Research Topic: Resource use of endangered bats in Fiji

Abstract:

While the islands of Fiji are relatively small, knowledge of cave bat colonies is still incomplete and ecological studies are in their infancy. Almost all Fijian bat species are threatened with extinction. I am applying a multi-disciplinary approach combining temporo-spatial acoustic-based surveys and genetic analyses to address critical ecological knowledge gaps for the native and endangered Fijian free-tailed bat *Chaerephon bregullae* across fragmented landscapes and islands of Fiji. I am investigating how bat activity and the seasonal availability of food resources vary with land-use types, and cave colony connectivity. I am exploring a single-species derivative of the Habitat Amount Hypothesis (sensu Fahrig 2013) to help understand foraging activity in fragmented systems. The acoustics-based study of foraging habitat preference and diet is centered on the only known colony of *C. bregullae* at Nakanacagi cave, and population genetic work will sample other areas of occurrence outside the putative nightly flight range of the species to examine population structure. The outcomes are critical for conservation strategies for island bats and will inform long-term forest management and conservation policy in Fiji and the wider Pacific region.

Research Questions/Objectives

1. What habitats do Fijian free-tail bats use for foraging?;
2. What is the diet of Fijian free-tail bats, and does this change with the seasonal availability of insects in different habitat types?;
3. Are Fijian free-tail bats found across two islands connected and do they originate from the only known roosting cave (Nakanacagi cave)?

Activities, methods and specific study sites:

Research Question 1:

I will first characterize landscape features (land use type, patch size, patch isolation, total habitat amount and matrix quality and structure) of habitats available for foraging bats in three distance categories: within 0–10 km, 10–20 km, and 20–30 km from the cave. This will be carried out using GIS tools in the

software Quantum GIS on relevant landscape mapping coverages. A multi-season field survey will then be conducted to identify which vegetation types are used by *C. bregullae* as foraging habitats on the island of Vanua Levu. Bat detector recordings will be used to quantify levels of activity and feeding behavior (from feeding buzzes). AudioMoth bat detectors will be deployed for 12 months at seasonality stations within chosen landscape study sites and will be monitored accordingly. Every two weeks, a trained person will visit the bat detectors to change the batteries, change the sd memory cards as well as set up the station again to keep recording for up to 12 months. Acoustic data will be collected and analyzed using a custom-written script in the R statistical computing language (modified from K. Armstrong unpublished) and visualized in the software Audacity. Finally, I will then quantify and compare which landscape features (land use type, patch size, isolation, matrix composition and structure, habitat amount) are important determinants of foraging focus for *C. bregullae* based on bat activity and feeding levels. Observation of crop growth within plantations will also be recorded during the study period and will be used for correlation analysis (bat activity, time, and crop growth) to determine if crop growth stages influence bat activity because of related prey (insect/pest) availability.

Research Question 2: The diet of bats will be sampled at the Nakanacagi cave colony roost site, rather than bats captured at particular feedings sites, so trends in diet will be based on a combined population sample. Bat guano pellets will be collected from plastic sheets spread under areas within the cave where the bats are known to roost. This will be done two hours from dusk (when bats have left the cave to forage). Samples will be collected the morning after, packing pellets into labeled vials with 100% ethanol for preservation. A metabarcoding DNA sequencing approach will be used to identify the prey items eaten by *C. bregullae* across preferred foraging habitats over the wet and dry season in Vanua Levu. Relevant mitochondrial DNA barcoding regions will be amplified using arthropod-specific primers developed to analyze the diet of insectivorous bats (Zeale et al. 2011), which provides sufficient taxonomic resolution of insects for bat dietary studies (e.g. Kolkert et al. 2020). DNA will be extracted from fecal samples using a QIAGEN Faecal DNA extraction kit. The identity of species or higher taxonomic level will be assigned to each haplotype using the NCBI

database nucleotide Basic Local Alignment Search Tool (Altschul et al. 1990) and analyzed further with the freeware MEGAN.

Research Question 3: A high-high throughput genetic sequencing ('DArTseq'; a commercial equivalent to the method 'RADseq' that is available in Australia) will be used to determine the population structure of *C. bregullae*, and whether there is evidence of movement between occurrences on the islands of Vanua Levu and Taveuni. Capture Harp traps will be used close to the Nakanacagi cave entrance to capture bats as they emerge. Canopy-mounted mist nets will be used at Taveuni, Waisali, and Natewa to capture *C. bregullae* since they, like most free-tailed bats, fly far above the canopy and hence will be difficult to catch with harp traps. Mist nets will be arranged in vertical layers in a configuration hung from a rope that is spread between two large trees. Supervisor Armstrong is highly experienced with this technique. To increase the chance of capture further, a custom-made acoustic lure will be used to attract bats into the net. A library of social calls from *Chaerephon jobensis* in Australia is available from Supervisor Armstrong. The lure device is a micro-computer (Raspberry Pi Zero) that is configured to play calls in a continuous loop. The canopy net and acoustic lure method have been used as an effective means for capturing high-flying bats in northern Australia (Hill et al. 2015).

Bat measurements and tissue collection: Once collected from the net, morphological measurements will be recorded, including sex, age (adult, juvenile, or pup), reproductive male or female (lactating). After measurements, a plug of wing membrane will be collected using a clean 4 mm biopsy punch and fixed in 100% ethanol. Genetic analysis Wing biopsy samples will be sequenced for Single Nucleotide Polymorphism (SNP) markers by Diversity Arrays Technology using their 'DArTseq' method, which represents a combination of the DArT genome complexity reduction method and sequencing on a next-generation sequencing platform (e.g. Illumina HiSeq; Kilian et al., 2012). DArTseq enables the production of a lower density of markers with higher coverage and less missing data in comparison to other analyses, including RADseq (Kilian et al. 2012). The data sets generated from the genotyping of individuals will be analyzed for patterns of population structure and connectivity using a simple Principal Coordinates Analysis, and population assignment tests STRUCTURE (Hubisz et al., 2009) and sNMF (Frichot et al. 2014). These analyses will establish whether there is

more than a single genetic group and whether there is likely to be some level of gene flow between them if more than one genetic group is identifiable.

Thermal video recording: In addition to understanding the population structure C.bregullae through latest genetic analysis methods, to help determine the population status, population trends and cave use over the year, thermal camera recording will be carried out at the mouth of the cave using a FLIR EX series thermal camera. Temperature thresholds from previous studies were used to define recording settings for this reason. Video recording will be carried out at dusk (from the first bat fly out) until after 2 hours or when bats are no longer observed leaving the cave. Data recorded will be analysed using both computed count method analysis and verified through manual counts to illustrate population trends throughout the year at the cave and will improve our understanding of how the only known cave is used throughout the year.

Precautions taken to avoid the spread of SARS-CoV-2 between people and lower risk of people exposing bats during research activities?

The "MAP" (Minimize, Assess, Protect mitigation strategy) measures designed by the IUCN Bat Specialist Group (BSG) will be adopted to prevent human-to-bat transmission. The following precautions have been considered in the project activities and work plan. Prior to any fieldwork, the team will undergo training to become familiar with the MAP mitigation strategy and appropriate protective gear will be available for fieldwork. Team members collecting samples will be tested (for COVID) up to 2 weeks before fieldwork. The project largely employs the use of acoustic detectors to understand how landscape features and arrangements affect bat activity and resource use across the year. Most of the data collected and analyzed for the project will be acoustic data. As such, there will be very little contact with bat species. Guano and tissue collection will be delayed until the latter half of next year (2021) when we expect more information to be available or a vaccine provided. Should this not be possible, and sample collection cannot be delayed further then: 1. Guano will be collected with sheet preparation ending a minimum of 1 hour, 2 hours after the period of emergence, and sheet contents collection at least 1 hour after sunrise. Prophylaxis (masks and gloves) will be worn when working within 100 meters of the cave entrance. 2. Wing biopsies will be collected at only four sites (reduced from 8 considered initially). Masks, gloves, and face shields will be worn by all personnel handling and observing bats.

Expected Outcomes:

1. A better understanding of critical habitat and factors that affect the ability of bats to effectively use these resources.
2. A better understanding of their dietary needs and trends observed across season based on food availability and correlating plant phenology
3. A better understanding of connectivity across subpopulations and an indication if there are other caves available that we do not know about and should manage.

Significance:

1. New critical ecological information acquired. Filling in these essential ecological knowledge gaps about critical habitat and landscape factors, diet, as well as population status and structure significantly increases the efficiency of future conservation plans and efforts to save this species from further population decline and even extinction.
2. A better understanding of the effects of landscape factors on this bat's survival will help inform future sustainable development of forests and resources (contribute to forest management strategies).
3. Understanding their diet will help improve our understanding of their role in agricultural and forest systems (pest reduction services?). This will help strengthen conservation messages and may encourage increased "buy-in" from farmers and local resource users and policymakers in the area.