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ABSTRACT

Animal behavior is strongly influenced by the threat of predation. This has led to the development of complex communication systems between predators and prey. In bats, many types of ultrasonic calls have been studied in depth, but low frequency distress calls have rarely been examined. In order to analyze which variables might be able to predict whether a given bat performs a distress call, I captured 36 bats and recorded their behavior and the decibel power of their calls. I found that bats of a smaller mass were more likely to engage in distress calls while bats with a greater forearm length emitted calls with greater decibel power, indicating greater energy and range. However, these findings may be biased by which species I captured. I also found that nursing bats were less likely to call than non-reproductive males and females, implying some unknown risk associated with distress calling that nursing bats needed to avoid. My findings suggest that while smaller bats may be more likely to call, the calls of larger bats are more acoustically powerful and therefore would likely be more effective at attracting bats from long distances. There is likely an unknown cost associated with distress calling in nursing bats that merits further study.

Efectos del tamaño y sexo en las llamadas de socorro de murciélagos en Monteverde

RESUMEN

La depredación afecta el comportamiento y la evolución de las especies. Existen sistemas complicados de comunicación entre depredadores y presas. Los murciélagos emiten sonidos ultrasónicos que se han investigado durante más tiempo, pero sus sonidos audibles a humanos y otros animales han sido mucho menos estudiados. Estos ruidos de baja frecuencia incluyen las llamadas de socorro. Analicé 36 murciélagos capturados en redes de niebla, evaluando cuáles características pueden predecir si un murciélago va a emitir llamadas de socorro. Encontré que los murciélagos de tamaño pequeño hacen más llamadas de socorro que los murciélagos más grandes, y también que los murciélagos con alas más largas emiten sonidos más poderosos. Encontré que las hembras en periodo de lactancia emiten menos llamadas. Hay algún factor negativo en emitir llamadas de socorro, siendo posible que tenga que ver con la energía que cuesta emitir estos sonidos mientras el murciélago está dando de mamar, que es también muy caro energéticamente. Puede ser que las llamadas de socorro, indiquen algo más, además del peligro a los otros murciélagos. Pienso que existen costos aún no descubiertos de las llamadas de socorro que merecen mucho más estudio.

One of the driving factors of the natural world is predator-prey interaction. Predation pressure can cause the evolution of toxins, spines, warning coloration, and so much more (Lima, 2002). In the evolutionary arms race between predator and prey, communication can play a vital role through behavioral signaling or coloration. A prey item communicating that it is a bad choice, because of its toxins or fitness or other antipredator defense, can be important in determining survival and behavior of both species involved (Caro, 2005).

There are 1,240 species of bats belonging to the order Chiroptera and a large diversity of natural history traits associated with them. Bats can feed on nectar, fruit, insects, or even blood; can range in size from 2 grams to 1,200; and can be highly social or completely solitary. The vast majority of these bats are eaten by birds of prey and other animals, and some have evolved different antipredator behavior (Lima et al., 2013). While extensive work has been done on ultrasonic communication between bats, I believe that a scientifically neglected example of antipredator behavior is audible (not ultrasonic) calling or screeching.

Audible distress calls have not been the focus of very much study in bats, but ornithologists have made fascinating and relevant discoveries about their role in bird predator-prey interactions. Laiolo et al. (2004) found that short-toed larks in good body condition used anti-predator calls when caught that ranged across more frequencies than birds in poor body condition. For birds, the calls function as honest signals. There has not been an effort to find out the potential function of these calls in bats. This is a particularly interesting topic because not all bat species are known to be equally noisy, suggesting different selective pressures acting on different species.

In bats, distress calls have been found to induce mobbing behavior (Caro, 2005). Mobbing behavior consists of many bats being attracted to the calling bat and circling it, occasionally diving at the predator. This has been found among unrelated individuals and across many species, including some found in Costa Rica such as *Dermanura spp.* and other phyllostomidae (Russ et al., 1998; August, 1979). However, there has not yet been a study into which bats use low frequency (audible to humans) distress calls and which characteristics might be able to predict these calls.

In this paper, I seek to analyze a set of variables to see if there is an effect of sex, age, reproductive status, mass, species, and forearm length on likelihood to call for a given bat. I believe that this will elucidate some of the selective pressures that could lead to the rise of audible defensive calling. Determining which of these variables predicts defensive calling can uncover much about the function and perhaps evolutionary origins of defensive calling in bats.

MATERIALS AND METHODS

To address this question, I set up mist nets to capture many species of bats over a period of two weeks (12 May, 2019 through 30 May, 2019). I had access to five nets of different lengths. Mist netting occurred at the Monteverde Institute and in the Bajo del Tigre neighborhood (10.3052704, -84.8096583 and 10.3056389, -84.8144494 respectively).

Once captured in the net, I recorded the encounter with the bat to see if it made audible calls during untangling and processing. To do this, I used a Zoom HN4 audio recorder. Then, I ran the audio recording through the program RavenLite developed by the Cornell Ornithology Lab. I analyzed the single most powerful syllable of the bat's call and noted frequency and power density. Power density indicates the amount of energy in watts needed to produce this sound.

I analyzed simply whether or not a bat called and compared this to variables like sex, mass, species, and reproductive status using chi square tests. I separated sex into male, female, pregnant female, and nursing female for analysis. I determined that a female was pregnant by palpating the stomach and that she was nursing by noting exposed teats and a lack of pregnancy.

RESULTS

Overall, we caught and obtained distress call data for 34 bats, 20 of which called and 14 of which did not. We caught a total of 12 species, but the vast majority (16) were *Myotis pilosatibialis* (Table 1). This small species called 15 out of 16 times. Other species frequently caught included *Dermanura tolteca* (6) and 3 species of the *Carollia* genus (7).

I did not have sufficient data points to draw conclusions about whether species affected likelihood for distress calls, with the exception of *M. pilosatibialis*, which called 93.75% of the time (N=16). Typically, we found about 3 bats of each species except *M. pilosatibialis*, which was not enough to run accurate data analysis.

The variables that were able to predict whether a bat emitted a distress call were mass and sex separated by reproductive status. Overall, smaller bats called more than larger bats (fig. 1, chi square p=0.0315, N=34). Mass (which indicates both size and body condition) was a better predictor of call likelihood than forearm length (which indicates purely size and was insignificant).

Additionally, the single *Desmodus rotundus* we caught did call and had a mass of 41 g. If I excluded *D. rotundus* from analysis, the correlation between mass and likelihood of calling was even more robust (p=0.0024). It would be useful to capture more of this species and other very large bats (>35 g) to determine if calls are actually common in the species or in very large bats, or if this individual was an outlier.

However, it's worth noting that the majority of bats were of a single, very small species. When we eliminate these bats, the trend is much weaker (fig. 6). Sex was also able to predict calling. I chose to separate female into three reproductive categories: (non-reproductive) female, pregnant female, and nursing female. I did this because I wanted to see if reproductive stakes might alter an individual's anti-predator behavior. I did indeed find that males and non-reproductive females were similarly likely to call, while nursing females in particular stayed silent (fig. 2, p=0.0326). Pregnant females seemed to call more than nursing females, however, I noticed that 2/3 of the pregnant females who called were *M. pilosatibialis*, a species that called 93.75% of the time. In contrast, none of the nursing females were *M. pilosatibialis.* To test whether species was a source of error here, I analyzed sex versus calling again, this time removing *M. pilosatibialis* from the data (fig. 3). I found that the trend of nursing bats calling at lower rates still exists when this species is not considered.

When I looked at average power density, I found an association between forearm length and power (fig. 4). The association appeared strong before isolating just *M. pilosatibialis* to get a better idea of whether forearm strength had an impact within species (fig. 5). Thus, I found that species may have more to do with power density than forearm length itself.

Table 1. Species of bats caught (sex, measurements) with records of their distress calls and mean power density of these calls.

Species name	Sex	Forearm length (mm)	Mass (g)	Distress call	Average Power Density (dB)
Carollia perscpicillata	Male	42	19	No	
Myotis pilosatibialis	Male	35	6.5	Yes	
Sturnira hondurensis	Nursing Female	44	20.5	No	
Carollia sowelli	Pregnant Female	40	23	Yes	
Carollia nicaraguensis	Pregnant Female	44	22.75	No	
Micronycteris schmidtorum	Male	35	8	Yes	
Myotis pilosatibialis	Female	35	7.5	No	
Dermanura tolteca	Nursing Female	42	19	No	
Sturnira hondurensis	Nursing Female	44	20	No	
Myotis pilosatibialis	Male	37	4.5	Yes	
Myotis pilosatibialis	Female	36.5	6	Yes	
Dermanura tolteca	Nursing Female	41	16.5	No	
Carollia perscpicillata	Male	40	19	No	
Carollia perscpicillata	Male	43	18	Yes	
Myotis pilosatibialis	Male	35	6.5	Yes	
Dermanura tolteca	Nursing Female	42	16.5	No	
Myotis pilosatibialis	Male	36	6.5	Yes	
Myotis pilosatibialis	Male	36.5	5.5	Yes	
Myotis pilosatibialis	Pregnant Female	36	7	Yes	-39.1
Carollia sowelli	Nursing Female	41	19	Yes	-43.5

Sturnira hondurensis	Nursing Female	43	23	No	
Carollia sawelli	Male	40	17	Yes	
Myotis pilosatibialis	Pregnant Female	36	6.5	Yes	-40.8
Myotis pilosatibialis	Male	36	5.5	Yes	-41.1
Myotis pilosatibialis	Male	35	3	Yes	-40.9
Glossophaga soricina	Male	37	11	No	
Carollia sowelli	Male	43	20	No	
Myotis pilosatibialis	Female	37	6	Yes	
Dermanura tolteca	Pregnant Female	41	17	No	
Myotis pilosatibialis	Male	37	7	Yes	-39.8
Desmodus rotundus	Male	60	41	Yes	-23.6
Dermanura tolteca	Male	44	18	No	
Platyrrhinus helleri	Male	38	16	Yes	
Dermanura tolteca	Male	41	14	Yes	-40.6

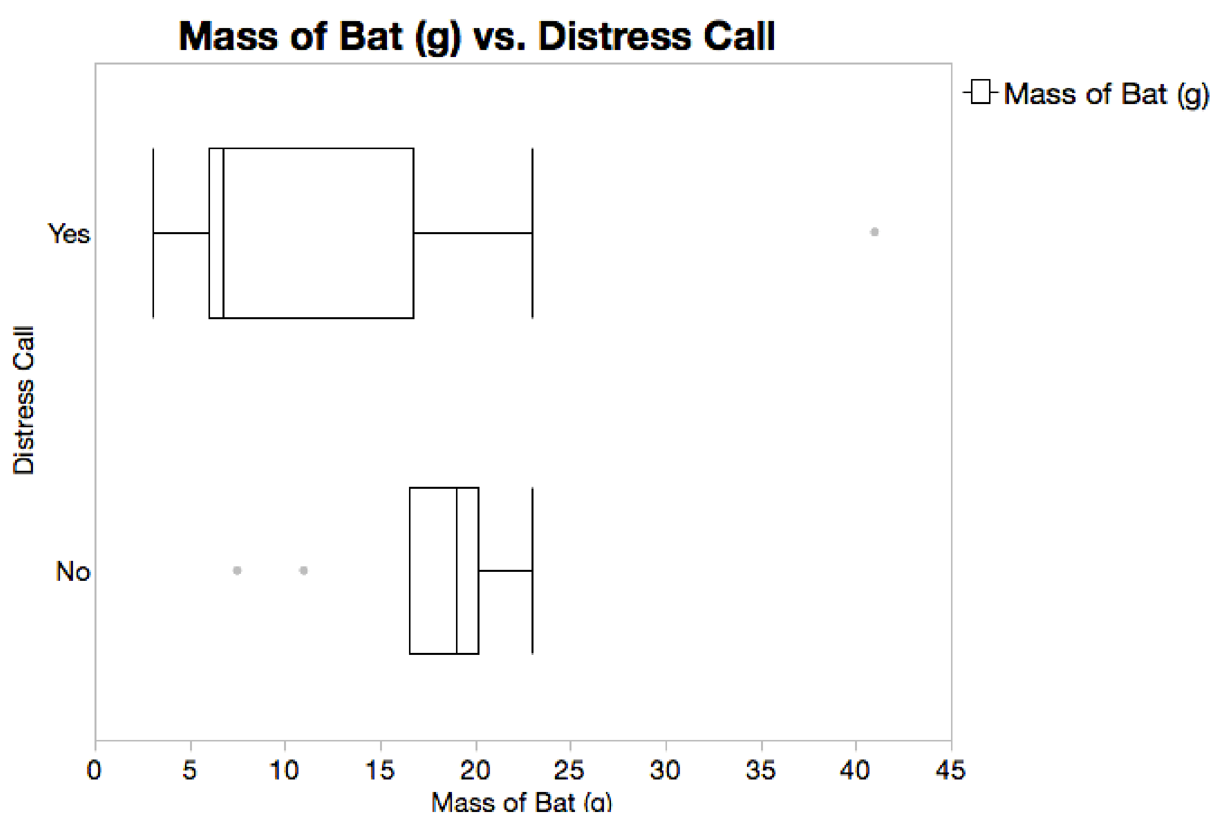

Fig. 1. Smaller bats called significantly less than larger bats (chi square, p=0.0315). Bats that did call showed a greater range, including one outlier at 41 grams.

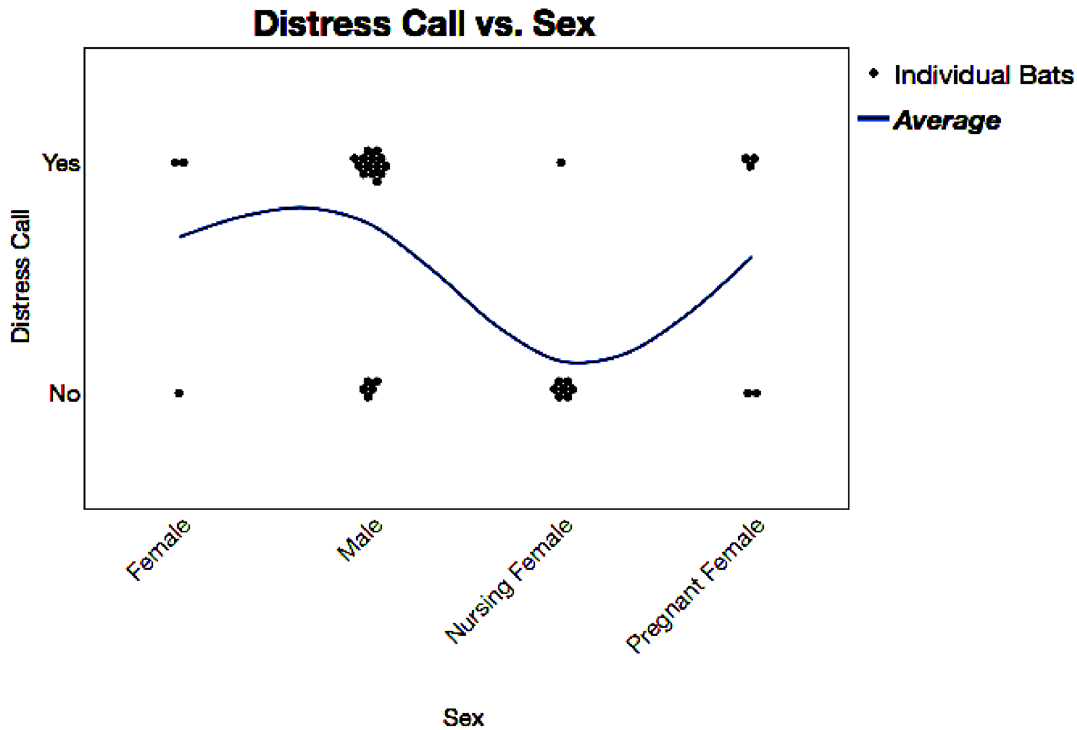

Fig. 2. Nursing females were significantly less likely to call than males and females of different reproductive stages (chi square, p=0.0253, N=35). Males were the most likely to call of any sex class. The average line allows us to better see how female, male, and pregnant female show similar "yes" versus "no" results, compared to nursing female which favors "no."

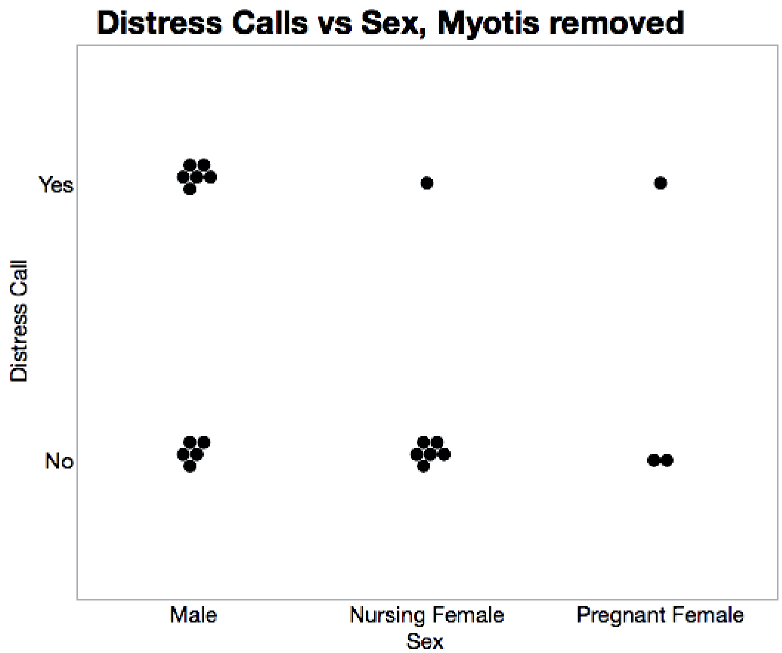

Fig. 3 When *Myotis* is removed, we still see the same trend of nursing females calling less (chi square, p=0.2028, N=19). The data are not statistically significant; however, this is likely impacted by the low sample size of pregnant females. The quantity of males calling has gone

down without *Myotis* but remains much higher than females calling. Non-reproductive female is not included in the graph because all non-reproductive females were *Myotis*.

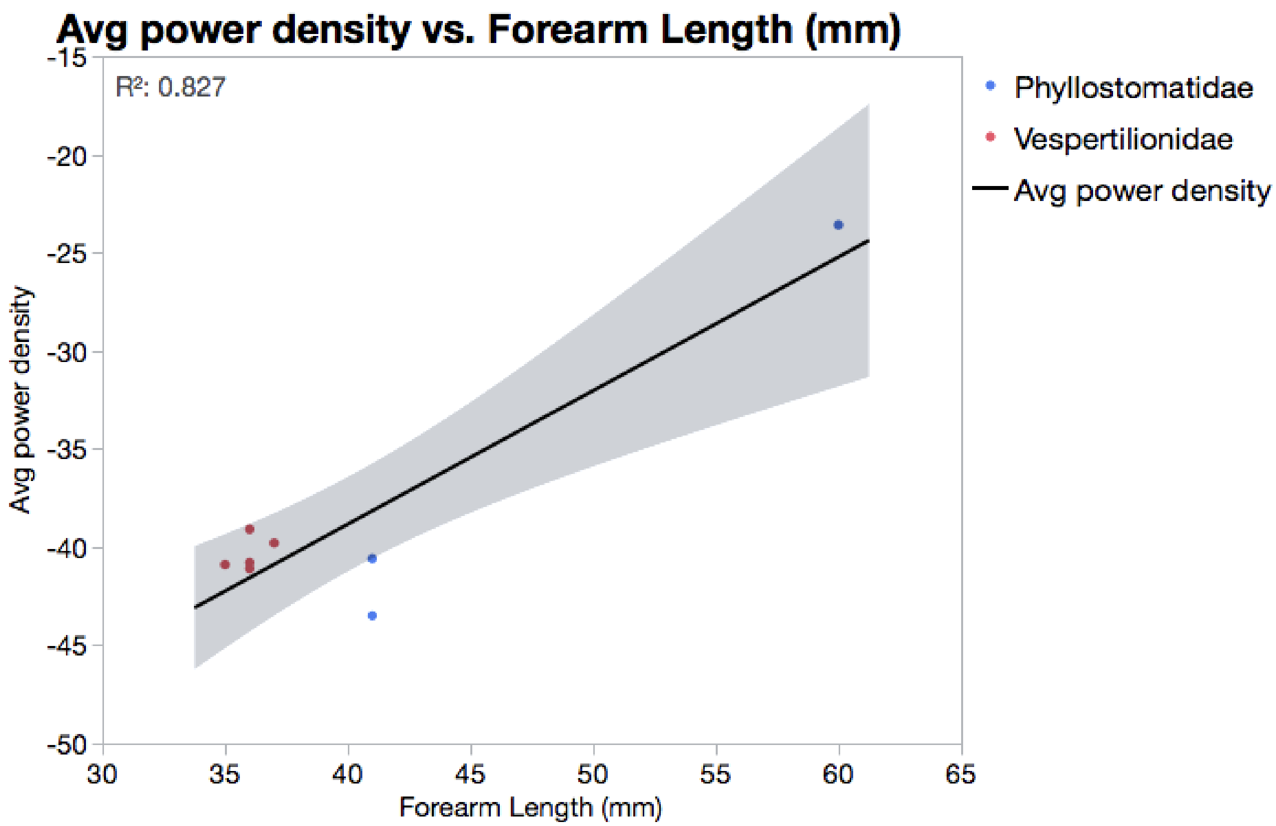

Fig. 4. Average power density appears to increase with forearm length. As bats get larger, the amount of power put into their calls seems to increase, meaning they are more energetically expensive. Red points indicate bats from the family Vespertilionidae while blue indicates the family Phyllostomatidae. There is only one Vespertilionidae species that we caught: *M. pilosatibialis*. There is a very high likelihood that this graph is impacted by the outlier *D. rotundus*, seen at the top right corner of the graph.

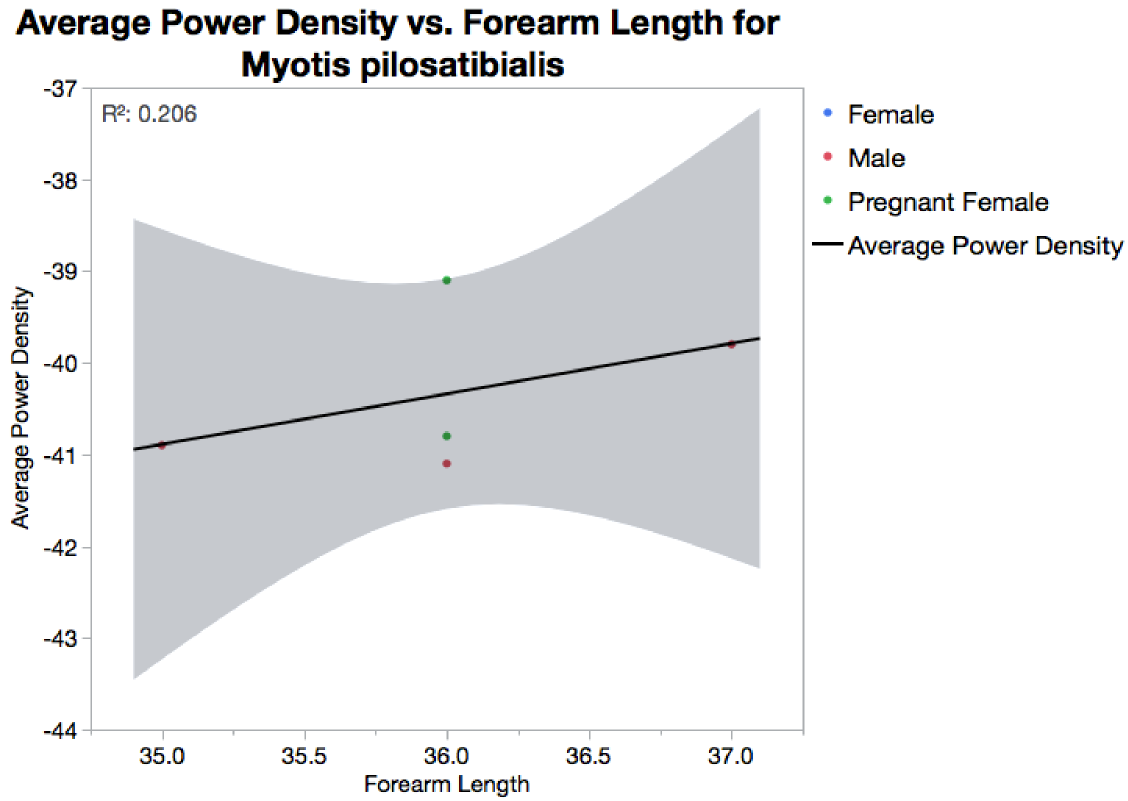

Fig. 5 Here we see power density versus forearm length isolated for just one species, *M. pilosatibialis*. The trend is very weak if it exists at all (R^2=0.206). Sexes are denoted with colors, and there seems to be no pattern there either.

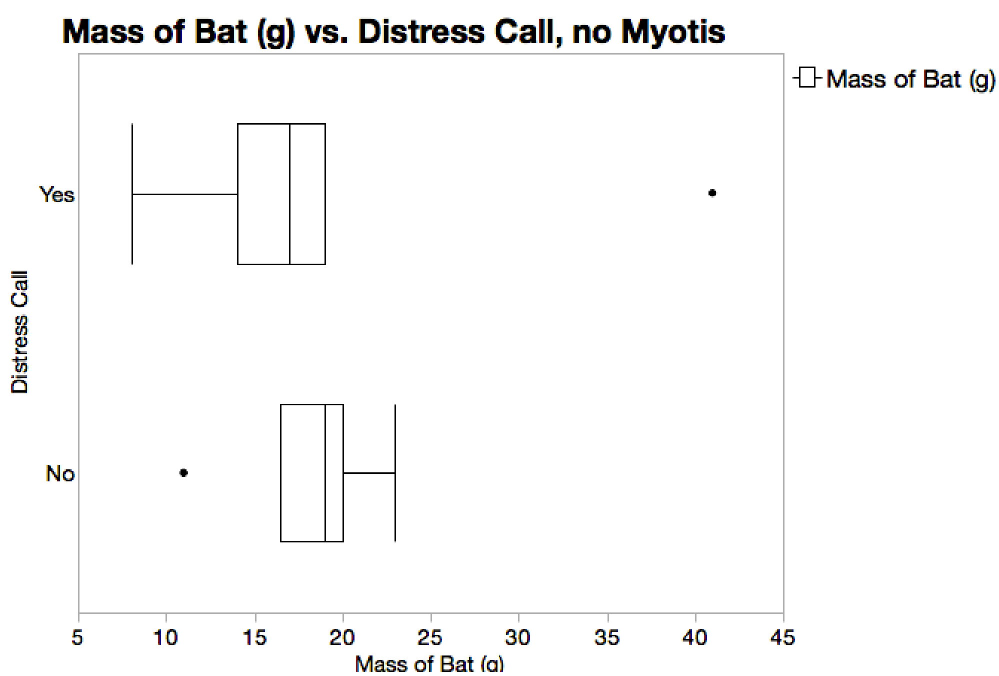

Fig. 6 When we remove *M. pilosatibialis* from the dataset, the trend is much less clear (chi square, p=0.8492). There does not seem to be much of relationship between distress call and mass of bat when we eliminate the overrepresented *Myotis*.

DISCUSSION

Mass initially seemed to be a reliable predictor of distress call likelihood. Overall it is true that smaller bats were much more likely to call than larger ones. What's unclear is whether the effects of mass are clouded by the effects of the species *M. pilosatibialis*, or whether the perceived effects of the species *M. pilosatibialis* on distress call likelihood are actually the effects of mass. The individuals we caught of *M. pilosatibialis* ranged in size from 3 to 8 grams and were the smallest species by far. Because there was only one species in this size range, it is difficult to separate the effects of mass from potential species-specific effects.

I attempted to do this by analyzing mass versus distress calls with *M. pilosatibialis* eliminated. Once I did this, I did indeed find that mass had a much, much weaker association with distress calling. This is very interesting because it points to species itself being a factor in whether an individual chooses to call. Unfortunately, I did not have the dataset necessary to analyze species, but this is an essential next step for bat distress call research. My study suggests that there is something about the species *M. pilosatibialis* beyond obvious variables that is causing them to call at disproportionately high rates. A future study that involved high sample sizes across many species could get a more accurate answer for the question of which species call the most.

Abiotic weather factors were disregarded as potential sources of variation because bats could not be caught in the rain due to heightened mist net visibility, and thus the weather was very similar on most nights that we were able to catch bats.

Sex was also an adequate predictor of distress call likelihood. Males and non-reproductive females were found to be the most likely to call (fig. 2, p=0.0253). However, nursing and, to a lesser extent, pregnant females called much less. It is possible that this is due to a decreased desire for risk-taking in females with dependent young. In order to understand why females, call less when reproductively engaged, we must first understand the purpose of the distress call.

Lima et al. write about the function of distress calls and claim that the call can either act as a personal risk to warn other bats, or as a way to attract other bats to mob a predator (2013). Each of these interpretations has different implications for the lack of calling in nursing bats. If distress calls act as a somewhat altruistic warning to other bats at personal cost, it stands to reason that a nursing bat, whose fitness depends now on the survival of her dependent young, would not want to make that sacrifice for her roost-mates.

However, if the second interpretation is correct, then it does not make sense that such a vulnerable demographic would decline to call for help. In this case, my data may be skewed by the season in which my study was conducted. Of the pregnant females we caught, half were *M. pilosatibialis* and the other half were of the *Carollia* genus. LaVal and Fitch found temporal differences in bat reproductive cycles indicating that *M. pilosatibialis* should not yet be giving birth, which is supported by my data (LaVal and Fitch, 1977). *No M. pilosatibialis* and only one *Carollia* was nursing, which could impact data since *M. pilosatibialis* was found to be noisier as a species. Other species such as *Dermanura tolteca* and the *Sturnira* genus were only captured while nursing, not pregnant. This could create a false pattern if *D. tolteca* and *Sturnira* are simply less likely to make noise, not necessarily because nursing bats as a class make less noise.

To test this, I analyzed sex data after removing all *M. pilosatibialis* to see if the nursing pattern remained. While the results were less robust, I still found a disproportionate refusal to make distress calls by nursing bats (chi square, p=0.2028). This indicates that the pattern is real and not being created by the presence of *M. pilosatibialis,* as was the case with my mass data.

When it comes to the bats that did make calls, I found that bats with longer forearms emit more powerful sounds (R^2=0.827). This is a more reliable measure of size than mass because it does not take food or pregnancy into account. In particular, the biggest bat that called was a *D. rotundus*, or common vampire bat. We observed that the bat had fed recently and was bloated with blood, meaning that its mass was much higher than it would be even a few hours later.

However, when I isolated *M. pilosatibialis*'s calls and compared their power to each forearm length, there was not a strong relationship at all (R^2=0.206). This is not conclusive but means that we need more data to confirm this fact, and that a single point by *D. rotundus* may be disproportionately represented. We also have a very small sample size for audio analysis, and it's possible that with more points this relationship could change.

The idea that larger bats seem to invest more energy in their sounds, knowing that producing sound is energetically expensive, suggests that there is a benefit to producing the most powerful sound possible in the face of a predator. In the context of predator-prey interactions, this means that sound power is a worthwhile expense and serves an anti-predator function.

What exactly the calls are conveying is more difficult to understand. Fenton et al. found that in *Myotis lucifigus*, closely related to *M. pilosatibialis*, bats responded to distress calls of conspecifics by flying more actively and even circling a speaker (1976). They were able to conclude that distress calls specifically attract other bats in this species. Even heterospecifics are capable of being attracted by acoustically similar calls (Huang et al., 2018). This leads me to believe that larger bats, who are capable of producing more powerful sounds, have their distress calls heard and responded to from farther away.

Combined with the results we saw comparing mass and likelihood of calling, I propose that smaller bats could be more likely to call because of their heightened risk for predation and that there is some hidden cost shown by the lack of calling in nursing bats. More study should be conducted to draw more robust conclusions about the effect of sex on call likelihood and quality.

Low frequency distress calls in bats provide us with a novel way to study the relationship between predator and prey in an animal that is usually hidden by darkness. The decision to call or not to call is not as straightforward as it might seem, and by isolating mass and sex as key variables we can begin to understand the antipredator behavior of these highly social mammals.

Future work might include analysis of a much larger sample size of bats across a longer period of time. This could help clarify the effects of phylogeny and species on likelihood to call.

With a large enough sample size, and captive bats, we could even study whether individuals have personalities that make them more likely to call. Additionally, future work should include the playback of low frequency distress calls for other bats to see how they react.

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