Alice McCosh Report

Introduction

Difficulty in gathering ecological information on tropical mammals has resulted in the IUCN being unable to classify the conservation status 861 species and instead labelling them as “data deficient” (Schipper *et al.*, 2008). This clearly illustrates the need for an expanded set of tools that ecologists can employ to gather data on species occurrence in the tropics. Genetic sampling, such as invertebrate-derived DNA (iDNA), is one such method emerging as a valuable non-invasive tool for monitoring single species and biotic communities.

iDNA utilises coprophagous, necrophagous and haematophagous insects (Calvignac-Spencer, Merkel, et al., 2013) and leeches (Schnell et al., 2015) to sample DNA from the wider ecosystem. Once the invertebrate has been collected, PCR and metabarcoding techniques can be carried out to identify the species that the invertebrate has been feeding on. Whilst robust protocols have been developed for the use of live-capture, camera trapping and line transects to assess mammalian occupancy, to date there are no such protocols for collecting iDNA samples in the field. Previous studies have used vastly different sampling methods and analysis techniques meaning comparisons between studies are difficult to draw, and studies are difficult to replicate. In order for iDNA to be used more widely the field techniques must be optimised and standardised to limit cost and effort and develop a competitive, repeatable method.

The key focus of this project is to develop robust field protocols for using invertebrate-derived DNA (iDNA) to assess mammalian occupancy and ecology with a particular reference to elusive and rare species. Depending on the investigation and target species it may be beneficial to collect different invertebrate samples, just as you would choose different standard monitoring techniques for rodents and elephants. Previous studies such as Drinkwater *et al.*, (2018) and Weiskopf *et al.*, (2018) have compared the feeding preferences of different leech species. No study to date has compared the effects that different dipteran feeding strategies have on iDNA studies. Grant money from the Alice McCosh trust has enabled an initial study of these feeding preferences to be carried out at zoos across the UK. The results have provided me with good baseline data which I can now expand on.

Methods

Money was used to carry out an initial pilot study to explore the most effective method for catching engorged female mosquitos. Most standard mosquito traps use baits and lures however these largely capture unfed females which would be of no use in iDNA studies (Silver, 2008). Resting boxes and gravid traps were built and set overnight in 9 locations. The mosquitos were aspirated from the resting boxes and the gravid traps before being sorted into engorged and unfed. The gravid traps returned the best results as they trapped a higher percentage of engorged to unfed mosquitoes. They work using stagnant water as a lure which female mosquitoes are attracted to after they have fed to lay their eggs. Once near the water a tube and fan draws them up into a collection box (figure 2).

Grant money from the Alice McCosh Trust was then used to collect invertebrate samples from zoos around the UK. Zoos provided an excellent artificial system where mammals with a range of diets inhabit a small area, enabling the comparison of invertebrate feeding preferences. Three mosquito traps, and three canopy traps baited with fish to attract blowflies were placed in Exmoor, Paignton, Newquay, Welsh Mountains and Chester zoos, giving 15 trap days per invertebrate. Invertebrate samples were collected and dried with silica before being transferred to the lab at the University of the West of England. DNA was then extracted from the invertebrates by cryogrinding them with liquid nitrogen and subsequently using a DNA extraction kit from Zymo. After the first round PCR using 16S mammal primers was carried out at UWE, the samples were sent to Source Bioscience for sequencing on their Illumina Miseq.

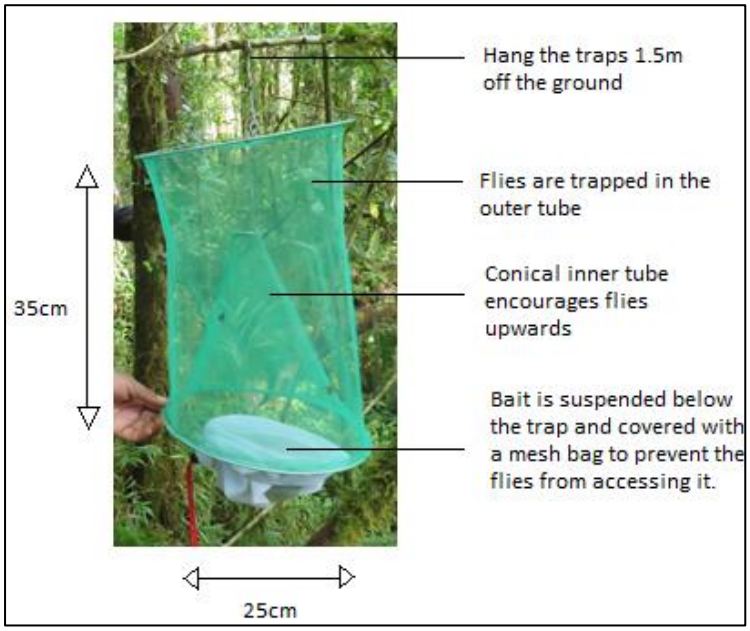

Figure 1 Canopy trap used to collect blowflies

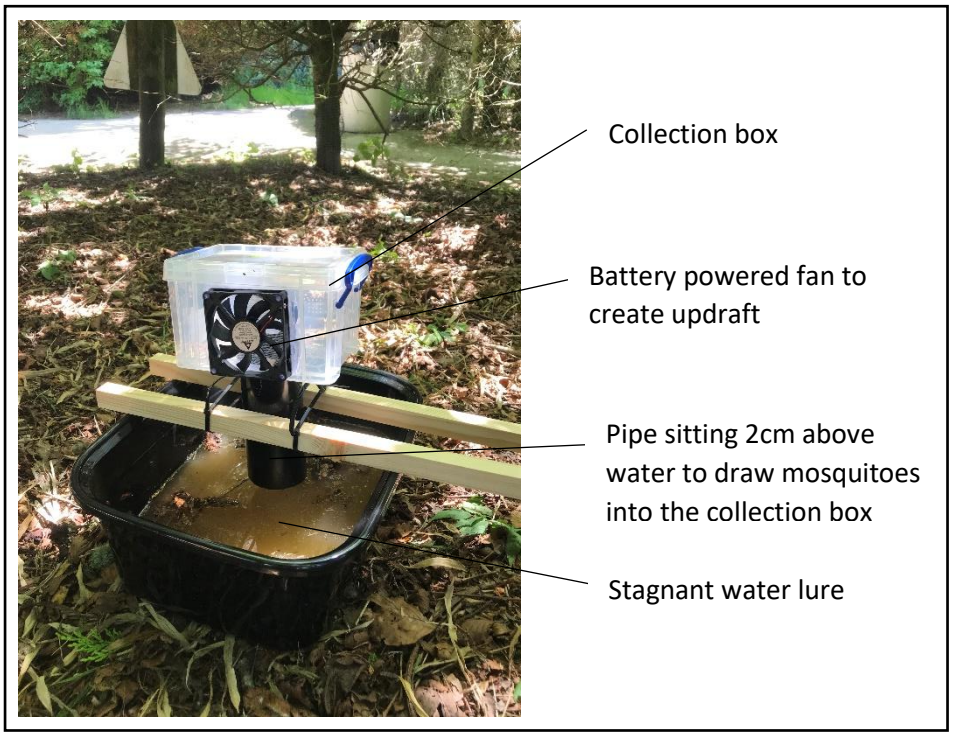

Figure 2 Gravid trap used to collect mosquitos

Results

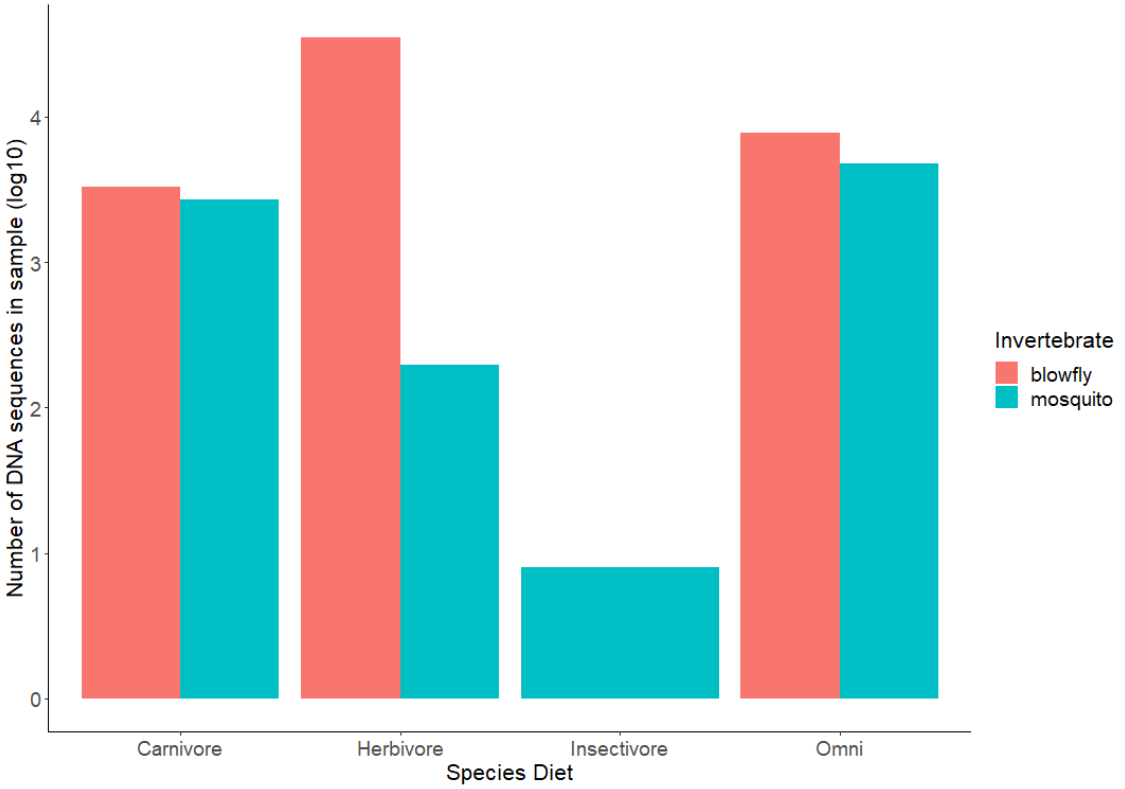

Figure 3 A comparison of invertebrate feeding preferences in relation to the diet of the mammal they have fed from. Overall, more DNA was recovered from blowflies, however mosquitoes fed on a wider range of mammals.

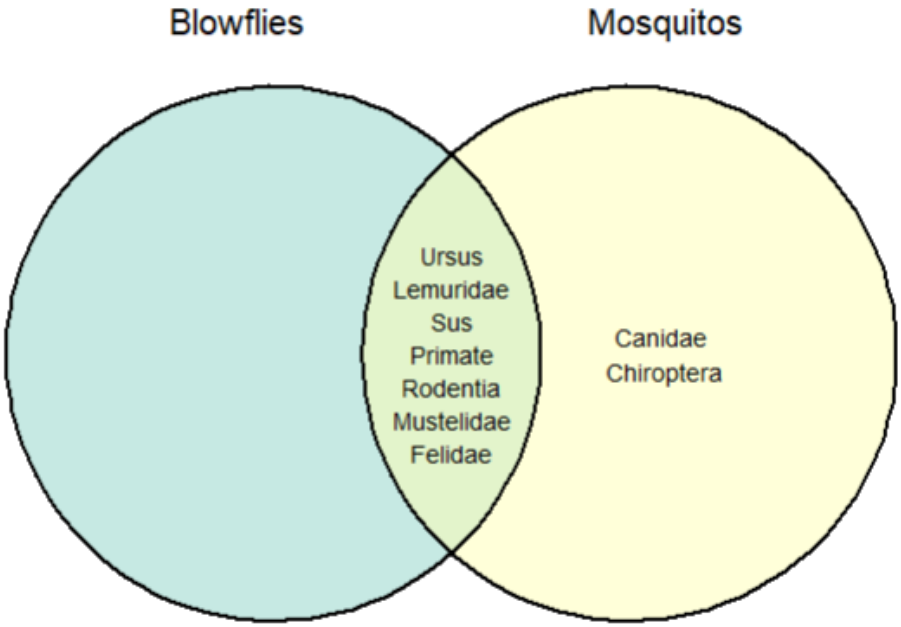

Figure 4 A Venn diagram illustrating overlap in the families that both invertebrates fed on. Only mosquitoes were found to have fed on canids and bats

Discussion

The data displayed in figure one illustrates that mosquitoes have less a less specific diet as they fed from more species (n=13) compared to blowflies (n=10) Blowflies were easier to catch and therefore provided more data, however mosquitoes fed on a wider range of species, suggesting that with increased sampling effort mosquitoes would be a more effective invertebrate for sampling mammalian communities in the field. The greatest difference in feeding was seen in the herbivore group where blowfly samples returned significantly more DNA. This is likely because herbivore scat is very attractive to blowflies compared to the other groups, which include species such as bats and canids, which have smaller, less attractive, scat.

The Venn diagram (figure 4) illustrates more clearly the wider range of species that mosquitoes fed on in comparison to blowflies. Blowflies didn't feed on any families that mosquitoes didn't, whereas mosquitoes fed on both bats and canids. Despite mosquitoes being more difficult to catch, this research has shown that they are a better invertebrate sampler for understanding mammalian diversity as they fed on a wider range of species. Increased sampling effort will enable more mosquitoes to be caught and lead to higher data yields.

Unfortunately, the 16S mammal primer used was only able to identify species to family level. Literature suggests that combining multiple primers would enable higher taxonomic resolution of the invertebrate's diets. This work, enabled by the Alice McCosh grant, has led to further funding being granted and the research continuing. The samples will now undergo additional sequencing to include 2 more primers. The higher taxonomic resolution of the results will then allow for more detailed analysis.