Final Evaluation Report

Your Details	
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Project Title	Will Neotropical insectivorous bats adapt to climate change? An Approach from a physiological standpoint
Application ID	30289
Date of this Report	02/05/2022

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Determine the upper thermal maximum (Tuc), or the highest temperature, at which our target species maintain basal metabolic rates		✓		It was partially achieved because bats did not show extreme changes in metabolic rates with the increase in temperature. However, the maximum temperature used for the experiments is related to the maximum temperature predicted by climate change scenarios. We decided not to increase the temperature further than that because it was unnecessary or irrelevant. The intention was to keep the bats alive.
Teach undergraduate students from the University of Costa Rica in Golfito (UCR-Golfito) about bat ecology, and train them to lead with us the education activities in communities		✓		It was partially achieved because, due to COVID-19, I had to return to my home country. Due to economic issues, I was able to move to Costa Rica to do the experiments, but I was not able to stay longer. However, during fieldwork, I also trained my field assistant on research methods that were relevant to her. Also, I gave talks and showed the experimental design to undergraduate and graduate (MSc) students attending a course in the field station while I was there doing my research.

2. Describe the three most important outcomes of your project.

To measure bat metabolic rates, we weighed the bats and put them into the corresponding chambers in bath water to begin the experiments with all chambers at 27.5°C of ambient temperature (Ta). We pumped air (250-270 ml min^{-1}) through a mass flow controller (FB8, Sable Systems International, Las Vegas, NV, USA) into the 0.5 l respirometry chambers. Excurrent air from the chamber and baseline air were subsampled sequentially using a respirometry multiplexer (MUX3-1101-18M, Sable Systems International, Las Vegas, NV, USA).

We recorded data on O_2 consumption, CO_2 production, and water vapor pressure (WVP) directly with the Expedata software via the data acquisition interface (Sable Systems) and the Field Metabolic System (FMS, Sable Systems), which is a fully integrated respirometry system optimized for field use.

We kept the bats at 27.5°C for one hour and increased the Ta by two °C every hour until we reached 39.5°C, the maximum temperature at which we measured the bats (Table 1). We measured bat body temperature (Tb) via PIT tag (BioThermo13, Biomark, Inc.) every 3 minutes using an antenna (HPR Plus, Biomark) in the insulated chambers.

We recorded data for 33 insectivorous bats of the target species *Myotis pilosatibialis* and *M. riparius*. Half of the individuals were males, and the other half were females, which allows us to compare species' responses by sex.

Table 1: Describes the meaning of each block of temperature during the experiments.

temp_block 0	Starting temperature, bats getting used to the chamber (27.5°C)
temp_block 1	Starting temperature, bats get used to the chamber (27.5)
temp_block 2	approximate 2 degrees increased from temp_block 1
temp_block 3	approximate 2 degrees increased from temp_block 2
temp_block 4	approximate 2 degrees increased from temp_block 3
temp_block 5	approximate 2 degrees increased from temp_block 4
temp_block 6	approximate 2 degrees increased from temp_block 5
temp_block 7	approximate 2 degrees increased from temp_block 6

*This helps understand the graphics

1) **Bats are not showing changes in metabolic rates with rising temperatures.**

To test thermal tolerance, we performed open flow respirometry. We connected a metabolic chamber to an air pump with 250-270 ml min^{-1} of airflow. We connected the chamber to the Sable System gas analyzer to measure oxygen (O_2) consumption and carbon dioxide (CO_2) production. Thanks to this system, we test up to three bats simultaneously (Fig. 1)

We recorded four variables to measure metabolic rates: maximal oxygen consumption (VO_2), maximal carbon dioxide production (VCO_2), Respiratory Exchange Ratio (RER, CO_2 production/O_2 uptake), and energy expenditure (EE). All these variables say relatively constant during the experiments disregarding the ambient temperature (Fig 2-5). However, Tb showed a constant increase until temperature block 5. In blocks 6 and 7, Tb stayed constant. This suggests that bats increase Tb with Ta until they reach a limit that cannot surpass.

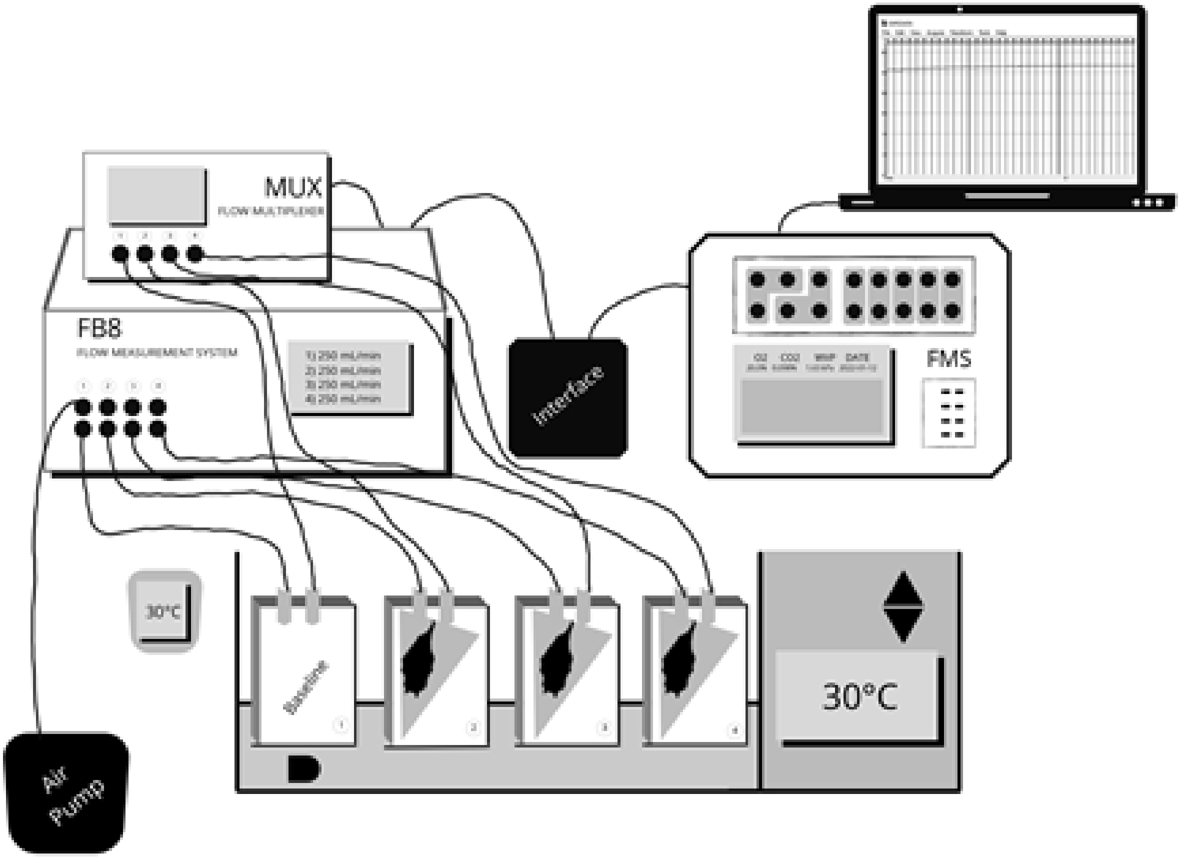

Figure 1: Diagram of the experimental design. A baseline chamber helped to measure data without any bat to have a control of measurement.

During the experiments, we recorded body temperature (Tb) and air temperature (Ta) every 3 minutes.

a. VO_2 – Maximal oxygen consumption

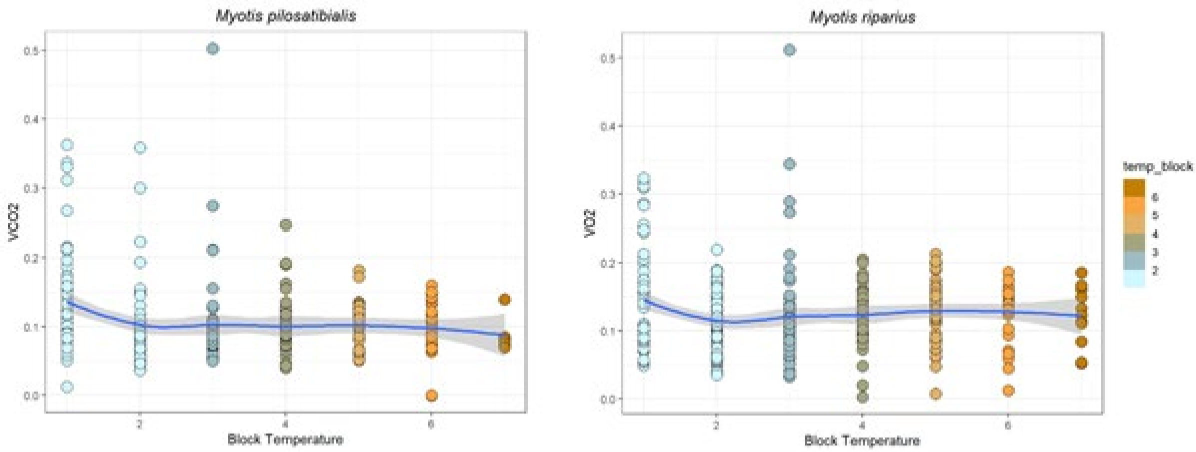

Figure 2: For both species of Myotis, the oxygen consumption stays relatively constant in all the temperatures experimented.

b. VCO_2 – Maximal carbon dioxide production

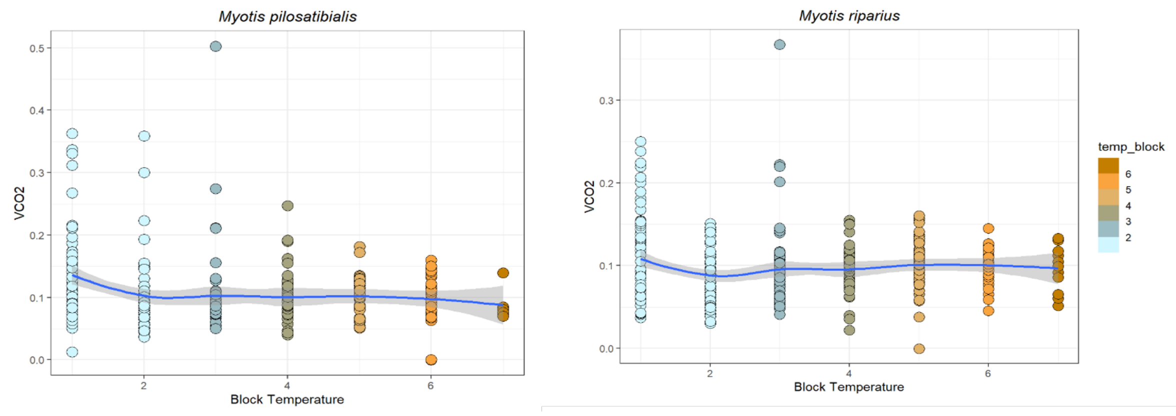

Figure 3: For both species of Myotis, the carbon dioxide consumption says relatively constant in all the temperatures experimented.

c. Respiratory Exchange Ratio (RER, CO_2 production/O_2 uptake)

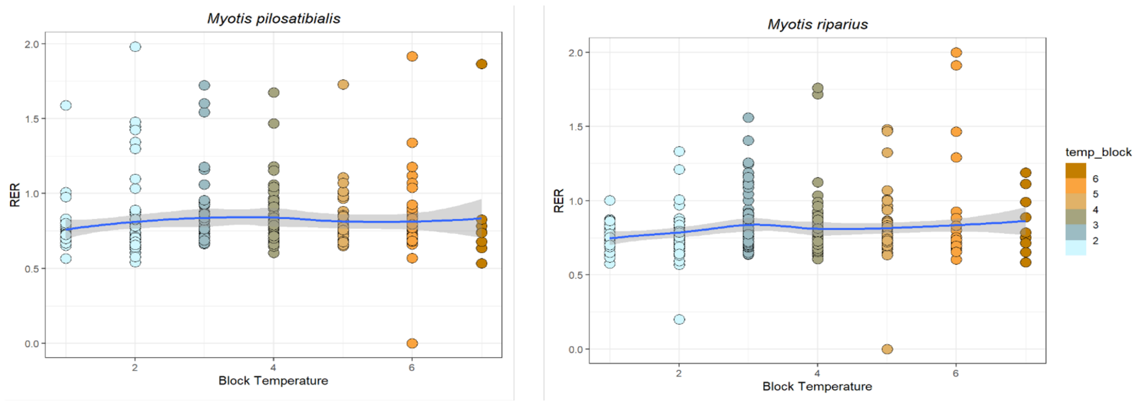

Figure 4: For both species of Myotis, the RER stays relatively constant.

d. Energy Expenditure

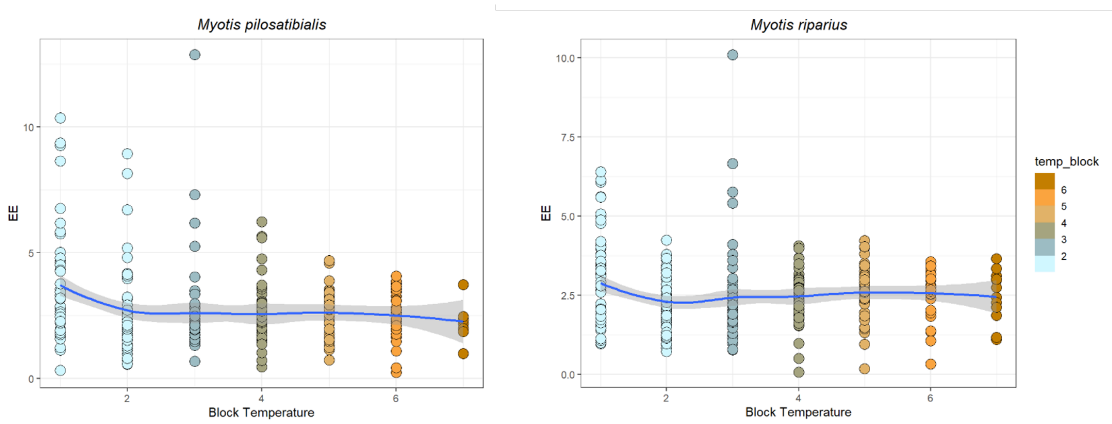

Figure 5: Both Myotis kept constant energy expenditure. The increasing temperature during the experiments did not the energy used on bats.

2) **Bats showed an increase in Water Vapor pressure which can be related to Evaporative Water Loss. This means that this could be the way bats respond to increasing temperatures.**

Bats increased water vapor pressure with Ta (Fig. 6). This suggests that bats are increasing the Evaporative Water Loss (EWL), which we still need to analyse. However, it is possible that bats are responding to increasing temperatures by losing water to dissipate heat.

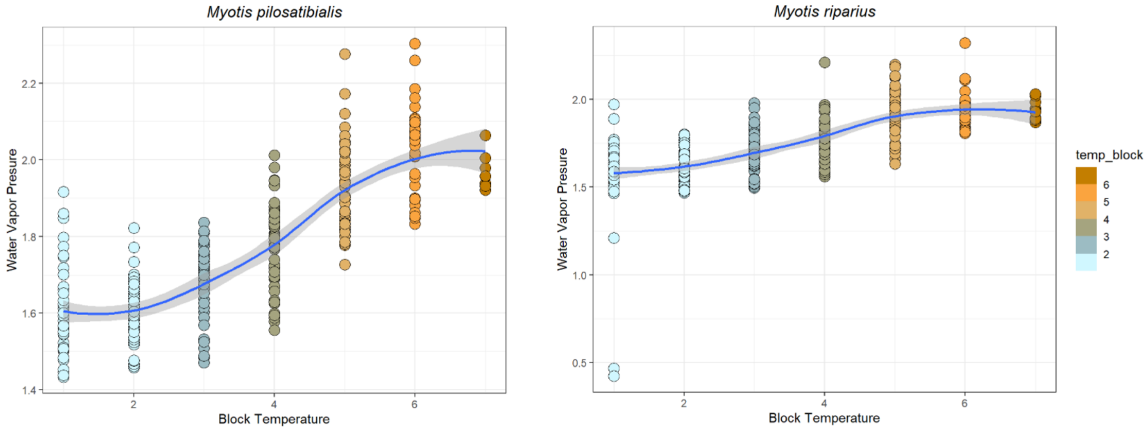

Figure 6: Water Vapor Pressure increases for both species of bats with increasing temperatures during the respirometry experiments.

3) **I did not find big differences in metabolic rates among females and males.**

Myotis species are difficult to capture with mist nets in the forest, so we decided to work with both females and males to have a higher number of individuals to test. Luckily, we captured the same number of individuals for each sex in the two species of *Myotis*. This helped to compare results between the sexes.

Figure 7 shows the trends in VO_2 with increasing temperatures. For the other variables we tested during the respirometry experiments, we obtained similar results, so graphics are not depicted here.

Figure 8 shows trends in WVP with increasing temperatures with similar results for males and females.

This is important to mention because, in physiological experiments, it is not rare to find different reactions depending on the sex of the individual. Since we did not find differences between sexes, we can use all the dataset to analyses the responses of species to increasing temperatures.

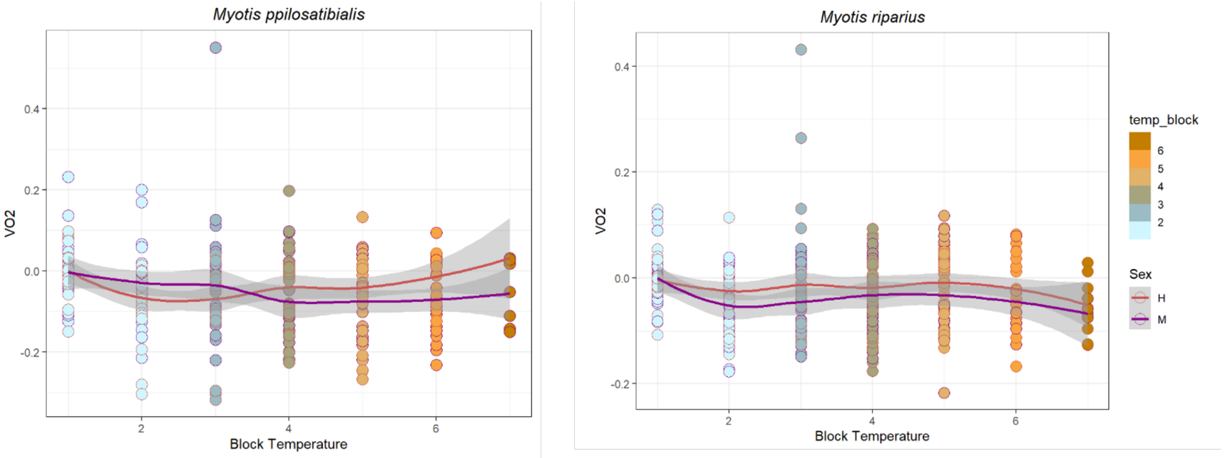

Figure 7: Oxygen consumption of individuals divided by sex. H=males, and M=females. The trend obtained is similar for both sexes, and we did not find significant differences.

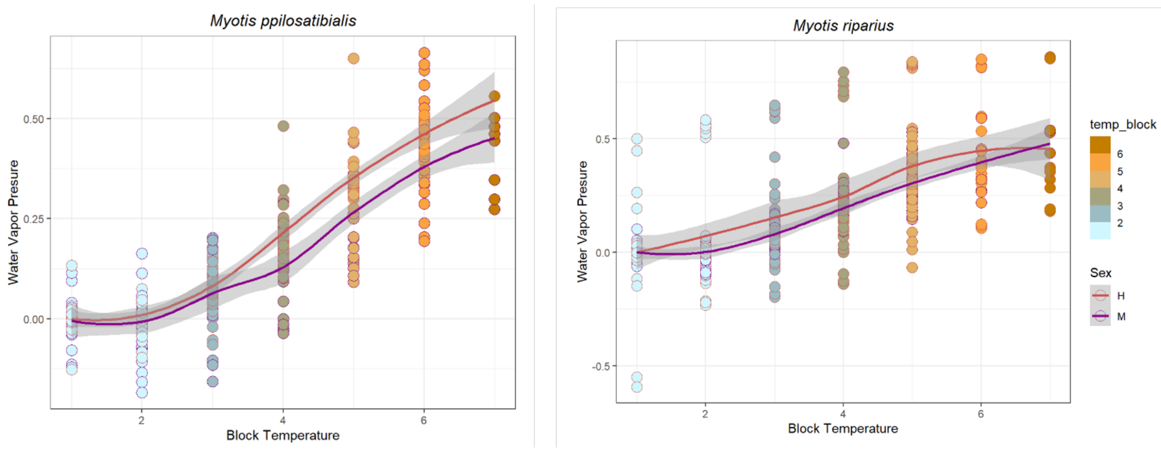

Figure 8: We show here results of Water Vapor Pressure per species by sex. H=males, and M=females. The trend obtained is quite similar for both sexes, and we did not find significant differences.

3. Explain any unforeseen difficulties that arose during the project and how these were tackled.

The main issue was a big delay in carrying out the experiments. Due to COVID-19 it was forbidden for several months to work with bats in the field. I had to come back to my home country for over a year.

This made it difficult to achieve our aim of giving talks to communities. Initially, it was not allowed, and then, I had a shorter timeframe to finish the experiments. My student card was expired, as an Ecuadorian, I can stay in Costa Rica as a tourist for no more than 30 days. The schedule was very tight for learning how to use the equipment and perform the experiments.

The budget was limited for the outreach to communities because we changed the methodology looking forward to more accurate results. We added to the experimental design the use of driedite to dry the air incoming in the metabolic chamber, tubes, air pump, and PIT tags from BioThermo13, Biomark, Inc, to record the body temperature of bats in a more accurate way.

4. Describe the involvement of local communities and how they have benefitted from the project.

Local communities from nearby the Biological Station were not involved, but undergraduate and graduate (MSc) students were. I had the chance to interact with students during fieldwork. Some of them were interested in learning about my research. We did together night sampling activities to catch bats, where I could explain to them bats' characteristics and the main ecological roles. I showed them different morphological features to help them identify different bat families.

During fieldwork, I was also hired for 4 days as an invited professor in a biology undergraduate course. There, I was also able to do some outreach about my project and explain the importance of understanding the ecological responses of animals to climate change.

Finally, my field assistant was very interested in following a scientific path. I trained her on different ways to do experimental design, and I hope this was relevant for her later decision to pursue a master's degree in UCR. I believe that her experience was also relevant for the authorities to make the final decision to accept her into the programme.

5. Are there any plans to continue this work?

The publication of a peer-review paper is in progress. Once it is published, I will also prepare a poster with the main results and make it available for visitors at Las Cruces Biological Station.

We will use these results to determine the sensitivity of bats under specific environmental conditions. We will calculate the sensitivity index using a logit model. Future climate models will be our main source of information to determine areas from the current distribution ranges of the target species that might be less suitable for them according to the sensitivity calculated. For this part, we will work together with Professor Hugo Hidalgo from UCR, who is an expert in climate change and climate variability.

6. How do you plan to share the results of your work with others?

I hope to present the results at an International Congress.

7. Looking ahead, what do you feel are the important next steps?

My project results could explain how insectivorous bats respond to increasing temperatures. Physiological experiments are scarce on neotropical bats since insectivorous bats are heterothermic, they are a great study model to understand their responses to increasing temperatures better.

The fact that the target species in this research are not significantly changing the metabolic rate is an interesting result, as bats may tolerate important increases in temperature. However, we need to do extra analysis to understand why the Water Vapor Pressure (WVP) significantly increases. Bats might be able to tolerate higher temperatures and dissipate heat by eliminating water. Further research will be needed to confirm the effect of temperature on metabolic rates.

8. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

Yes, I have done a few local oral talks to students where I used The Rufford Logo, and in some other talks where I have shared my experience as a PhD student and my path as a science communicator.

I would like to add here that I have done several talks to guide graduate students on how to apply for grants, funding, and stipends during their academic careers. I showed them some relevant questions that organisations ask during the application process. While doing these talks/workshops, the logo of The Rufford Foundation was present permanently.

9. Provide a full list of all the members of your team and their role in the project.

Gloriana Chaverri, Ph.D.
Gloriana is my academic advisor at UCR-Golfito and has been involved in the project since I started my career. She was a great help during experimental design and data analysis. Her experience as a bat researcher has been extremely valuable in guiding me to organize research projects step by step. I would not be able to carry out this project without her wisdom and genuine interest in teaching research strategies and problem-solving.

Natalia Sandoval, Ph.D.
Natalia has had a key role in this project. She was pursuing her Ph.D. at the University of Toronto at Welch's lab (she is graduated now) and has vast experience with physiological experiments. Thanks to her, I could use the Sable System equipment, which gathers accurate data on animals' metabolic rates. This equipment is also perfect for fieldwork as it is designed to work well in humid places. Natalia showed me how to use the equipment and helped me to install, to test it, and gave me guidance on how to solve issues while recording data in the field.

She has also been a great support during data analysis, and I don't exaggerate when I say that she has been my cornerstone in this project.

Ken Welch, Ph.D
Ken is a professor at the University of Toronto, and he runs Welch's lab. He's an expert in physiology, metabolism, energetics, and locomotion. I could contact him thanks to Natalia, who gave the green light for me to use the equipment and have Natalias' help to learn how to use it. He was a great support during experimental

design in solving problems with scripts for data analysis using the Sable Systems software (ExpeData). He is also involved in data analysis for the peer-review publication.

Mariela Sánchez
Mariela was my field assistant. She was an incredible help during fieldwork because she was always keen a ready to sample bats at night, even at places that were at least 45 minutes' walk from the field station. She was very attentive during metabolic experiments, writing comments, body temperature data from the bats, and the perfect alarm clock if something was happening with the equipment. Everything went well in the field, and I am very grateful to her.

Adarli Romero Vásquez, Ph.D.
Adarli is a professor of animal physiology at UCR. She shared with me knowledge about the main concepts and methodologies that are important to understand when working with animal physiology.

10. Any other comments?

Thanks for your patience. I know that I have been slow in sending this report. I hoped to have the final graphics for the peer-review publication before I sent this, but it took longer than expected. Thanks for trusting me and allowing me to carry out this research.