertebrates [27], which rest on the vegetation in the dense understory of the tropical forest. This species hovers over vegetation and picks up prey from a leaf [28], therefore it is classified as a gleaning bat [11]. It uses only echolocation for the detection, classification and accurate localisation of its silent and motionless prey in a highly cluttered environment [28] and thus is also defined as an active gleaning hunter [11].

Molossus sp. are free-tailed bats (*Molossidae*) that use echolocation to hunt flying insects in open space [29-31]. They forage for a relatively short time period and emerge from their roost around sunset and sometimes again around sunrise [22, 25, 29].

As both species rely on echolocation for hunting, their hunting success might be strongly influenced by heavy rain. The impact of raindrops on the foliage of the forest could produce noise that masks the echolocation calls of *M. microtis*. Also, the additional clutter, formed by raindrops, could reduce their ability to detect prey. *Molossus* sp. normally does not encounter clutter in open space, therefore the sudden clutter formed by raindrops should interfere heavily with their ability to detect small insects. In open space, *Molossus* sp. is also entirely exposed to the rain, which should increase their metabolic rate considerably.

1.5 Research Questions

As bats delay their emergence and activity for various reasons when it rains, how do they make these decisions? **Do bats use the noise of rain as a cue to delay their emergence?** This is the first research question. Because bats rely heavily on their acoustic sensory system for evaluating their environment, and because rain forms a great challenge to bats, I predict that they delay their flight until the rain noise has stopped.

Since bats are so diverse in habitat, food source exploitation, behavioural, sensory-, and motor systems, the second research question is: **do two bats with a different foraging strategy and habitat react differently to the noise of rainfall?** As the effect of rain differs with different environments (e.g. raindrops are fewer but larger in the understory of a forest) and different prey might react differently to rain, I predict that two bat species with different foraging strategies do react differently to the noise of rainfall.

2. Methods

Since relatively little is known about how bats are affected by rain, the first step is to study how bats experience rain when they are inside their roost. Do they decide to delay their emergence based on the cue of rain noise, and do two species with a different hunting strategy and habitat react differently to the noise of rain? To test this, playback experiments were conducted on natural roosts of two different bat species, *Micronycteris microtis* and *Molossus* sp.

2.1 Preparation

Audio file pairs, containing ambient noise and rain noise, were created. This was done using existing audio files and audio editing software. Each bat roost included in this study was located at least three days prior to the experiment. The species or genus was confirmed by a second observer. Then the roosts were prepared for the experiment and monitored for three days.

2.1.1 Sound file preparation

Ten audio recordings of heavy rain, preferably around sunset (≈18:30), were selected from an existing database created by Wouter Halfwerk. The database contained audio files of ambient sound that were recorded with an acoustic monitoring system (Song Meter SM2BAT, Wildlife Acoustics, Massachusetts) at twelve different sites along Pipeline Road, Soberania National Park, Panama in 2012-2013. See appendix 6.1 for the location of the recordings. Each audio recording of rain was paired with a audio recording of ambient sound, absent of rain, which was recorded at the same location around sunset within three days from the rain noise recording. All selected files covered the sonic as well as the ultrasonic frequency range. For an overview of the audio files see appendix 6.2.

Each original audio recording had a duration of 15 seconds. With audio editing software (Audacity 2.0.6) the original recordings were looped until they had a duration of 3 min. Hereafter the amplitude was normalized to −3.0 dB. Each pair of audio files was randomly assigned to a bat roost of each species. The playback of the audio was tested in an empty drainage tunnel. The audio files were played back at 80dB at the entrance of the tunnel and was measured with 72dB at 6.2 m inside the tunnel. See appendix 6.3 for a full description of the audio files tests.

2.1.2 Roost location

Micronycteris microtis

To locate *M. microtis* roosts, drainage pipes were examined before and along pipeline road, Soberania National Park. For the locations of the roosts see appendix 6.1. Around Gamboa, drainage pipes are known to be frequently used as roosting locations by different bats species (pers. com. Inga Geipel). The bats hang from the ceiling of these pipes and can be spotted easily. For an overview of the selected roosts see appendix 6.4.

Molossus sp.

To locate *Molossus* sp. roosts, residences in Gamboa were inspected. The houses were checked for possible entrance holes and feces. Hollows in buildings are common roosting sites for *Molossus* sp. For an overview of the selected roosts see appendix 6.4.

2.1.3 Roost preparation for playback experiments

In case of multiple entrances to a roost, all but one entrance were temporally blocked at least three days prior to the start of the experiments. Large entrances (*M. microtis*) were sealed using insect mesh and small entrances (*Molossus* sp.) were blocked with soft foam. The following three days, before noon, the roost was monitored and the number of bats was estimated each day. These observations continued during the days of the experiment. For an overview of the observations see appendix 6.5. Monitoring the number of *Molossus* sp. was not possible because roost entrances were too small to allow for accurate counting of individuals. *Molossus* sp. roosts were monitored occasionally using a video camera with infra-red light (IR) function (DCR-SR45, Sony, Japan) and IR-lights (HVL-IRM, Sony, Japan) to confirm that the bats were still occupying the roost.

2.2 Playback experiment

Between the 19th of April and the 29th of September 2015 playback experiments were conducted at 10 *M. microtis* and 10 *Molossus* sp. roosts. The experiments lasted three consecutive nights for each single roost. On the first night the baseline of the bat emergence behavior was established. The following two nights playback experiments were carried out. During one of these two nights the noise of heavy rainfall was played back, during the other night the sound of ambient noise was played back. The order of the audio files was randomized. If it rained during the experiment, it was canceled and continued on the first following rainless night.

2.2.1 Baseline

To record a baseline of the bat emergence behavior, a video camera with an IR-function (DCR-SR45, Sony, Japan) and two IR-lights (HVL-IRM, Sony, Japan) were mounted on a tripod. The tripod was placed either over the entrance of the roost with the camera facing down, or under the roost with the camera facing up, depending on the height of the roost entrance. A dummy speaker (≈14x14x14cm) was mounted on a tripod and placed more or less 1 m from the roost entrance. In cases where this was not possible, due to the height of the roost locations, the dummy speaker was placed as close to the roost entrance as possible and the distance was noted. For the exact distances between speaker and roost see appendix 6.4. The recording started approximately 30 minutes before sunset and ended approximately 90 minutes after sunset.

2.2.2 Playback

During the playback experiments the camera and the IR-lights were set up in the same way as during the baseline recordings. An ultrasonic speaker (ScanSpeak, Avisoft Bioacoustics, Germany) was mounted on a tripod and placed at the same position as the dummy speaker was during the baseline recording, with the front of the speaker directed towards the entrance of the roost. The speaker was connected to an amplifier (UltraSoundGate Player 116, Avisoft Bioacoustics, Germany) and the amplifier was connected to a laptop (Thinkpad T420, Lenovo, China). The sound files were played back at 80dB, measured at 10cm distance from the entrance of the tunnel, using software (RECORDER USGH, Avisoft Bioacoustics, Germany) compatible with the amplifier. The sound level was measured with a sound pressure level meter (Digital Sound Level Meter 33-2055, RadioShack, United States). Video recordings started approximately 30 minutes before sunset and ended approximately 90 minutes after sunset. Audio playbacks started approximately 30 minutes before sunset and stopped approximately 60 minutes after sunset. For the exact start and stop times of the playbacks see Appendix 6.4.

2.3 Video and statistical analysis

After recording a baseline or a playback experiment, the video files were transferred to a computer (Compaq, 6910p, Hewlett-Packard Company, Unites States) for analysis. The data was analyzed with event recording software (Solomon Coder 15.03.15). Every time a bat entered or emerged from the roost the event and the video time were documented with an accuracy of 1/20 seconds. During playback experiments the start and stop of the noise playbacks were recorded as well. The event recordings were exported as CSV-files. Then all the event recordings of a single roost were copied into a XLS-file. With spreadsheet software (Microsoft Excel 2010) the video time of the event recordings was converted into actual time. The actual time was converted into seconds after sunset (SASS).

One *M. microtis* roost and one *Molossus* sp. roost were excluded from statistical testing. At the *M. microtis* roost, too few bats emerged during the rain noise treatment to be compared to the baseline. The *Molossus* sp. had too few emerging bats during the entire experiment.

From the total number of bats that flew out during the baseline, the median was determined. This 'median bat' was used as a reference point for the following nights, regardless of the number of bats that emerged during those nights. The time of emergence of the 'median bat' was determined for each treatment. The value of the emergence time of the 'median bat' was labeled 'medianB'.

The effect of noise treatment on medianB was compared by using linear mixed models in statistical computing software (R, lme4 package). A null model was made with roost-ID as random factor. This model was compared to models, wherein species-ID (*Molossus* sp. or *M. microtis*), noise treatment and medianB were added as a fixed factor. Models were compared with a likelihood ratio test. Post-hoc independent contrast was used to follow up on any significant effect of noise treatment or the interactions between noise treatment and species identity.

Due to insufficient data on temperature, humidity, wind and cloudiness, these factors are present as noise in the data. after sunset

3. Results

To test whether the noise of rain has an effect on the emergence behavior of bats, the emergence behavior of bats from ten *M. microtis* and ten *Molossus* sp. roosts was observed and the roosts were submitted to noise playback experiments. One *M. microtis* roost and one *Molossus* sp. roost were excluded from the analysis due to insufficient numbers of individuals.

3.1 Emerging behavior

Micronycteris microtis generally emerged earlier than *Molossus* sp. during baseline observations without any sound playback. The average first *M. microtis* individual emerged −386 (±442) seconds after sunset (SaSS hereafter) and the average first *Molossus* sp. individual emerged 507 (±315) SaSS. The average median *M. microtis* emerged 215 (±376) SaSS and the average median *Molossus* sp. 1065 (±180) SaSS.

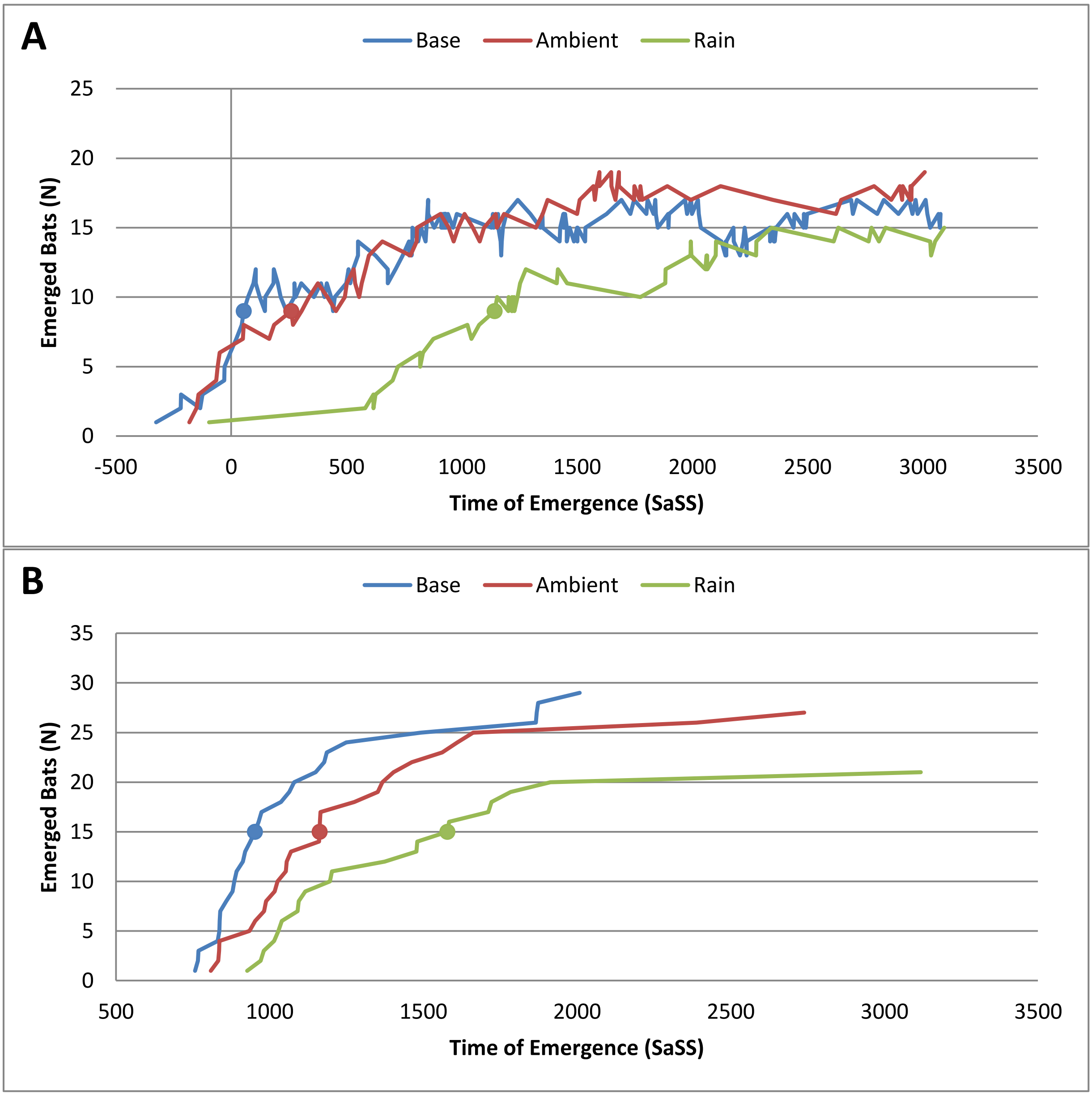

Fig. 1 Number of bats that emerged from the roost during the baseline treatment (blue), ambient noise treatment (red), and rain noise treatment (yellow) for one *M. microtis* roost: Roost-15 (A) and one *Molossus* sp. roost: Mol-03 (B). The large dots indicate the medianB bat for each treatment.

After emerging from the roost, *M. microtis* often made a short turn to immediately fly back into their roost. They sometimes make several of these 'examination-flights' before leaving the roost. This behavior was observed during all treatments, although it seemed more expressed during the baseline treatment. *Molossus* sp. did not display this behavior, after coming out of the roost entrance they left for an extended period of time. Examples of emergence patterns for both species are shown in **Error! Reference source not found.**. Emergence patterns for all roosts of both species can be found in appendix 6.5.

3.2 Effect of rain noise

To determine the effect of rain noise on emergence behavior of bats, the time of emergence of the median bat (medianB) was calculated and compared between the different noise treatments. Bats of both species delayed their emergence depending on noise treatment (GLMM, $X^2 = 38.71$, df = 2, $p < 0.0001$, n = 18). During the baseline treatment the average emerging time of the 'median bat' of both species was 640 (±517) SaSS, during the ambient noise treatment it was 705 (±553) SaSS and during the rain noise treatment the average emerging time was the 'median bat' was 1189 (±797) SaSS (**Error! Reference source not found.**).

Bats emerged later during the rain noise treatment when compared to the ambient noise treatment (post-hoc test, z-value = 4.80, p<0.0001) and the baseline treatment (post-hoc test, z-value = 5.20, p<0.0001). There was no difference in time of emergence between the nights of the ambient playback and baseline condition (t-test, $X^2 = 0.91$,df = 17, p = 0.11) see **Error! Reference source not found.**.

On average, during all treatments, *Molossus* sp. emerged 15.7 min later than *M. microtis* (GLMM, $X^2 = 33.15$, df = 1, $p < 0.0001$), but the two species did not react differently to the noise treatments (post-hoc test, $X^2 = 0.11$, df = 2, p = 0.95).

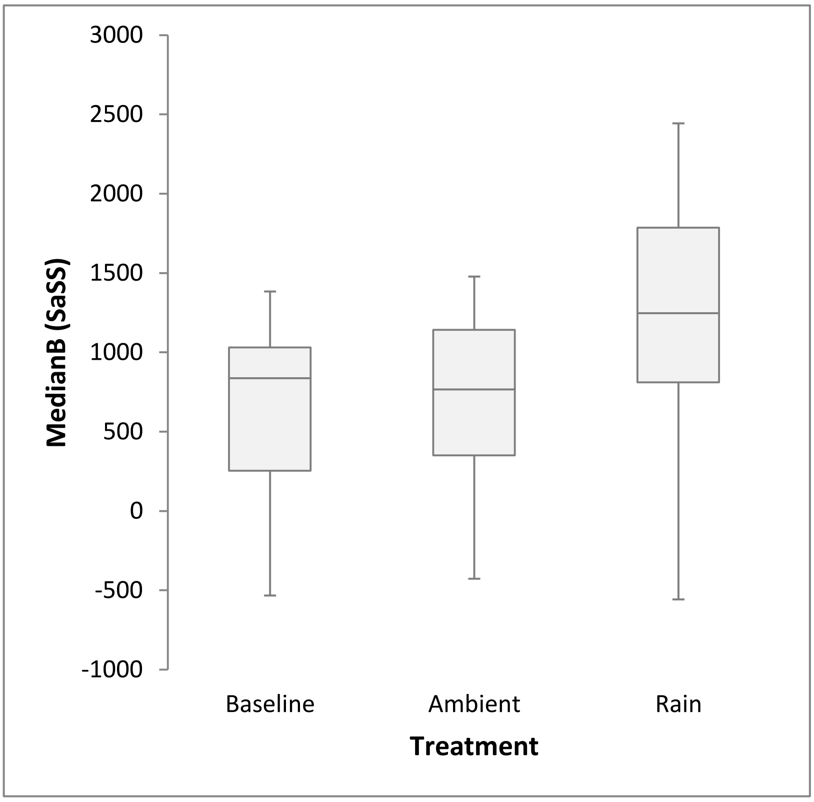

Fig. 2 Emerging times of the median bat during the experiments in seconds after sunset (SaSS) of both *M. microtis* and *Molossus* sp. for the three playback treatments.

4. Discussion:

Bats are very diverse in habitat and foraging strategies and rely heavily on their acoustic sensory system for orientation and food finding. Noise can mask important acoustic signals like echoes of their own echolocations calls. Rain is an important abiotic source of noise. Therefore I predicted that 1. bats use the noise of rain as cue to delay their flight and that 2. two bat species with different foraging strategies and habitat react differently to the noise of rain. This study confirms the first prediction. The bats did delay their emergence during the playback of rain noise, but they did not delay their flight during the playback of ambient noise, absent of rain noise. However, the second prediction was contradicted by the results of this study. The two bats species with a different hunting strategy and habitat did not react differently to the playback of rain noise. In other words, both species showed the same amount of delay in emergence during the playback of rain noise and did not delay their emergence during the playback of ambient noise, absent of rain.

4.1 Delay

There are several possible reasons for bats to delay their flight. First of all, it has been shown that when bats get wet, they suffer increased metabolic costs [21]. Secondly, rain-produced noise could mask bats' echolocation calls. For bats that rely on sounds generated by their prey to locate their prey, rain could also mask prey generated sounds. Further, rain drops produce additional echoes, this is known as acoustic clutter. For bat species that hunt small insects in open space, like *Molossus* sp., this clutter might make it impossible to distinguish raindrops from prey. For species like, *M. microtis*, that use echolocation to locate prey in an already cluttered environment, like the dense understory of the forest, additional clutter of rain might make it impossible to locate prey. Finally, prey availability might be a cause for bats to delay their flight. Although, contradicting statements can be found in literature, it is plausible that (at least certain) flying insects are less available during rain. [22-25].

In field experiments the bats did indeed delay their emergence during the playback of rain noise, while they emerged at a similar time during the ambient noise play back, as they did during the baseline. The rain noise and ambient noise where played back at the same intensity (80 dB). However, due to the reach of the sound pressure level meter, this intensity was measured in the 0-10 kHz range. In this range, the intensity of the ambient audio files is on average 8 dB louder than the intensity of the rain audio files. Therefore, to reach the same intensity in the 0-10 kHz range, the rain audio files were played back 8 dB louder than the ambient audio files. As a results, the intensity of the sound during the rain noise playback was around 10 dB higher in the 10-35 kHz range and around 6 dB higher in the 35-60 kHz range, than it was in during the ambient noise playback. The intensity was more or less the same in the 0-10 kHz and 60-140 kHz range (Fig. 3)

Molossus sp. echolocation calls are relatively low in frequency, 34-46 kHz [32, 33]. Therefore, this species could have experienced more masking of their echolocation calls during the rain noise playback than during the ambient noise playback, due to the higher intensity in the 35-60 kHz frequency range. This could be a reason why this *Molossus* sp. delayed emergence, besides recognizing the noise of rain. However, *M. microtis* has relatively high echolocation calls of 50-150 kHz [28, 34] and should therefore barely have experienced more masking, of their echolocation calls, during the rain noise playback than during the ambient noise playback. Still, both species show the same delay when exposed to rain noise playbacks.

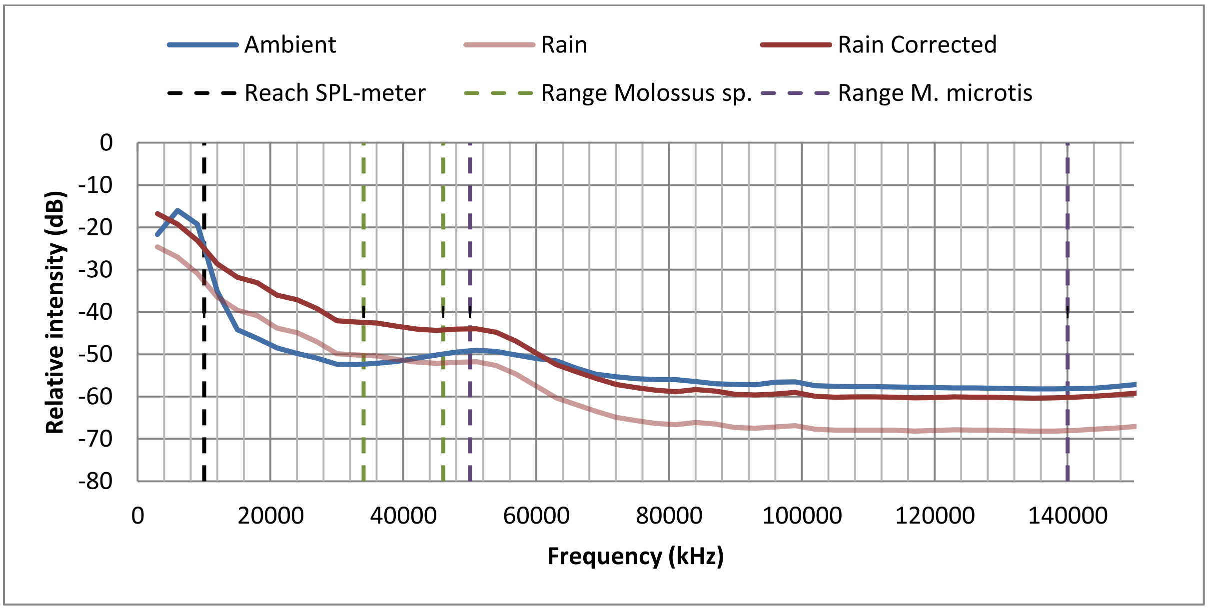

Fig. 3 Average intensity of all rain- (pink) and ambient (blue) audio files, the reach of the sound pressure level meter (black dotted line), call range of *Molossus* sp. (green dotted line) and call range of *M. microtis* (purple dotted line). The red line shows the expected intensity of the rain noise relative to the intensity of ambient noise during the actual playback.

4.2 Emergence

Although the bats delayed their emergence, they did not delay emergence until the rain noise had stopped. During the playback of rain noise, *Molossus* sp. emerged after delaying for 11.06 (±8.80) min. *Molossus* sp. only has two short foraging periods per night where they take in their required calories [25, 35] and since bats are small mammals they have a relatively high metabolic rate [36]. This means that, relative to larger mammals, they require many more calories to maintain their body temperature. Therefore, for *Molossus* sp., delaying emergence until rainfall has stopped could seem to be more costly than getting wet. Still, it is likely that this species can skip at least one night of foraging, without risking starvation. *Molossus* sp. go into a 'torpid' state [25, 37], which lowers their metabolic rate when resting [22]. Ekert [38] observed that *Molossus* sp. emerges considerably later or not at all during rainfall. Yet, Chase et al. [25] observed that the only instances that *Molossus* sp. emerges outside their regular foraging periods is during or immediately after light to moderate rain. Only during heavy rain they quickly returned to their roost. *Micronycteris microtis* also emerged, during the playback of rain noise, after delaying emergence for a relatively short time (7.25 (±8.63) min.). But in contrast to *Molossus* sp., they are active throughout the night, catching around 1.1 prey items per hour [26].

A possible reason why both bat species did not delay their emergence until the rain noise had stopped, is that they figured out that it was not actually raining. The directionality of the sound could have been a cue that the rain noise playback was not actual rain. Bats have strong directional hearing [39]. The playback of the rain noise came from one source (a speaker) and was therefore more or less directional, while actual rain noise is not. This might have been a cue for the bats that it was not actually raining. Still, it is doubtful that bats can hear the direction of the sound source when they are inside the roost, as the sound comes from outside the roost. Once the bats had emerged from the roost they would have been able to detect the directionality of the sound.

Besides the directionality of the sound, a lack of additional cues might have been the reason that the bats figured out that there was no actual rainfall. There was no sudden drop in temperature and increase in humidity, nor were there winds associated with actual rain. During all treatments *M. microtis* was observed to make 'examination flights' before foraging, that means, that they flew out and into the roost several times before leaving for an extended period of time. *Molossus* sp. emerged from the roost and did not return until much later, but has been observed by Chase [25] to emerge during rain and to quickly return to the roost during heavy rain. Possibly, both species emerge, after delaying for a short time, to inspect their environment. Since there was no actual rainfall during the playback experiments, there was no cause for the bats return or further delay emergence.

4.3 Conclusion

In conclusion, two bats species with a different foraging strategy and habitat (*Micronycteris microtis* and *Molossus* sp.) delayed emergence from their roost during playback of rain noise, but not during the playback of ambient noise, absent of rain. Increased metabolic rate and possibly masking of echolocation calls, additional clutter and decreased availability of prey are causes for bats to delay their flight. During the experiments the higher intensity of the sound pressure, in the 10 – 60 kHz range, during the playback of rain noise might have been a cause for *Molossus* sp. to delay their flight, but this does not explain why *M. microtis* showed the same response.

Even though the bats delayed their flight, they emerged from their roost before the rain noise had stopped. It is unlikely that *Molossus* sp. needs to emerge to take in enough calories to maintain its body temperature, because it has adaptions that reduce its metabolic costs while resting and it has been documented to skip foraging bouts. It is also doubtful that both species detected the directionality of the sound while inside the roost. However, the absence of additional cues, like a sudden drop in temperature and increase in humidity, might have been the reason why the bats emerged before the rain noise had stopped. Possibly, both species emerged from the roost to examine their environment. Once outside the roost the bats would have easily noticed that there was no actual rainfall and had no reason to return to the roost or to delay their flight any longer.

Although, it remains unknown what factors cause bats to be reluctant to forage during rain to what extent, the results show that bats are clearly able to discriminate between rain noise and ambient noise (absent of rain) and that they use rain noise as a cue to delay their flight. Further research is needed to understand specifically why bats are reluctant to leave their roost during rainfall and how it affects their hunting success.

4.4 Implication and application

One fifth of all bats species are threatened [40]. Increased global warming causes a change in weather patterns, which results in increased rainfall in tropical regions [1-3]. As rainfall forms a challenge for bats, an increase in rainfall might contribute to an increase in threatened bats species.

Another thing to consider is that traffic noise seems to be very similar to rain noise. It has already been shown that bats avoid traffic noise and that it decreases their hunting efficiently [15, 41], but it is unknown whether traffic noise causes bats to delay their emergence. If bats are indeed unable to discriminate between rain noise and traffic noise, traffic noise might cause them to delay their emergence. This will lead to a reduction in food intake, which will results in an overall decrease in vitality. If the on the other hand, bats could become habituated to traffic noise, they might emerge during rainfall and be exposed to the negative effects of rain .

To mitigate the negative effects of traffic noise on bats (and wildlife in general), these effects should be taken into consideration during infrastructure planning. Natural areas should be avoided as much as much as possible and the construction of wildlife crossings should be considered when making such plans. One type structure that seems to be beneficial for bats are underpass tunnels [42].

To alleviate pressure on the bat population in general, creation of artificial roosting structures are an option. All roosts, used in this study, were found in manmade structures (drainage pipes and residences). Drainage pipes are a very common roosting place for bats, even in the forest (personal observation). Specially designed bat boxes are also preferred roosting places in forests [43] and can be beneficial to bats [44, 45]. These kind of structures are relatively inexpensive and can easily be integrated in natural-, rural- and urban environments.

Besides providing roosting places for bats, creating awareness among the local people is important to reduce pressure on local bat populations. Bats that roost in or nearby residences are often exterminated or excluded, because people are concerned about stains, odors and diseases [46].

Although (some) bats might benefit from the measures mentioned above, they do not reduce the challenges that bats face due to increased rainfall. After all, it is increased global warming that results in a change of weather patterns and increased rainfall in the tropics. To tackle this problem, long term commitments, on an international, regional, and local levels must be made to reduce the emission of gasses and stop increased global warming.

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6. Appendix

6.1 Map of Study Area

6.2 Audio Files

Recording Site	Source Rain Audio File	Recording Date	Recording Time	Source Ambient Audio File	Recording Date	Recording Time	New Rain Audio File	New Ambient Audio File
ESP1	A-_20120728_191100	28-07-2012	19:11:00	A-_20120727_191200	27-07-2012	19:12:00	**ESP1_Rain**	**ESP1_Ambient**
ESP3	A-_20121225_235947	25-12-2012	23:59:47	A-_20121226_183800	26-12-2012	18:38:00	**ESP3_Rain**	**ESP3_Ambient**
ESP4	A-_20120824_190100	24-08-2012	19:01:00	A-_20120825_190100	25-08-2012	19:01:00	**ESP4_Rain**	**ESP4_Ambient**
ESP7	A-_20121006_183700	06-10-2012	18:37:00	A-_20121007_183600	07-10-2012	18:36:00	**ESP7_Rain**	**ESP7_Ambient**
ESP9	A-_20121111_192515	11-11-2012	19:25:15	A-_20121114_182500	14-11-2012	18:25:00	**ESP9_Rain**	**ESP9_Ambient**
ESP9	A-_20121113_182500	13-11-2012	18:25:00	A-_20121110_182500	10-11-2012	18:25:00	**ESP9_Rain2**	**ESP9_Ambient2**
ESP10	A-_20121205_230032	05-12-2012	23:00:32	A-_20121206_182900	06-12-2012	18:29:00	**ESP10_Rain**	**ESP10_Ambient**
ESP10	A-_20121214_015817	14-12-2012	1:58:17	A-_20121213_183100	13-12-2012	18:31:00	**ESP10_Rain2**	**ESP10_Ambient2**
ESP11	A-_20121018_183100	18-10-2012	18:31:00	A-_20121019_183000	19-10-2012	18:30:00	**ESP11_Rain**	**ESP11_Ambient**
ESP12	A-_20130209_015817	09-02-2013	1:58:17	A-_20130209_185700	09-02-2013	18:57:00	**ESP12_Rain**	**ESP12_Rain**

6.3 Playback Test

To compare the sound of the playback at the entrance of the roost to the sound inside the roost, where the bats would be, we tested the playback at an uninhabited drainage tunnel similar a bat roost.

Audio files containing rain noise, white noise and a sweep were played 1 m from the tunnel entrance with an ultrasonic speaker (ScanSpeak, Avisoft Bioacoustics, Germany), mounted on a tripod, facing the entrance of the tunnel. The speaker was connected to an amplifier (UltraSoundGate Player 116, Avisoft Bioacoustics, Germany) and the amplifier was connected to a laptop (Thinkpad T420, Lenovo, China). The audio files were played back using the RECORDER USGH software (Avisoft Bioacoustics, Germany). The different sounds were measured with a sound pressure level meter (Digital Sound Level Meter 33-2055, RadioShack, United States) the audio files were played back and measured at the entrance (1m) and 6.2 m inside the tunnel. The playback was also recorded at those distances with a handheld audio recorder (PMD661, Marantz, Japan) equipped with a power module (K6-C, Sennheiser, Germany) and a microphone head (ME 62, Sennheiser, Germany).

The rain noise was played at 80dB at the entrance of the tunnel (1m distance to speaker) and was measured with 72dB at 6.2 m inside the tunnel. The equipment only allowed the white noise to be played at 78dB, at the entrance of the tunnel, during the first try and at 77dB during the second try. Inside the tunnel it was measured at 68dB. See Fig. 4 for a power spectogram of the white noise recordings. The sweep was played with the same output settings as the white noise, but because the sound pressure level meter only covers the sonic range and the modulated structure of the sound, the pressure level could not accurately be measured.

During the playback of the white noise the equipment overloaded due to lack of output power of the laptop. The maximum pressure level that would not cause overloading was 74 dB, measured at the entrance of the tunnel.

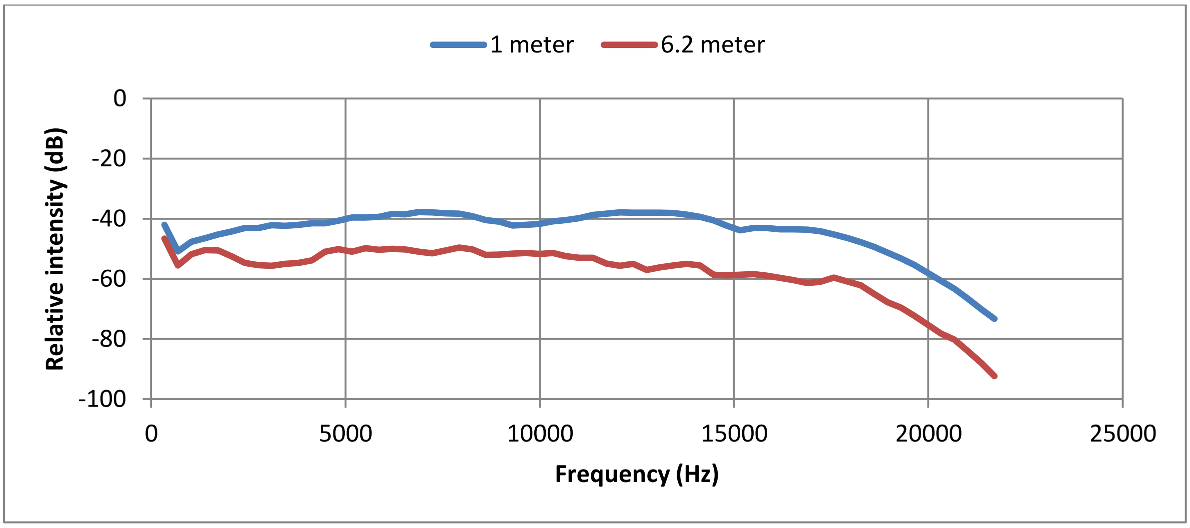


Fig. 4 Power spectrogram shows the relative intensity of the white noise recordings at the entrance of the roost (1 m away from the speaker) (blue) compared to the recording 6.2 m inside the tunnel (red).

6.4 Roost Overview

Roost ID	Species	Baseline Date	Ambient Date	Rain Date	Audio File Pair	Distance Speaker	Included	Notes
Roost 06	*Micronycteris microtis*	19-04	20-04	21-04	ESP10	100cm	x	
Roost 07	*Micronycteris microtis*	24-04	25-04	26-04	ESP1	100cm	x	
Roost 08	*Micronycteris microtis*	27-04	28-04	29-04	ESP9	100cm		Too few bats during rain playback
Roost 10	*Micronycteris microtis*	02-05	03-05	04-05	ESP7	100cm	x	
Roost 09	*Micronycteris microtis*	08-05	09-05	10-05	ESP12	100cm	x	
Mol 01	*Molossus* sp.	11-05	13-05	12-05	ESP10_2	115cm	x	
Roost 11	*Micronycteris microtis*	14-05	16-05	15-05	ESP7	100cm	x	
Roost 13	*Micronycteris microtis*	31-05	01-06	03-06	ESP10_2	100cm	x	
Mol 02	*Molossus* sp.	06-06	08-06	10-06	ESP7	140cm	x	
Mol 03	*Molossus* sp.	11-06	12-06	13-06	ESP9	100cm	x	
Mol 04	*Molossus* sp.	20-06	21-06	22-06	ESP3	110cm	x	
Mol 05	*Molossus* sp.	25-06	27-06	26-06	ESP10_2	100cm	x	
Mol 06	*Molossus* sp.	12-07	13-07	14-07	ESP9_2	120cm	x	
Roost 14	*Micronycteris microtis*	31-07	02-08	01-08	ESP1	100cm	x	
Roost 15	*Micronycteris microtis*	03-08	05-08	04-08	ESP3	100cm	x	
Roost 16	*Micronycteris microtis*	18-08	19-08	20-08	ESP11	100cm	x	
Mol 09	*Molossus* sp.	22-08	25-08	24-08	ESP1	130cm	x	
Mol 10	*Molossus* sp.	26-08	28-08	27-08	ESP11	120cm	x	
Mol 11	*Molossus* sp.	02-09	03-09	04-09	ESP10	115cm	x	
Mol 12	*Molossus* sp.	29-09	-	-	ESP4	100cm		Too few bats

6.5 Survey

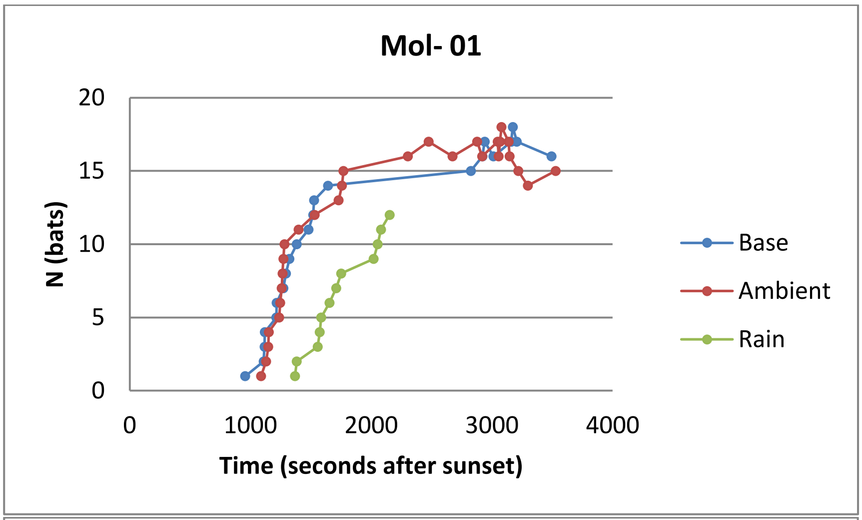
Unblocked roost	Day of blocking	Blocked roost	Day of experiment	Rain

	Roost- 06	Roost- 07	Roost- 09	Roost -10	Roost- 11	Roost- 13	Roost- 14	Roost- 15
15-apr	15+	1					10	12
16-apr	8	5					12	11
17-apr	8	5	7				11	10
18-apr	9	6	7				12	11
19-apr	8	5	8				13	10
20-apr	9	4	0				12	11
21-apr	11	6	0				13	10
22-apr	10	4	0					
23-apr		4						
24-apr		5			2			
25-apr		5						
26-apr		5	7	4				
27-apr			8	4				
28-apr			8	3				
29-apr			7	3				
30-apr			8	4				
1-mei			7	4				
2-mei			0	4				
3-mei			0	4				
4-mei			0	4				
5-mei			0					
6-mei					5			
7-mei			7					
8-mei			7			7		
9-mei			7					
10-mei			7		4			
11-mei					1			
12-mei					4			
13-mei					4			
14-mei					4			
15-mei				4	4			
16-mei					4			
17-mei								
18-mei								
19-mei								
20-mei						0		
21-mei						5		
22-mei						7		
23-mei								
24-mei								
25-mei						6		

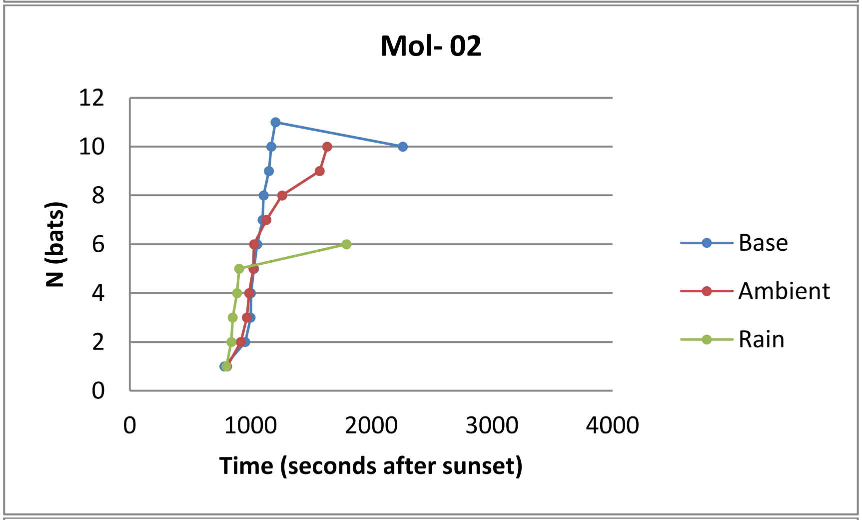
26-mei	6
27-mei	7
28-mei	
29-mei	7
30-mei	
31-mei	7
1-jun	
2-jun	7
3-jun	7

6.6 Emergence Behavior

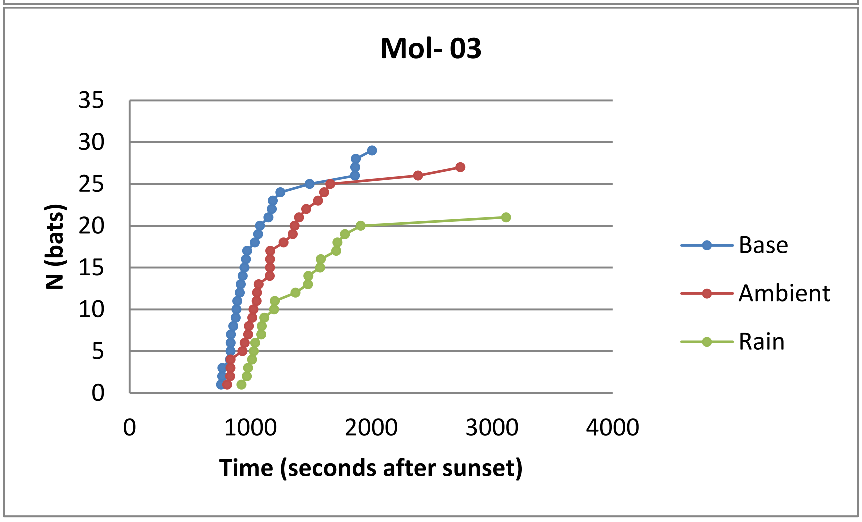
Mol 01	Baseline	Ambient	Rain
Date	11-05	13-05	12-05
Total Bats	18	18	12
First Bat	956	1088	1369
Median_B	1383	1282	2054
Delay	-	-101	670
Noise Start	-	-1793	-1599
Noise Stop	-	3585	3575



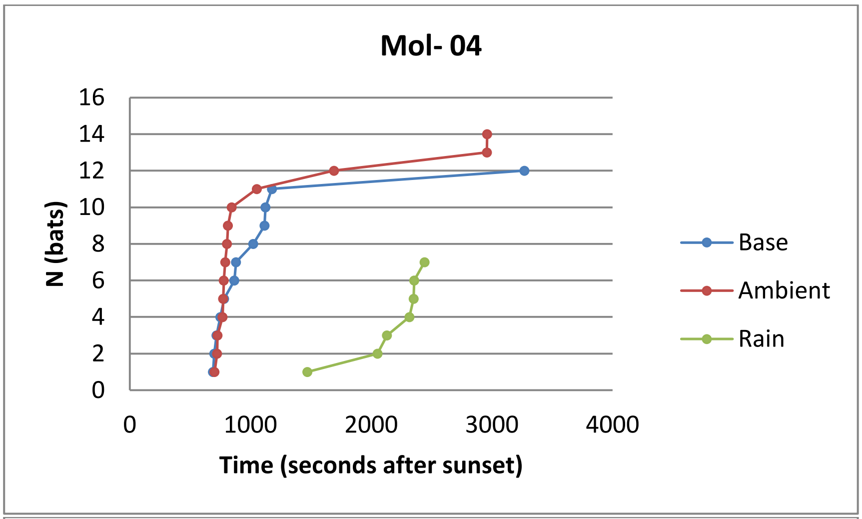
Mol 02	Baseline	Ambient	Rain
Date	06-06	08-06	10-06
Total Bats	11	10	6
First Bat	785	806	801
Median_B	1057	1030	1796
Delay	-	-27	739
Noise Start	-	-2217	-2252
Noise Stop	-	3229	3110



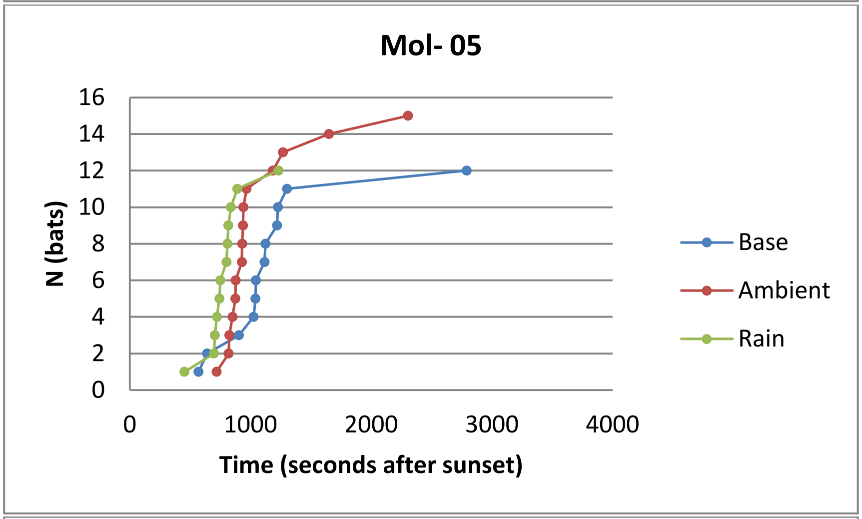
Mol 03	Baseline	Ambient	Rain
Date	11-06	12-06	13-06
Total Bats	29	27	21
First Bat	758	808	927
Median_B	952	1163	1578
Delay	-	211	626
Noise Start	-	-1580	-2045
Noise Stop	-	3208	3124



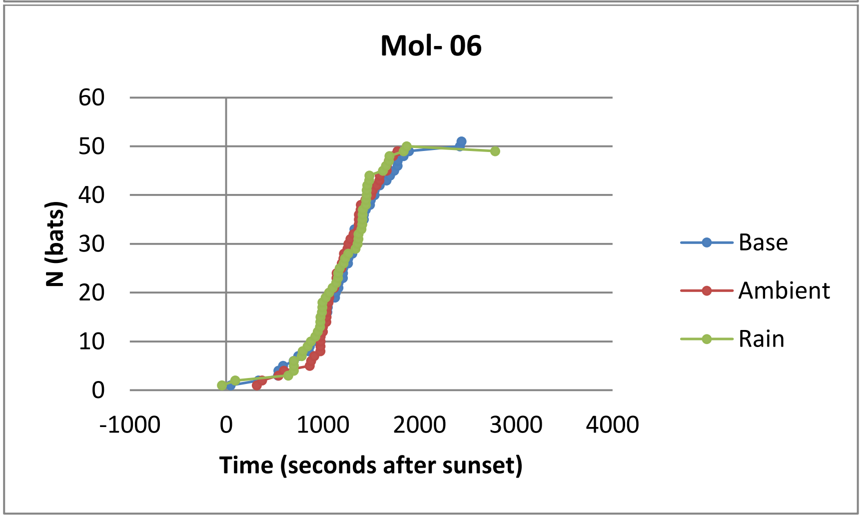
Mol 04	Baseline	Ambient	Rain
Date	11-06	12-06	13-06
Total	12	14	7
First	689	702	1472
Median_B	881	792	2443
Delay	-	-89	1563
Noise Start	-	-1325	-1801
Noise Stop	-	3515	3369



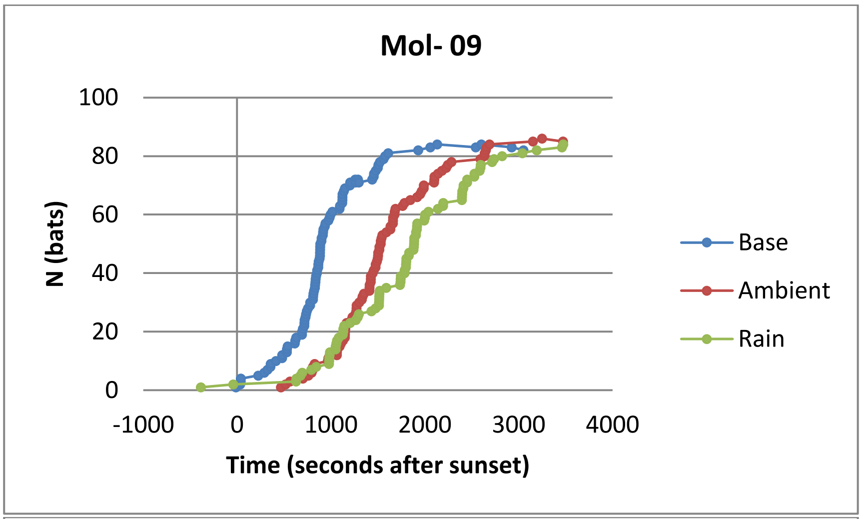
Mol 05	Baseline	Ambient	Rain
Date	25-06	27-06	26-06
Total	12	15	12
First	570	720	453
Median_B	1117	930	802
Delay	-	-188	-315
Noise Start	-	-2290	-2302
Noise Stop	-	3006	3042



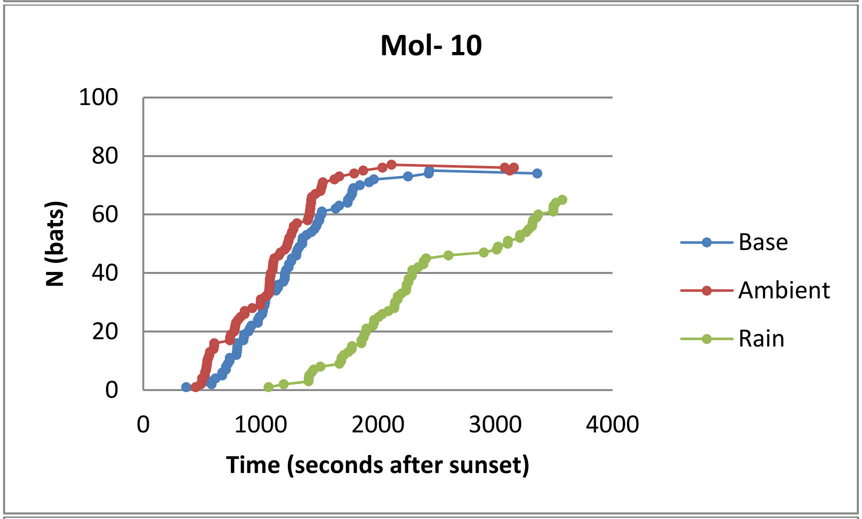
Mol 06	Baseline	Ambient	Rain
Date	12-07	13-07	14-07
First Bat	45	316	-45
Total Bats	51	49	50
Median_B	1261	1196	1215
Delay	-	-64	-46
Noise Start	-	-2509	-2752
Noise Stop	-	2859	3002



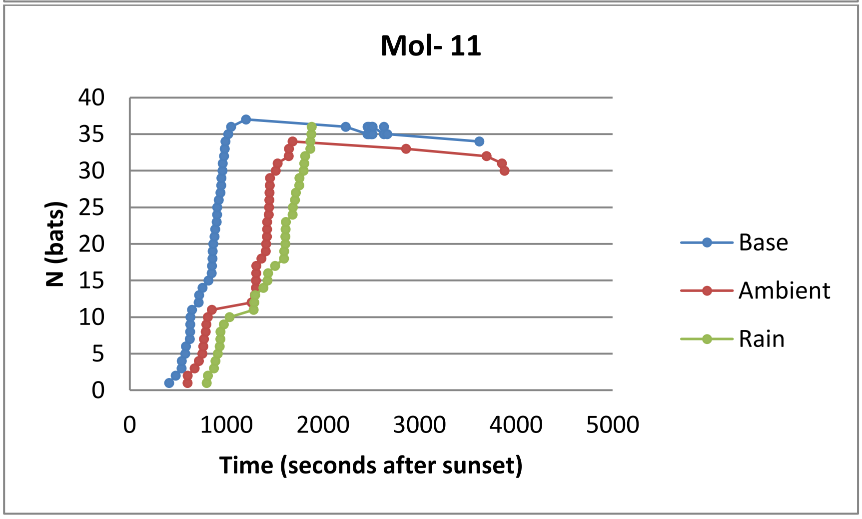
Mol 09	Baseline	Ambient	Rain
Date	22-08	25-08	24-08
Total Bats	84	86	84
First Bat	-12	467	-386
Median_B	866	1477	1800
Delay	-	611	935
Noise Start	-	-1729	-1290
Noise Stop	-	3657	3718



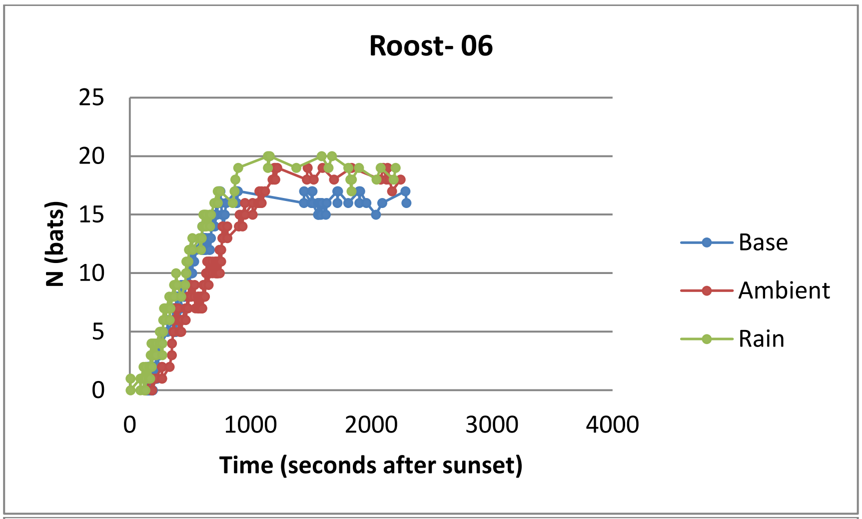
Mol 10	Baseline	Ambient	Rain
Date	26-08	28-08	27-08
Total Bats	75	77	65
First Bat	366	446	1068
Median_B	1206	1080	2263
Delay	-	-126	1057
Noise Start	-	-1703	-1567
Noise Stop	-	3774	3776



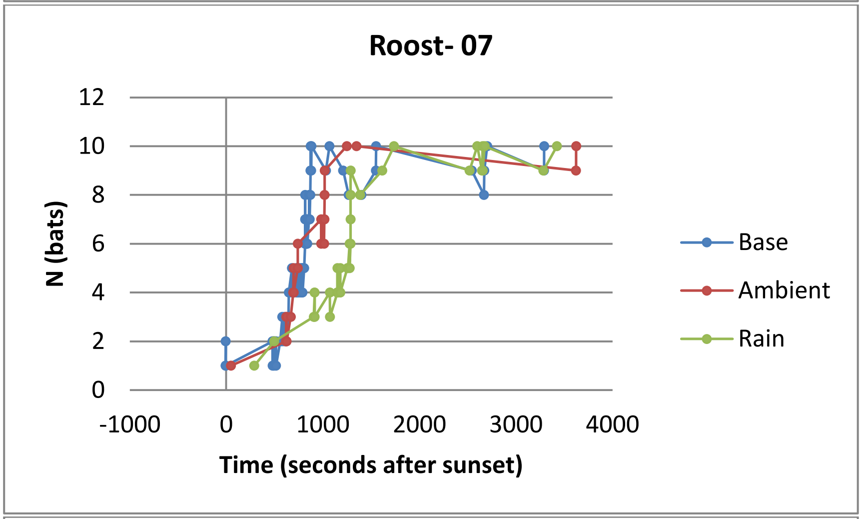
Mol 11	Baseline	Ambient	Rain
Date	02-09	03-09	04-09
Total Bats	37	34	36
First Bat	408	599	797
Median_B	859	1408	1600
Delay	-	549	741
Noise Start	-	-1244	-1070
Noise Stop	-	3949	4010



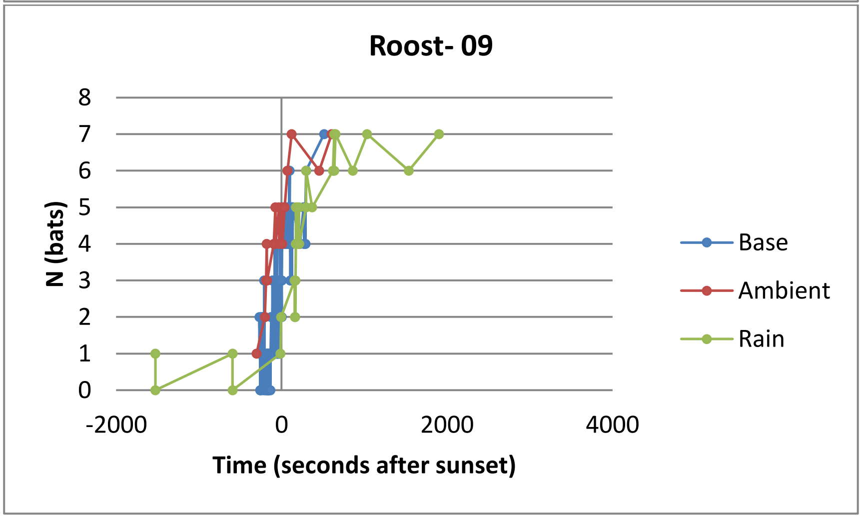
Roost 06	Baseline	Ambient	Rain
Date	19-04	20-04	21-04
Total Bats	17	19	20
First Bat	137	127	7
Median_B	427	495	368
Delay	-	68	-59
Noise Start	-	-1574	-1595
Noise Stop	-	3791	2361



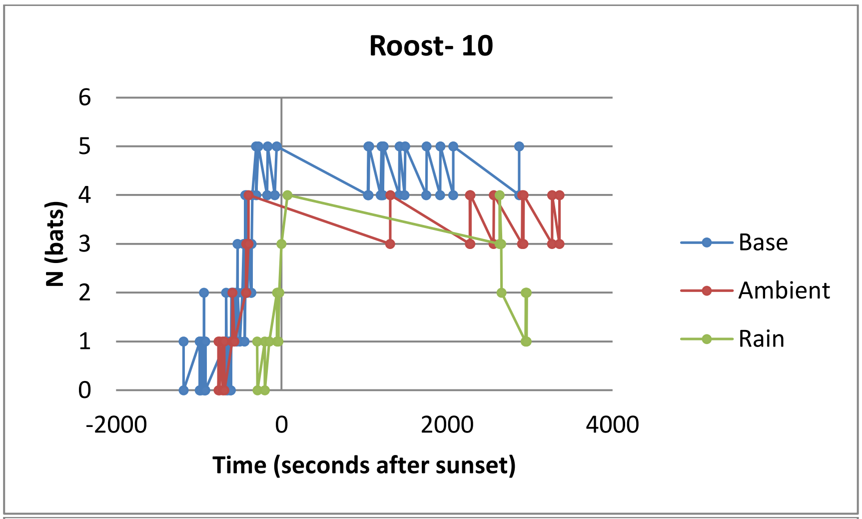
Roost 07	Baseline	Ambient	Rain
Date	24-04	25-04	26-04
Total Bats	10	10	10
First Bat	-7	49	290
Median_B	816	741	1278
Delay	-	-75	462
Noise Start	-	-1763	-1637
Noise Stop	-	3730	3783



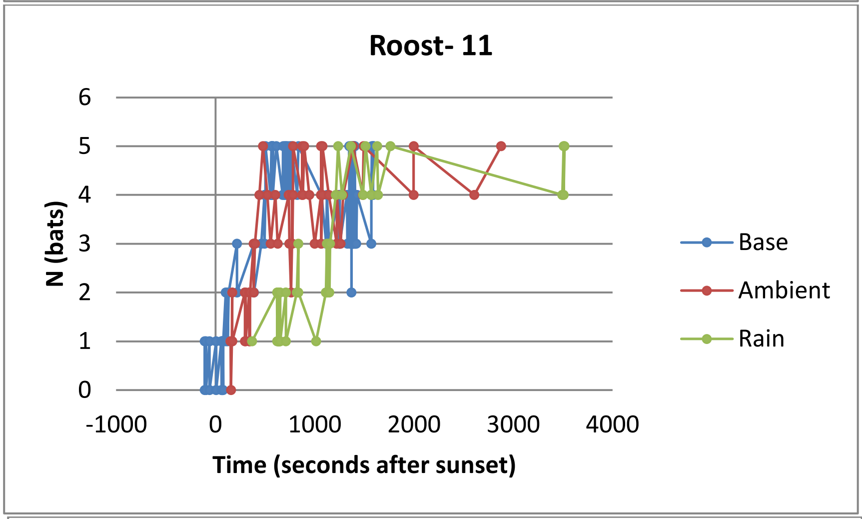
Roost 09	Baseline	Ambient	Rain
Date	08-05	09-05	10-05
Total Bats	7	7	7
First Bat	-268	-303	-1527
Median_B	-85	-180	172
Delay	-	-96	257
Noise Start	-	-1591	-1633
Noise Stop	-	3600	3600



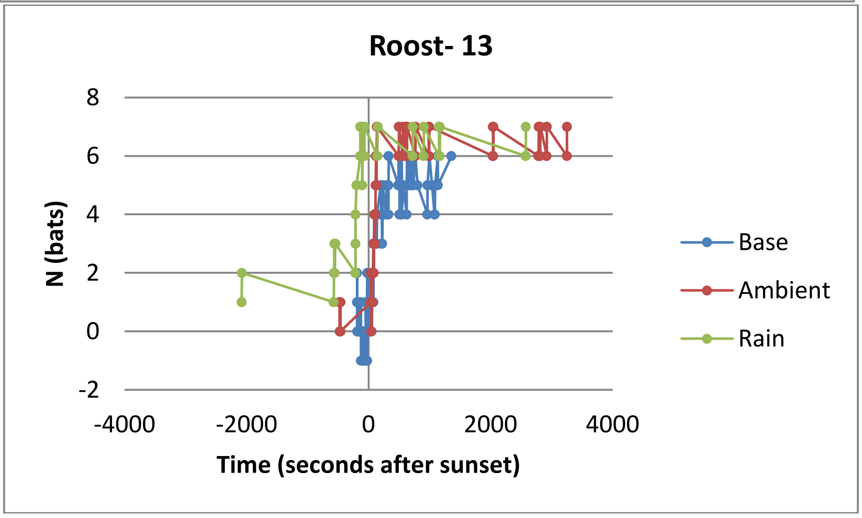
Roost 10	Baseline	Ambient	Rain
Date	02-05	03-05	04-05
Total Bats	5	4	4
First Bat	-1185	-763	-296
Median_B	-533	-427	-2
Delay	-	106	532
Noise Start	-	-1535	-1722
Noise Stop	-	3655	3617



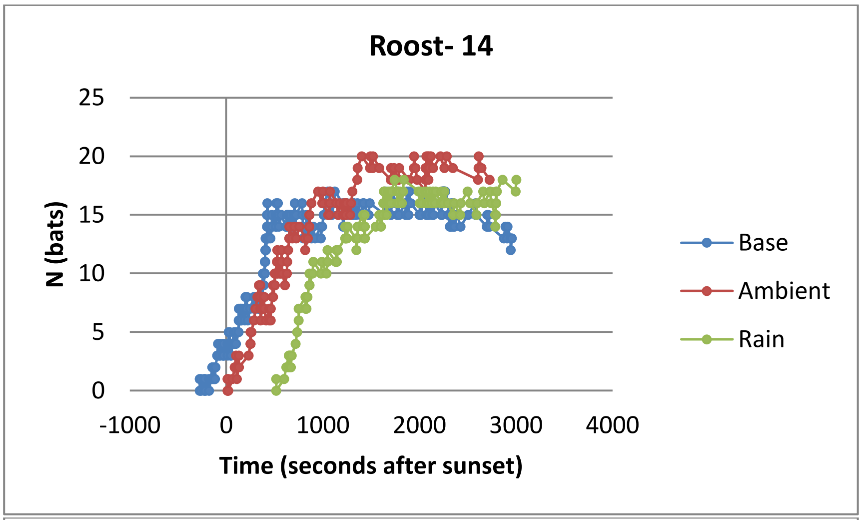
Roost 11	Baseline	Ambient	Rain
Date	14-05	16-05	15-05
Total Bats	5	5	5
First Bat	-108	151	369
Median_B	217	385	835
Delay	-	168	618
Noise Start	-	-1381	-1371
Noise Stop	-	3922	3563



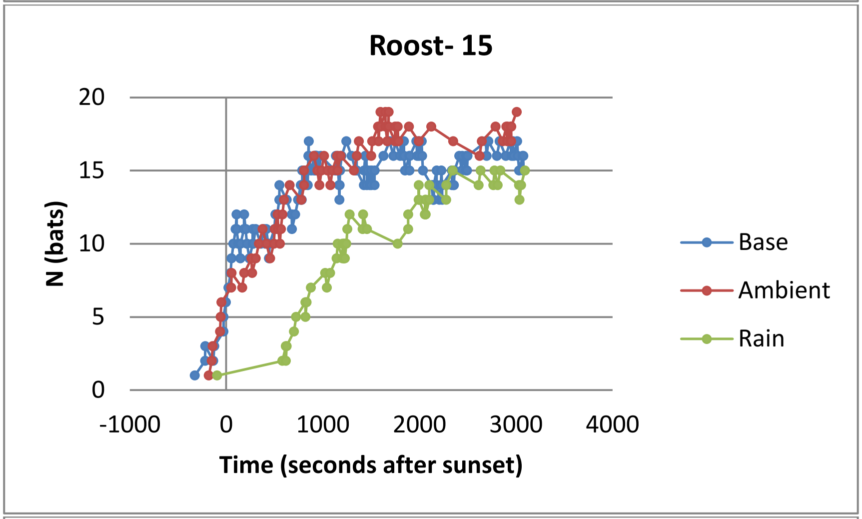
Roost 13	Baseline	Ambient	Rain
Date	31-05	01-06	03-06
Total Bats	6	7	7
First Bat	-188	-477	-2079
Median_B	78	83	-558
Delay	-	5	-635
Noise Start	-	-1979	-2090
Noise Stop	-	3288	3269



Roost 14	Baseline	Ambient	Rain
Date	31-07	02-08	01-08
Total Bats	17	20	18
First Bat	-275	13	517
Median_B	362	338	861
Delay	-	-24	499
Noise Start	-	-2359	-2504
Noise Stop	-	3014	3042



Roost 15	Baseline	Ambient	Rain
Date	03-08	05-08	04-08
Total Bats	17	19	15
First Bat	-326	-182	-95
Median_B	54	260	1143
Delay	-	206	1089
Noise Start	-	-2267	-1768
Noise Stop	-	3197	3200



Roost 16	Baseline	Ambient	Rain
Date	18-08	19-08	20-08
Total Bats	16	12	10
First Bat	-1254	-163	226
Median_B	600	633	1753
Delay	-	33	1153
Noise Start	-	-1430	-1526
Noise Stop	-	3781	3541