



Operation Wallacea Cusuco National Park, Honduras 2015: End of Season Report

20 January 2016

This end of season report is submitted as a review on the summer 2015 season and the research activities of the Operation Wallacea research teams in Cusuco National Park during that season. This report contains a summary of the methodologies and surveys employed, in addition to the data collected during that time, and a complete analysis of that data as part of this complete report.

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1. Introduction

This report provides an overview of the results of the Operation Wallacea research programme in Cusuco National Park to date. Here we present a summary of the survey effort completed during the 2015 field season and provide a complete report of the data collected and analysed from this season and present ways forward for our research in the coming summer of 2016.

Each year, the Operation Wallacea research teams survey Cusuco National Park (CNP) in North-Eastern Honduras, where a select group of taxa are monitored in a standardised way to evaluate ecosystem quality and change. Complementary observations on selected other taxa are collected, striving towards a more complete overview of biodiversity in CNP. Additional research projects are completed to better our understanding of the cloud forest ecosystems and its ecology. Cloud forests are hydrologically and biologically unique ecosystems with high diversity and endemism. CNP has been identified as one of the world's top 100 irreplaceable protected areas for conservation of amphibians, birds and mammals (le Saout, 2013). Despite this world wide importance, large parts of cloud forest biodiversity remain unstudied and unknown and cloud forests are one of the most threatened habitats in Central America. In Honduras all mountain habitats above 1800m have been legally protected since 1987, based on a decree that was issued to protect the source of drinking water in Honduras. The established National Parks in Honduras, however, often lack effective protection, and this is, unfortunately, true for Cusuco National Park.

After a reconnaissance expedition in 2004, Operation Wallacea established an annual research project in CNP that centres around a monitoring program of selected cloud forest taxa. Monitoring data is collected on sampling points along transects equally divided over seven camps. Sites are selected to cover as broad a range of habitats in CNP as possible, but with the main focus on the mid to high elevation forests. Monitored taxa include dung beetles (Scarabeinae), jewel scarab beetles (*Chrysina* sp.), Sphingidae and Saturnidae moths, amphibians, reptiles, birds, large mammals with special attention for Baird's tapir, small mammals, bats and plants. Additional projects include bromeliad associated aquatic invertebrates, dragonflies, spiders and their allies, crabs and epiphyte communities among others. In addition to the monitoring, specialised research studies are completed to generate data facilitating the management of the Park. These include a wide range of projects, such as the development of an aquatic biotic index that can be used in the Merendon mountain range to monitor water quality. Another project is focussed on the incidence and possible methods of transmission of the Chytrid fungus (*Batrachochytrium dendrobatidis*) between amphibians.

The monitoring data, up to 2010, have been combined with information gathered from buffer zone communities, collected during the 2008-2012 field seasons, and remote sensing data to produce a Natural Forest Standard (NFS) report for Cusuco National Park. NFS is a voluntary carbon standard that integrates social, biodiversity and carbon values for REDD natural forest projects. This report will document the state of CNP in terms of carbon tonnage and biodiversity, but will also outline plans and associated budgets for forest patrols to protect the remaining forest and biodiversity as well as a sustainable development project with buffer zone communities, aimed at combating poverty and reducing community reliance on forest resources.

2. Camps and transects

Eight camps are/have been used in Cusuco National Park, two in the 'buffer zone' and six within the core area of CNP. At each of the camps three to four transects have been installed and sample sites positioned along these route (Figure 1). The steep terrain posed limitations on the sample site locations, so sites were installed wherever possible as long as they were a minimum of 200m apart. For labelling purposes, the camps have been identified with a two letter code (BA = Buenos Aires, BC = Base Camp, CA = Cantiles, CP = Capuca, CO = Cortecito, DA = Danto, GU = Guanales and ST = Santo Tomas). An additional camp used in the 2011 season, in the water protection zone (LP = Las Piñitas) is no longer surveyed and Santo Tomas is only subject to very limited surveying since 2014. The transects are numbered (1-4) and on each of the routes the sites are numbered sequentially starting from the camp. Thus BA3/3 is the third site along transect 3 at Buenos Aires. Close up maps of each camp and associated transects and survey sites are provided in appendix 1.

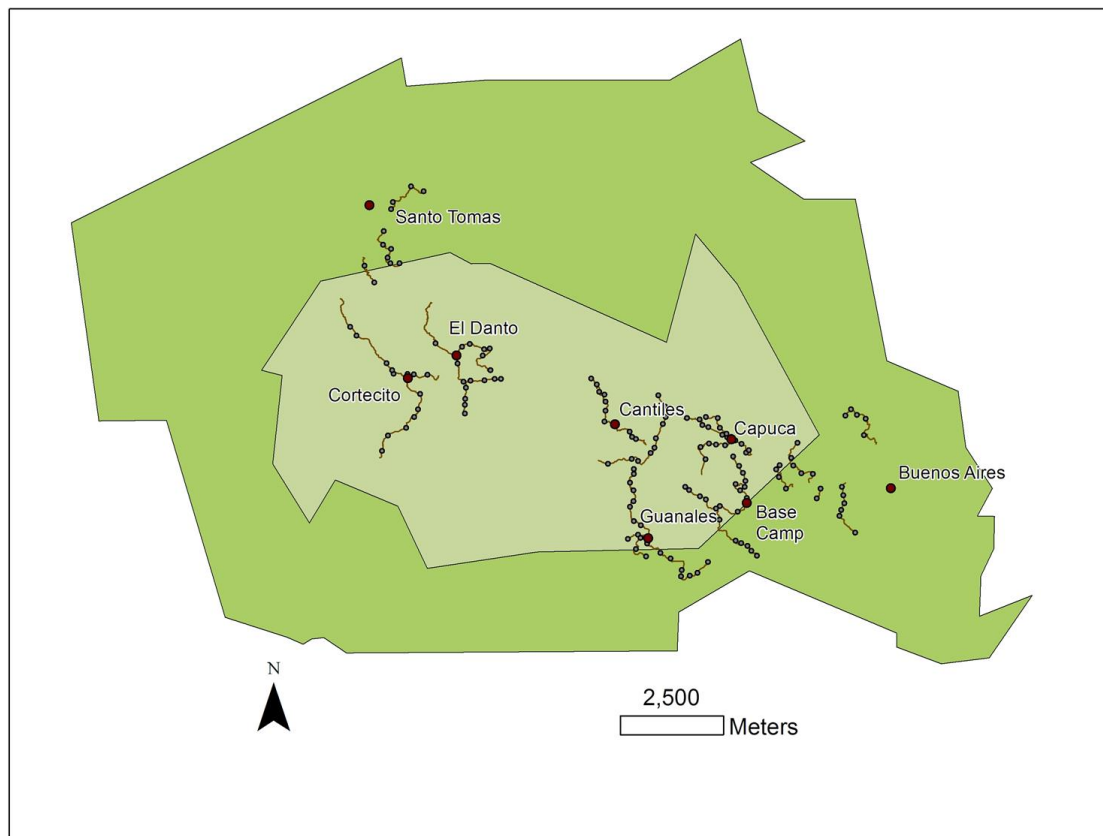


Fig.1. Map of Cusuco National Park Buffer Zone (outer green area) and Core Zone (inner green area), showing Operation Wallacea caps (red circles), transect network (lines) and standardised survey locations (small green circles).

3. Climate and habitat assessment

3.1 Climate data

Every camp has a rain gauge and a HOBO temperature and humidity data logger deployed during the period that the camp is being operated. The precipitation in the rain gauge is measured every 12 hours (once at 7.00AM and once at 7.00PM). The data logger records values every 30 minutes.

3.2 General habitat assessment

Environmental data are collected at the established Sample Sites (SS) and at Habitat Plots (HP) along transects to characterise the habitats. Measured variables characterise the soil (leaf litter depth, soil horizon width and soil density), epiphyte density, number of saplings and the vegetation density in the plots. The vegetation is categorised as none (open), broadleaved, pine, palm, bamboo, fern, dwarf pine and tree diameters are recorded. Canopy cover and epiphyte density is recorded. More information can be found in the habitat and environmental data collection protocol.

3.3 REDD+ carbon assessment

As part of the general habitat assessment a stratified sample of at least 120 habitat plots are surveyed throughout CNP. Habitat plots are located along the transects. Each habitat plot is 20m x 20m in area. Within each plot, every standing tree (alive or dead), fallen trees and cut stumps over 15cm in circumference are measured. Tree diameter at breast height (DBH) is measured over bark at 1.3m above the ground. Tree height is calculated using a clinometer and a measuring tape to calculate the distance from the base of the tree and the angle from this point to the tree top. A full description of the measurements taken can be found in the Habitat Survey Protocol. For each tree measured, the corresponding tree species is identified and the state of the tree (alive or dead) recorded. If tree species cannot be determined, then trees are identified to the most accurate level of classification possible (genera or family).

For each tree (live and dead, upright and fallen) in each habitat plot, the DBH and height values are used to calculate tree volume. By referencing published wood density tables, it is possible to determine the density of each tree species recorded. Using these data, it is possible to calculate carbon biomass for each tree and thus for each habitat plot. Once the carbon biomass for the 120 different habitat plots has been determined an estimation of total carbon biomass of the study area can be calculated based on the mean carbon biomass value for a given forest type and the proportion of these forest type present in the study area.

4. Biodiversity monitoring

The main purpose of the monitoring program is to collect standardised data on focal taxa to document changes in the ecosystem over time. Surveys follow a standardised protocol and data collected during the field season is entered in the CNP MS Access database before the end of the season. A brief overview of survey methodologies is presented here. Please consult individual survey protocols for details on the recorded variables.

4.1 Amphibians and reptiles

Amphibian and reptile data are collected on transect surveys during the day, opportunistic night walks and with opportunistic pitfall traps. Population densities of some species are calculated from data collected with capture-recapture surveys in selected river stretches. Specimens are only

collected if field identification is inconclusive and a voucher specimen is needed.

4.1.1 Distance sampling on transects

Each of the sample routes at all camps are searched for amphibians and reptiles during daylight hours, generally starting between 8:00-9:00h AM. For all observed animals the distance along the transect is recorded as well as the perpendicular distance to the centre of the transect. Snakes are preferentially identified from a distance. Remaining amphibians and reptiles are captured, whenever possible, in order to collect data on sex, weight, snout-vent length (SVL) and to photograph the specimen for later confirmation of the identification. Photographs are taken of the back, side and close up of head. The survey effort is quantified in time (marking start and end time for each survey), the number of participants and distance (length of the transect surveyed).

4.1.2 Night surveys

Additional observations are added to the day transects by opportunistic surveys both during the day as well as during the night. Additional time is used to search complementary optimal habitats not covered in the sample route surveys (e.g. rivers, forest edge) at night when amphibians are most active. The same information is recorded for each specimen as in the daytime survey. Total search time for each survey session is recorded as well as the number of participants.

4.1.3 Pitfall trapping

In addition to transect and opportunistic visual encounter surveys, an opportunistic pitfall trap is often installed near each camp and checked daily, each morning, over the 8-week survey period. This method produces records for fossorial species not recorded from other surveys.

4.1.4 Population density surveys

For a select group of species (*Plectrohyla exquisita*, *Plectrohyla dasypus*, and *Duellmanohyla soralia*) population densities are estimated based on capture-recapture data. A selected river/stream track (of about 200m) in each camp is surveyed three-four times at night during the season to estimate population densities. All animals encountered are caught and photographed (back, side and close up of head) so that individuals can be recognised from their unique patterns and markings. From the data collected during these surveys a population estimate for that area can be calculated. The survey effort is quantified in time (marking start and end time for each survey) and the number of participants.

4.2 Birds

Bird communities are monitored using a combination of point counts and banding of birds at fixed/constant effort mist netting stations. The combination of these two techniques provides a more complete overview of the bird communities present in CNP, as a detailed insight in the population fluctuations and also community structure across altitudinal and land-use gradients. Mist netting has an element of inherent bias, by only providing a sample of the species present in the understory (e.g. it will not sample canopy and mid canopy species adequately) and captures are unlikely to reflect relative abundance of non-understory communities. However, the use of mist nets provides important quantitative information for these species, including sampling species that are inconspicuous or seldom vocal and thus often missed in point counts. The use of mist nets minimises observer bias and produces results that are easily repeatable. Furthermore, the recent initiation of a constant effort mist-netting protocol (as of 2012) will provide important data on productivity, survivorship, phenology and longevity of a number of species.

Assessing bird diversity from point counts by recording all species detected requires a high level of observer skill, considering diversity in the park is high (250+ sp. recorded in CNP). Variation between observers can be substantial in this type of survey, dependent upon experience and skill. The initial week at Basecamp is spent training members of the bird team, where protocols for bird banding/mist netting and ageing/sexing neotropical bird species in the hand are discussed and practised. Subsequently, the team is split into three pairs of bird banders and single bird team members that will conduct point counts only. Bird team members will rotate between teams so must be proficient in each methodology (although individual strengths will also be utilised). Overall, a total of 5 fixed banding sites are present at the 5 camps. Banding teams work simultaneously in two or three camps, using ten 12-meter mist nets per camp. Each station must have at least 6 visits (banding days) per season. Banding is not conducted on successive days to remove observer effects of 'net shyness'. This allows relatively constant capture rates with birds experiencing less stress as a result (particularly regularly captured breeding individuals). Each banding day, ten nets is operated for 6 hours after opening time (dawn). This will make a total of 36 hours (360 net hours per week).

Table 1. Proposed bird indicator species for CNP

Common Name	Latin Name
Common Bush-Tanager	<i>Chlorospingus ophthalmicus</i>
Slate-coloured Solitaire	<i>Myadestes unicolor</i>
Grey-breasted Wood-Wren	<i>Henicorhina leucophrys</i>
Black-headed Nightingale Thrush	<i>Catharus mexicanus</i>
Chestnut-capped Brush Finch	<i>Arremon brunneinucha</i>
Yellowish Flycatcher	<i>Empidonax flavescens</i>
Slate-throated Redstart	<i>Myioborus miniatus</i>
Spotted Woodcreeper	<i>Xiphorhynchus erythropygius</i>
Spectacled Foliage-gleaner	<i>Anabacerthia variegaticeps</i>
Green-throated Mountain-Gem	<i>Lampornis viridipallens</i>
White-breasted Wood-Wren	<i>Henicorhina leucosticta</i>
Emerald Toucanet	<i>Aulacorhynchus prasinus</i>
Brown-capped Vireo	<i>Vireo leucophrys</i>
Collared Trogon	<i>Trogon collaris</i>
Highland Guan	<i>Penelopina nigra</i>
Resplendent Quetzal	<i>Pharomachrus mocinno</i>
Blue-crowned Chlorophonia	<i>Chlorophonia occipitalis</i>
Nightingale Wren	<i>Microcerculus philomela</i>

4.2.1 Point counts

A minimum of three 10-minute point counts must be completed at each of the survey points on each transect at all camps throughout the season. Point counts must be completed between 05:30am and 09:00am. In the event of heavy rains or strong winds that impede the accuracy of the survey, activities are cancelled. On all surveys the weather conditions at the time of the point count are recorded. On arrival, a settle period of one minute is allowed prior to commencing the survey. The count is subdivided in 2- 5 minute intervals where all species detected are recorded. For the duration of the count (10mins), for each contact observed, the following details are



recorded: species, audibly or visually detected, approximate distance from the observer (to the nearest meter) and any behavioural observations considered important.

To fulfil the objectives of the protocol and monitor the population trends of the avifauna with a variety of different team members, a number of indicator species have been identified that are potential cloud forest indicators specifically for CNP. These species were selected based on their presence and absence in habitat types and from prior knowledge and experience from ornithological team members. Additionally, they are readily and distinctively detected in the field visibly and audibly. Further analysis to ground-truth this is currently being conducted.

4.2.2 Bird banding

Bird banding is performed at permanent banding stations in each camp. Nets are checked every 40 minutes or less, dependent on climatic conditions. Captured birds are extracted and placed in individual cotton bags while waiting to be processed. Birds are banded with uniquely-numbered aluminium rings (size according to species). Important morphometric, condition and breeding status data are taken:

- Maximum wing chord
- Maximum Metatarsal length
- Tail length
- Mass and Fat Scores
- Breeding Status
- Age and Sex

Accurate ageing of species in the Neotropics is still challenging and largely understudied. As a result, banders will take some time in attempting to age each individual using the cyclical-based ageing 'WRP' terminology. Standardised sets of photographs for all captured birds are taken for data checking purposes and future reference. Birds are released close to the net site but far enough away to avoid their immediate re-capture. Abundance and community composition are compared between habitats and used to supplement data collected during point-counts. Bird welfare must always take priority. Occasionally, not all data can be collected on captured individuals. In such instances, important data (e.g. wing length and mass) will be prioritised. This is particularly the case for hummingbirds, considering their high metabolic rates and relative fragility.

All information is noted on the provided bird banding data sheets. Furthermore, separate data are collected on net-effort hours and opportunistic observations of non-captured species during banding hours. After a banding session, nets are furled or taken down. Nets are set-up on days prior to a banding cycle at a given camp and left furled overnight, easing early morning set-up times. Data are checked after each session for minor mistakes and entered as promptly as possible in the Base Camp system.

4.3 Bats

Bat communities are surveyed with mist netting at fixed netting stations (2 in each camp, and four in basecamp). Following an initial training week at Base Camp, mist net surveys will run 6 nights per week and will take place at up to four different camps simultaneously. At each camp, narrow (< 1 m wide) trails are cleared in suitable patches of forest to place five 6m long mist nets, each 2.5 meters high, providing a total netting area of 75m². Two permanent mist netting sites are used



per camp, each one as close to the main survey site as possible. Each mist netting location is marked and the GPS location recorded. Mist netting is conducted between 6:00pm and 12am giving rise to a netting effort per site per night of 450m^2 (6 hours x 75m^2). Therefore, the total netting effort for each camp in any given week is 36 hours or $2,700\text{m}^2$.

The nets are checked every 15 to 20 minutes during the first 3 hours of sampling and every 30 minutes for the last three. All the bats are extracted from the nets following standardized protocols so as to minimize the stress and are kept in a capture bags for 30mins, maximum. This time will vary depending on the size of the bat and the sex; pregnant females are measured and released. Bats are weighed, sexed, and the length of the forearm, feet and leg are measured. We will also continue to collect data on parasite-host specificity across the sites. Hair samples from the dorsal fur are taken (between shoulders) to correlate size of parasites and fur size. Small wing punches are also taken from some individuals and stored in ethanol for genetic analysis.

4.4 Large Mammals

4.4.1 Transect surveys

Large mammals are surveyed in the park along line transects using presence and absence methodology. Sample routes up to 3 km in length are surveyed over the season in accordance with the guidelines established by MacKenzie (2005). Large mammal occupancy is recorded through detection of dung, tracks, visualization, vocalizations, and characteristic leaf rustling. Surveys are focused on Baird's Tapir, but evidence of the presence of any large mammals are recorded. Digital images and GPS locations of tracks, spoor, and scat are also recorded. Scat and spoor samples are collected for different analysis including DNA sampling, parasitology, and seed dispersion projects. Survey teams will complete three replicates of each transect and complement the survey with off transect data collection. Survey is quantified by distance (measured by GPS). This multi-season multi-species analysis of the large mammal population will aid in understanding hunting and encroachment issues, and is a key component in conservation and management in the park. Any hunting platforms encountered, snares or encounters with groups of locals trekking through the forest should be noted as relative indicators of hunting pressure between years.

4.4.2 Camera traps

A total of 30 camera traps (Bushnell Trophy CAM HD) are placed along the ~3km transects associated with each of the 6-7 camps. Sampling effort is double in the core zone of the park (Cantilles, El Danto and Cortecito) than in the buffer zone (Buenos Aires, Basecamp and Guanales) as large mammal abundance and activity are significantly higher in more remote regions. After each inter-camp movement, as many camera traps as possible are erected within 2 days, and left *in situ* for only 3 days before collection in the last 2 days of each week prior to movement to another camp the following week. This highly intensive and physically demanding protocol maximizes the number of independent locations at which data are collected to contract to 2014 where camera was left *in situ* for four weeks. Large mammal detection rates will be compared between on and off transects and between the core and buffer zones of the park.

4.5 Dung beetles (Scarabaeinae)

Dung beetles are surveyed with the use of pitfalls traps set out on all transects during the season, aiming for a minimum survey effort of three weeks for each transect. Over the years OPWALL has accumulated probably one of the largest datasets of dung beetles with species level identifications in Central America, particularly valuable considering the elevational gradient covered.

Four dung baited pitfall traps are installed at every site in a 2x2 grid, separated by 5m from the edge and 10m from each other. Traps are buried in the ground so that the lip is flush with the soil surface. The cups that make up the trap are 4-5 inches in diameter, and two cups should be placed one inside the other to form a single trap, to make emptying traps easier. Cups should be $\frac{3}{4}$ filled with killing fluid mixture (either saturated salt solution or propylene glycol mixed with water and detergent). A plate should be placed over the trap opening, supported by twigs, to protect from rain. Bait should be suspended slightly above the trap, with no part of the bait touching the side of the cup. Bait should be formed from approx. 25g of fresh horse or mule dung, wrapped in muslin or similar fabric and tied to form a ball. Excess string from tying can be used to hang the bait. Especially fresh dung should be squeezed of excess water before bait-making. Dung should be no more than 24-36 hours old. Traps should be emptied by pouring through a fine strainer into another cup. Killing fluid may need to be returned to the trap and further pourings carried out to ensure all of the contents of the trap are collected. Some scarabs are less than 5mm in length, so care should be taken to ensure everything is collected - stubborn specimens can be collected using a fine brush or with a gentle stream of water. The strainer should then be carefully emptied into a suitably labelled Whirl-Pak bag, as above. Killing fluid should generally be reused, although if it has been excessively diluted by rain water or contaminated by rotting individuals, it should be discarded and replaced with fresh. Dung baited pitfall traps should be left for at least three days before collection and re-baiting. Each site should have a minimum of three collections over the season.

4.6 Jewel scarab beetles (*Chrysina* spp. and relatives) and moths (*Sphingidae* and *Saturnidae*)

Jewel scarabs and selected groups of moths are surveyed with light traps on a fixed location at each camp. Light traps consist of two 2m squared sheets and a mercury vapour bulb (125W) powered by the camp generator. One sheet is placed flat on the ground with approx. 10cm of the edges rolled inwards. The other sheet should be suspended about 1.5m from the ground, either from a tree branch or from a rope tied between two trees or sticks. The second sheet should form the vertical section of an L shape with the sheet on the ground, although slightly curved or diagonal to form an obtuse angle between the sheets. The light bulb should be suspended around 50-80cm in front of the vertical sheet, at a height of about 1 metre. The light trap should be run for about 2 hours in a single trapping session, from 7.00pm to 9.00pm. Light traps should be run at least 4 times a week at each camp more if time and weather allows. In Buenos Aires camp, a car battery and a 40W florescent tube should replace the generator and 125W MV bulb. Light collecting should be undertaken as far from the generator and centre of camp as the available wiring allows.

Jewel scarabs attracted to the sheets should be captured and placed in a container alive. During the session or at the end, jewel scarabs should be identified as far as is possible according to the provided guidebook and checked for marks. Any unmarked specimens for which a definitive identification cannot be achieved should be placed in a suitably labelled Whirl-Pak half filled with ethanol to kill the specimens. At the end of the trapping session, excess ethanol should be removed for later use and the Whirl-Pak bag closed and stored as above. Moths of the families *Saturnidae* and *Sphingidae* should be collected by hand or net from the sheet. Each specimen should be killed by injection of ethanol, then stored in a labelled envelope. Envelopes should be stored in a waterproof box and returned to the Base Camp fridge as soon as possible. Any other beetles of interest should also be collected in 75% ethanol, in particular longhorns and click beetles. Any relevant environmental conditions should be recorded in the logbook.

5. Additional biodiversity surveys

5.1 Small mammals

Sherman's small mammal traps are used to survey the small mammal communities in CNP. Transects of paired traps set at 5m intervals for 20 metres (i.e. 10 traps) are used. Peanut butter/oat mix is used for bait. In each camp one transect is placed in the forest and one along the river. Transects are run for four nights in each camp. The objective is to get standardised abundance data per year to look at temporal trends.

During the last four years, three specimens of an unknown water mouse (*Rheomys* sp.), a suspected new species to science, have been caught opportunistically on the Rio Cusuco near Basecamp. Captures were largely incidental in either crab traps or in traps baited with peanut butter or tuna set for other species (e.g. the Mexican deer mouse). During 2014, two specimens were caught quickly after using freshly prepared crab meat from animals collected nearby the trapping locations in new, clean traps. It seems likely this is the optimal strategy for this highly elusive species. Thus, an additional objective in 2015 was to focus trapping on small streams at all camps using crab meat as bait to establish the species' range and distribution and collect more specimens for species description.

5.2 Dragonflies (Odonata) and butterflies (Lepidoptera)

Dragonflies and (day) butterflies are collected by hand net whenever encountered along transects and rivers. GPS coordinates for all animals are recorded. Every year species are added to the list and work has been put in progress to create a field guide of the Odonata from CNP and a check list of butterflies with distribution maps from Cusuco National Park.

5.3 Orchid bees (Apidae, Euglossini)

Orchid bee collection will make use of chemical attractants set up at bait stations in a site. Each bait station consists of a paper towel/cotton wool ball suspended from a tree branch. Each bait station should receive 20 drops of an attractant at the beginning of the sampling session, and 10 drops each half hour thereafter. Bees attracted to the baits are collected using insect nets, and placed in a suitably labelled Whirl-Pak bag half filled with alcohol. All individuals collected during a single trapping session should be collected together into a single sample. The number of bees attracted but not collected should always be recorded in the logbook. Sampling sessions should be carried out for 2 hours from 9.00am to 11.00am. Trapping methods for orchid bees will also be used. At each site, this will involve the placement of four different traps, baited with chemical attractants and using an alcohol or propylene glycol based killing fluid.

5.4 Longhorns (Cerambycidae) and click beetles (Elateridae)

Opportunistically and on light traps longhorns and click beetles are collected in CNP. Animals are collected by sweeping or light trapping and preserved in 70% and some in 98% ethanol. Data are collected to compose preliminary distribution maps of the species and notes are taken about host plants.

5.5 Harvest-spiders (Opiliones, Cosmetidae)

Collections of Opiliones are conducted along transects at night in CNP. Specimens are collected by hand from vegetation and superficial leaf litter. Individuals only from the family Cosmetidae will be collected, and stored in 98% ethanol.

5.6 Hooverflies (Syrphidae)

Hooverflies or syrphids are a family of Diptera typically found in open sunny spots or forest edges “hanging” in the air. Syrphids are collected opportunistically to contribute to the biodiversity surveys.

5.7 Botanic surveys

Botanical surveys are normally conducted every other year, however, this year no intensive vegetation recording was conducted. But, additional selected samples needed for identification were collected to complement the botanic surveys from last year. Most commonly this entails the collection of fertile specimens of species already recognised as distinct from prior survey work but not yet fully identified due to lack of flowers and/or fruits. The locations of some of these are known with precision, others only approximately. A number of these target species may be new to science. The aim is to make a reasonably full list of the species of trees and shrubs of Cusuco National Park. Specimen are dried in the field (sun or oven) and kept dry wrapped in newspapers. Flowers are collected in ethanol to preserve the structure.

6. Specialist Studies

6.1 Aquatic invertebrates in bromeliads

Since 2006 the aquatic invertebrate communities in bromeliads have been studied in CNP. This project is part of the biodiversity survey. Additionally, the bromeliad system provides a unique study system to research fundamental ecologic and evolutionary topics. The small and well delineated communities are easy to sample and have a large number of replicates over strong environmental and altitudinal gradients. Current research focuses on the identification and disentangling of community structuring factors and the role of habitat selection and dispersal frequency. This is achieved by a combination of collecting samples from bromeliads in the field and experimental set-ups with plastic cups attached to trees functioning as artificial phytotelmata. Collection of samples in the field includes the recording of a wide range of environmental factors. Together with every bromeliad sampled a considerable amount of information is collected. Before the bromeliad is collected, the height of bromeliad attachment on the tree, size of the plant, water collecting capacity, light intensity, exposure to direct rainfall and the regional richness of bromeliads is recorded. Subsequently bromeliads are collected in a 20 litre bucket with lid to prevent escape of organisms and transported to camp to dismantle. Back in the camp, core diameter, actual water content and maximum water content, number of leaves, weight of the washed leaves and weight of the detritus in the bromeliad are recorded. The plant is consequently taken apart leaf by leaf and rinsed in 64 micrometer filtered river water. All organisms are picked out alive, and preserved in 70% ethanol. Hypotheses based on observations from the sampling of bromeliads are tested with the experimental setups. As the communities are better documented, the research slowly shifts more and more towards an experimental side.

6.2 Status of Chytrid fungus and Ranavirus in CNP

Amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) is an emerging infectious disease which is causing catastrophic amphibian population declines throughout Mesoamerica, and is a serious threat to the amphibians of CNP (Kolby et al. 2010). To date, 12 amphibian species have now been found infected with *B. dendrobatidis* within this cloud forest fragment, threatening 40% of CNP's amphibian diversity. Furthermore, eight of these infected species are listed either as endangered or critically endangered by the IUCN Red List of Threatened Species. The chytrid

research project is focussed on two main areas: investigating the extent of chytrid infections in CNP and factors that affect infection rates (e.g. comparing infection rates across species, across different site elevations, or across different morphological states of amphibian), and possible dispersal mechanisms.

Last year we performed the first survey to determine whether Ranavirus is affecting the amphibians in CNP. Amphibian ranaviruses (genus *Iridovirus*) have also been responsible for significant amphibian die-offs worldwide (Gray et al. 2009) since first recognized in the 1960's. Ranaviral infections occur most frequently in tadpoles and recently metamorphosed juveniles, but may also infect adults. Clinical signs range from dermal erythema to sudden death without symptoms. The pathogen is highly persistent in the environment when independent of a host and transmission potential appears to be high (Pessier, 2002). Ranaviruses are known to jump hosts and classes, and can spread between amphibians, fish, and reptiles. A low number of samples were found positive and we collect additional samples to substantiate the presence of ranavirus in CNP.

Different species of amphibians are sampled along sample routes, rivers and streams at each of the field camps to provide a good cross section of species, habitat and elevations. For the detection of *B. dendrobatidis* infection, amphibians are swabbed using non-lethal protocols established by Hyatt et al. (2007). For adult amphibians and salamanders, the ventral surfaces of the legs, feet, and drink patch will each be swabbed five times, applying moderate friction. In the case of amphibian larvae, the swab is inserted into the oral cavity and twirled several times. Swab buds are broken off and stored in 2 mL microcentrifuge tubes containing 1 mL of 70% ethanol as a preservative. Samples will later be analysed by molecular analysis (PCR) to detect the presence of *B. dendrobatidis* DNA and to determine the infection status of each amphibian sampled. Swabs are collected across a range of different species and habitats. In addition, river water is sampled at a variety of locations to monitor the environmental presence of *B. dendrobatidis* across different amphibian habitats and between successive years. Some tadpoles will be collected for histologic research.

For the detection of ranavirus, amphibians were sampled using a non-invasive technique of swabbing the oral cavity (tadpoles) and cloaca (adult amphibians) as described in Grey et al. (2012). Swab buds are broken off in 2ml cryovial tubes and stored for subsequent PCR analysis. A fresh pair of Nitrile gloves are worn each time an amphibian is sampled for either *B. dendrobatidis* or ranavirus, to prevent any risk of cross infection. Any amphibian found dead is preserved for subsequent histological examination to investigate the cause of death.

Additionally, based on the positive results of a pilot study last year looking at the presence of chytrid in the digestive gut of freshwater crabs (Decapoda) this research is continued this year. Crabs are collected by hand during day and night, measured and collected to test the presence of chytrid. The abdomen is therefore cut with scissors at the base where it attaches to the carapace. The gut is incised using a scalpel, and cut open using scissors. To determine whether *Bd* was present, the inside of the gut is swabbed 20 times with moderate friction using individually wrapped sterile swabs with fine-tipped rayon buds. The swab is placed in a 1.5 ml Eppendorf tube with 1 ml of 99% ethanol and stored at room temperature prior to lab analysis.

6.3 DNA barcoding project of amphibians and reptiles in CNP

The aim of this project is to complete genetic barcoding of key taxa and to submit these data to GenBank. There are 7 endemic amphibians in Cusuco and none of these have been genetically described. Some of these species (from the genus *Craugastor*) and some species of *Anolis* (*Norops*) are suspected cryptic species. Special attention will go to fossorial species. Many neotropical reptiles and amphibians are soil- and compost-dwelling (fossorial) yet very little is understood about the ecologies or phylogenetic placements of them. It is therefore extremely important to document the species of a given area and a useful reference collection is vital to investigate the possible impacts of long term population change and/or disease. Surveys are conducted by digging with long-handled bladed or forked hoes. We will measure the abundance of fossorial reptiles and amphibians by recording the amount of time spent digging and/or the surface area of soil dug (following e.g., Gower et al., 2004). Habitat variables including canopy cover, soil temperature and soil texture are recorded. A recently developed non-lethal sampling method (Maddock et al. 2014) for caecilians and historically established methods for other reptiles and amphibians are used when voucher specimens are not required. For a limited number of samples, it may be necessary to collect voucher specimens when there is little data available or when identification is not possible. In these cases, tissue samples are collected from liver samples and specimens preserved. In our extensive experience, digging sometimes leads to accidental but likely lethal damage (e.g. broken backbones) of a small percentage of specimens. All such lethally damaged specimens are collected as vouchers and will form the bulk of the collections. Tissue samples from road-killed specimens (e.g., Pawlowski & Krämer 2010) can also be taken. Vouchers are fixed in 5% formalin, washed and stored in 70% ethanol for subsequent morphological analysis. Specimens will be deposited in the Natural History Museum, London and tissue samples in their Molecular Collections Facility. Few people carry out dedicated field studies of fossorial reptiles and amphibians and so there has been little development of guidelines for best practice. Guidelines developed for other amphibians will be applied as far as possible (e.g., the same anaesthetics work) and as a long term goal it may be possible to form best practice guidelines for sampling of fossorial reptiles and amphibians.

This project aims to collect DNA samples from multiple individuals of amphibians and reptiles in CNP to create a molecular database that can be used as a reference to identify potentially new species in the future. DNA samples are collected with swabs and preserved in 98% ethanol. Additionally, some samples are stored using FTA Whatman cards and exported for subsequent gene sequencing of the cytochrome C oxidase sub unit I (COI). To substantiate the molecular data, we also build on a reference collection of voucher specimen (one male and one female) for all amphibian and reptile species.

6.4 Canopy Invertebrate Communities

Selected tree individuals from forest camps are selected based on altitude and species. These trees are rigged with around 30 collecting funnels, each with a 1m² collecting surface. Funnels are placed in a single layer immediately beneath the canopy of the tree, and their location recorded. Rigging is undertaken by two trained climbers using recognised canopy access techniques. Each funnel will have a 1l collecting bottle attached. A thermal fogger is used to dispense 4.5l of 5% cypermethrin, a pyrethroid insecticide, into the canopy of the tree. Fogging will take place early in the morning, generally between 4am and 5.30am. Between 9am and 12am, the funnels are collected in and labelled bottles filled with 100% ethanol. Samples are sorted to remove large detritus and filtered into 50ml tubes with new 100% ethanol. These are stored at <5°C until

exported to the Natural History Museum, London, UK. Between 5 and 15 trees are sampled. Sampling will take place away from waterways and at times of low wind to reduce pesticide drift. Cypermethrin naturally breaks down in a short period of time, and has low general toxicity.

Additionally, malaise/FI trap sampling is carried out at two-five locations at base camp. The purpose of this is to collect large-volume invertebrate samples for the trialling of methods of DNA extraction for application to canopy fogging samples. Malaise traps are set up at locations selected for ease of access and to avoid interfering with other research. Pans are placed below the malaise trap to act as FIT collectors. Malaise trap collecting bottles are half filled with 100% ethanol. The trays are half filled with saturated salt solution. These are collected from and refreshed every 3-7 days (TBD). Samples are stored in 100% ethanol in the base camp fridge.

6.5 iBOL sampling

6.5.1 Global Malaise Program

Three Malaise traps are deployed at Base Camp (BC4-SS1), Guanales (GU4-SS1), and Cantiles (CA2-SS1) in an area which is subject to minimal disturbance. The Malaise trap should be assembled as securely as possible and securely pegging out the guy ropes. The trap should be set up correctly so that nothing blocks the potential flight path of specimens and should be checked frequently to ensure that it has not collapsed. The specimen collection bottle on each trap should be filled half full with 99% ethanol at the time of deployment. The catch should be removed each week. At this time, the specimen collection bottle should again be filled half full with fresh 99% ethanol. Once the weekly catch has been collected, it should be carefully poured through a mesh filter to allow the 'used' ethanol to be decanted and stored separately. The specimens should then be washed back into a Nalgene storage bottle with fresh ethanol and a label should be added indicating the collection details. The label should be written in pencil and placed inside the jar. The samples should be kept out of sunlight in a cool location.

6.5.2 Scarabaeinae

In this project barcoding is used to validate the morphological based identifications in the monitoring of dung beetle communities (section 4.5). Sub-sample per species per camp (10 specimen) from the standard collection will be isolated. Store samples in 99% ethanol with labels indicating species ID, identifier, collection ID and camp. The samples should be kept out of sunlight in a cool location.

6.6 Trophic ecology and population genetics of snakes of Cusuco National Park, with a particular emphasis on the Emerald Palm Viper (*Bothriechis marchi*)

Snakes are searched for during diurnal and nocturnal Visual Encounter Surveys (VES) by experienced herpetologists with experience of handling non-venomous and venomous snakes. All snakes encountered are captured and secured using appropriate techniques (snake hooks and clear plastic handling tubes will always be used for venomous species). Snakes are measured (SVL and tail), weighed, sexed and photographed. Up to three ventral scale clips are taken using a pair of sharp scissors and stored in ethanol in a 1.5ml plastic Eppendorf tube. Scales are retained as tissue samples for genetic and stable isotope analysis.

Tissues samples for genetic analysis are stored at the University of Kent for future population genetic and phylogenetic analysis. This analysis will give further insight into the genetic

distinctiveness of snakes (especially *B. marchi*) in Cusuco National Park as well as population structure within the park itself. Tissue samples for stable isotope analysis are returned to Queens University Belfast for processing and analysis. This analysis will provide insights into the diet of snakes in Cusuco NP and specifically if/how different species may be partitioning food resources or, conversely, be competing for the same resources.

6.7 Experimental evaluation of monitoring efficiency based on transects with plastic models of amphibians and reptiles.

The ability of volunteers to undertake different tasks and accurately collect data is critical for the success of many conservation projects. In this study, a simulated herpetofauna visual encounter survey is used to compare the detection and distance estimation accuracy of volunteers and more experienced observers. Plastic reproductions of amphibians and reptiles are placed along 200m transects close to camp in such way to answer particular hypotheses. Points of interest include among others the effect of group size, skill level of observers or specific particularities of the objects that affect it being recorded such as size, distance from the patch or height above the ground.

6.8 Investigation in the dependency and social engagement towards conservation of communities around CNP

This project is about reinforcing the conversation between the OPWALL research project and the communities surrounding CNP that are not immediately incorporated in the project. The main aims are to gain more insight in the dependency of communities living close to Cusuco National park, and increase our understanding of how to contribute to the amelioration of their livelihoods while in the meantime safeguarding the remaining forest in CNP. Information will be collected in the form of a questionnaire.

7. Full protocols available

More information on the survey methodology can be found in the following documents:

- * Bird banding protocol - Fabiola Rodríguez et al. - March 2012 - 23 pp.
- * Invertebrates team sampling protocol - Thomas Creedy - March 2012 - 8 pp.
- * Habitat survey protocol - Bruce Gareth & Merlijn Jocque - May 2014 - 7 pp.
- * Habitat and environmental data collection protocol - Thomas Creedy - April 2013 - 8 pp.
- * Amphibian and reptile survey protocol - Alex Laking - 2014 - 7 pp.

(please email info@opwall to request the most recent copies of these documents)

8. Reported results for 2015

Habitat monitoring- By Dr Danielle Gilroy

Habitat and forest structure data for Cusuco National Park were collected in the summer of 2015. For detailed descriptions of the methods used please refer to protocol document. 130 survey sites were visited between June and August 2015. Mean diameter at breast height (DBH), total number of saplings and trees, average canopy scores based on height and openness, number of cut stumps, number of saplings and altitude data were calculated for each survey site (Table 1).

Table 1. Site by site analysis of habitat and forest structure along transects for each satellite camp (BA = Buenos Aires, CA = Cantilles, CO = Cortecito, CP = Capuca, DA = Danto, GU = Guanales) and at basecamp (BC).

Survey Site	Aspect	Slope	Soil density	Sapling #	Tree #	DBH (m)	Canopy score	Cut saplings	Cut stumps	Broad-leaf %	fern %	palm %	pine %
BA1_SS1	NE	40	24	0.0	21	0.3	10	4	29	100	0	0	0
BA1_SS2	SE	14	27	0.8	12	0.3	13.4	6	19	100	0	0	0
BA1_SS3	SE	25	20	3.4	18	0.2	19	0	55	83	0	17	0
BA1_SS4	S	27	25.6	0.0	18	0.4	12.2	21	14	17	83	0	0
BA1_SS5	S	22	13.2	6.2	28	0.2	19	23	3	100	0	0	0
BA2_SS1	E	17	33.4	2.0	12	0.3	4.8	0	2	92	8	0	0
BA2_SS2	W	41	13.6	1.2	37	0.2	5.2	4	0	73	22	0	5
BA2_SS3	W	35	38	2.2	39	0.2	1.6	2	1	79	21	0	0
BA2_SS5	SE	34	33.2	0.0	31	0.2	7.4	4	2	100	0	0	0
BA2_SS6	N	21	49	4.0	46	0.2	9	5	2	46	54	0	0
BA3_SS1	NW	18	21	2.2	42	0.1	4.8	0	9	100	0	0	0
BA3_SS2	S	38	62	4.0	48	0.1	2.6	3	0	92	8	0	0
BA4_SS1	NW	33	48.2	8.4	33	0.2	9.8	13	1	52	48	0	0
BA4_SS2	SE	35	28	1.4	66	0.2	2.2	3	3	53	0	0	47
BA4_SS3	NE	22	37.4	3.0	83	0.2	0.4	0	0	77	0	0	23
BA4_SS4	SE	33	18.6	4.2	21	0.3	0	4	1	100	0	0	0
BA4_SS5	E	9	30	4.0	31	0.2	2.4	0	0	97	0	0	3
BC1_SS1	W	5	22.2	2.8	82	0.2	1.4	12	19	72	5	0	23
BC1_SS2	N	21	25.6	3.6	59	0.2	4	1	0	95	0	0	5
BC1_SS3	SE	41	22.4	5.0	56	0.2	5.4	2	0	89	0	9	2
BC1_SS5	E	20	34	1.2	55	0.1	2.8	0	1	75	0	5	20
BC1_SS6	E	11	8.2	5.0	74	0.1	7.6	24	5	97	0	0	3
BC1_SS7	NE	33	28	3.2	82	0.1	1	0	2	90	0	2	7
BC1_SS8	SE	20	32.6	2.2	37	0.2	3.2	8	1	86	0	5	8
BC2_SS1	N	33	33	2.6	74	0.2	2	0	0	86	0	1	12
BC2_SS2	E	20	41	5.4	51	0.2	5.4	20	2	86	2	0	12
BC2_SS3	SE	42	35.4	0.6	60	0.2	1.2	4	2	92	0	0	8
BC2_SS4	S	16	37.8	3.2	109	0.1	1.6	1	0	93	0	1	6

Survey Site	Aspect	Slope	Soil density	Sapling #	Tree #	DBH (m)	Canopy score	Cut saplings	Cut stumps	Broad-leaf %	fern %	palm %	pine %
BC3_SS1	NE	7	21.8	2.2	89	0.1	1.8	0	5	67	0	7	26
BC3_SS2	E	35	50.2	1.0	45	0.2	1.2	0	0	71	27	0	2
BC3_SS3	SE	14	19.6	6.4	44	0.1	6	6	1	77	18	0	5
BC3_SS4	E	31	26	7.4	56	0.2	5.8	4	1	84	0	0	16
BC3_SS5	SE	28	19.6	3.8	53	0.1	1.6	0	0	100	0	0	0
BC3_SS6	SE	15	41.4	6.8	95	0.1	0.8	5	2	100	0	0	0
BC3_SS7	SE	18	24	2.0	71	0.2	2.4	0	0	100	0	0	0
BC4_SS1	NE	5	24.4	3.0	61	0.2	3.4	0	0	93	0	0	7
BC4_SS2	E	3	35	3.8	75	0.1	1.8	5	5	99	0	0	1
BC4_SS3	S	30	43.2	0.6	33	0.2	3.4	2	1	91	0	0	9
BC4_SS4	SE	3	38	3.8	42	0.2	1.6	10	1	95	0	5	0
BC4_SS5	SE	3	48.8	2.6	51	0.2	0.8	12	1	80	0	10	10
BC4_SS6	SW	3	34.8	4.8	64	0.2	5.8	0	7	83	0	0	17
CA2_SS1	SW	28	23.2	0.8	93	0.1	1.4	4	3	18	0	15	67
CA2_SS2	SW	32.5	14.8	6.2	79	0.1	0.4	9	0	100	0	0	0
CA2_SS3	W	21	22.6	1.6	116	0.2	8.6	0	0	47	0	0	53
CA2_SS4	W	46	36.8	3.2	124	0.1	9.4	1	0	60	0	0	40
CA2_SS5	E	25	36.8	3.4	73	0.2	5.6	2	11	77	0	0	23
CA2_SS6	NE	34	23.6	2.2	91	0.1	4.6	4	11	48	0	0	52
CA2_SS7	N	16	19.8	4.2	111	0.1	6	6	10	51	0	0	49
CA3_SS1	NW	29	73.8	4.8	80	0.1	2	1	4	56	0	1	43
CA3_SS2	N	42	47	3.8	137	0.1	2.4	1	7	32	0	0	68
CA3_SS3	NW	32	28.8	3.6	170	0.1	3	9	3	33	0	2	65
CA4_SS1	S	31	22	3.6	88	0.1	1.4	4	7	45	0	0	55
CA4_SS2	SW	35	42.6	2.0	56	0.1	7.4	1	4	89	0	4	7
CA4_SS3	SW	37	29.2	3.2	72	0.1	0.8	0	1	42	0	14	44
CA4_SS4	SW	8	24	4.8	74	0.1	6.4	0	1	46	0	18	36
CA5_SS1	SW	15	16.1	5.7	78	0.2	1.9	6.0	6.0	92	0	5	3
CA5_SS2	S	36	16.8	0.8	69	0.1	4.2	3	2	59	0	0	41
CA5_SS3	WNW	32	37.4	5.8	251	0.1	2.7	1	8	59	0	2	40
CA5_SS4	NW	33	53.2	13.2	134	0.1	3	0	1	69	0	0	31
CA5_SS5	W	22	28.6	12.4	54	0.2	6.8	0	3	76	0	6	19
CA5_SS6	W	19	18.8	3.8	95	0.1	6.6	3	1	48	0	8	43
CA5_SS7	S	36	24	11.0	67	0.1	0.8	2	1	72	0	0	28
CA5_SS8	SW	35	10	2.6	109	0.1	10	4	7	46	0	0	54
CO1_SS1	SW	8	13	0.0	46	0.2	25	35	43	91	0	9	0
CO1_SS2	SW	27	28.8	5.6	78	0.1	1.2	3	5	81	0	19	0
CO1_SS3	SW	15	23.8	2.8	74	0.1	3.4	13	12	46	0	53	1
CO1_SS4	SW	36	27.6	5.8	115	0.1	1.4	1	3	66	0	26	8
CO1_SS5	SW	24	30.4	4.8	87	0.1	2	10	5	70	0	3	26
CO2_SS1	SW	34	36.8	3.2	64	0.2	1.8	4	8	78	0	20	2

Survey Site	Aspect	Slope	Soil density	Sapling #	Tree #	DBH (m)	Canopy score	Cut saplings	Cut stumps	Broad-leaf %	fern %	palm %	pine %
CO2_SS2	SW	48	33.8	2.6	90	0.2	2	5	14	86	0	14	0
CO2_SS3	N	19	46.8	5.0	42	0.1	1.6	9	1	79	0	19	2
CO3_SS1	W	46	32	1.2	85	0.2	3	4	1	66	0	8	26
CO3_SS2	W	34	28.4	4.6	45	0.2	2	0	0	87	0	13	0
CO3_SS3	S	22	24	1.4	49	0.2	3.4	20	4	94	0	4	2
CO3_SS4	N	29	26.8	4.0	106	0.2	4.6	4	24	88	0	6	7
CO3_SS5	SE	36	23.4	8.4	64	0.2	4.6	0	0	89	0	2	9
CO3_SS6	W	38	33.4	5.2	36	0.2	0.8	0	5	67	0	0	33
CP1_SS1	E	12	28.8	4.0	49	0.1	0	6	15	35	0	6	59
CP1_SS2	SE	12	20	1.3	102	0.1	0.8	0	0	28	0	16	56
CP1_SS3	SW	22	17.6	2.5	91	0.1	2.4	0	0	40	0	0	60
CP1_SS4	SW	20	26	3.4	65	0.1	1.8	0	0	52	0	3	45
CP1_SS5	SW	35	30.6	6.0	89	0.1	2.4	5	1	45	0	1	54
CP1_SS6	S	11	45.2	2.4	75	0.1	4.2	0	0	37	0	1	61
CP2_SS1	SW	31	18	3.0	49	0.1	0.2	0	3	49	0	6	45
CP2_SS2	S	30	32.8	3.0	74	0.1	0	10	2	59	0	0	41
CP2_SS3	W	22	39.2	3.0	106	0.1	3.2	4	2	45	0	0	55
CP2_SS4	SW	26	43.2	1.6	93	0.1	1.4	2	0	23	0	11	67
CP2_SS5	N	12	58	0.4	71	0.2	2	2	0	55	8	0	37
CP3_SS1	NW	19	40	5.2	130	0.1	1.2	3	6	77	0	5	18
CP3_SS2	E	43	41.8	1.6	44	0.2	0.6	3	1	86	0	0	14
CP3_SS3	E	35	36.6	3.4	38	0.2	9.2	1	0	71	0	0	29
CP3_SS4	E	33	5	2.8	70	0.1	3	0	4	27	1	0	71
CP4_SS1	SW	23	35.6	3.6	114	0.1	1.2	0	0	39	0	8	54
CP4_SS2	S	31	29	1.8	86	0.1	1	1	1	64	0	6	30
CP4_SS3	E	17	56.4	2.8	102	0.1	7	1	3	84	0	11	5
CP4_SS4	E	25	34	2.6	32	0.2	4.6	10	18	88	0	3	9
DA0_SS1	W	29	50.6	3.2	66	0.1	4.2	1	0	71	0	0	29
DA0_SS2	E	27	23	9.2	98	0.1	2.6	5	2	78	2	6	14
DA0_SS3	E	13	31.2	4.6	77	0.2	2.2	0	0	61	0	0	39
DA0_SS4	N	35	33.2	2.8	172	0.1	1	3	1	45	0	9	46
DA1_SS1	NW	18	38.2	3.2	163	0.1	1.6	8	1	29	0	15	56
DA1_SS2	W	37	37	4.6	88	0.1	3	15	20	56	0	17	27
DA1_SS3	NW	27	41.2	8.4	43	0.3	2.6	0	0	56	0	5	40
DA1_SS4	S	5	35.6	6.6	101	0.1	16.8	9	2	54	2	24	20
DA1_SS5	SE	12	28.8	5.6	47	0.2	2.8	0	0	68	0	6	26
DA1_SS6	S	22	45.6	11.0	58	0.2	0.8	2	0	100	0	0	0
DA2_SS1	S	31	25	3.4	188	0.1	1.4	2	1	37	0	12	51
DA2_SS2	SE	14	19.4	1.2	120	0.2	9.6	3	0	47	0	21	33
DA2_SS3	SE	29	42.8	2.4	88	0.2	10.6	17	4	69	0	22	9
GU1_SS1	S	21	29	3.8	61	0.2	2.2	1	1	98	0	2	0

Survey Site	Aspect	Slope	Soil density	Sapling #	Tree #	DBH (m)	Canopy score	Cut saplings	Cut stumps	Broad-leaf %	fern %	palm %	pine %
GU1_SS2	NW	14	16.6	1.3	116	0.2	2.3	0	0	97	1	2	0
GU1_SS3	SW	36	17.8	2.4	74	0.2	3	1	2	80	0	20	0
GU1_SS4	W	1	22	0.8	38	0.2	9.4	0	0	63	13	0	24
GU1_SS5	NW	33	51.8	3.8	33	0.3	0.8	0	0	97	0	0	3
GU1_SS6	N	21	18.6	2.0	54	0.1	2.4	0	0	52	0	13	35
GU1_SS7	SW	33	34.8	3.8	82	0.1	2.2	0	0	46	0	6	48
GU1_SS8	SW	29	44.6	2.4	39	0.2	1.6	1	1	87	0	10	3
GU2_SS1	W	10	28.4	0.6	61	0.1	2.6	4	4	100	0	0	0
GU2_SS2	W	38	29	2.0	77	0.2	1.4	0	0	96	4	0	0
GU2_SS3	NW	39	34.2	21.8	52	0.2	7	0	0	98	0	2	0
GU2_SS4	N	40	33	4.4	61	0.2	4.4	1	0	100	0	0	0
GU2_SS5	NW	6	22.4	14.2	39	0.1	2.2	0	0	97	0	0	3
GU2_SS6	S	19	17.8	3.2	108	0.1	2	1	0	100	0	0	0
GU2_SS7	NE	1	45.6	3.2	96	0.1	1.6	0	0	92	0	0	8
GU2_SS8	NW	27	62	2.2	69	0.2	1.4	0	0	77	16	0	7
GU3_SS1	NE	19	10.8	5.0	31	0.2	2.2	1	0	87	0	13	0
GU3_SS2	W	55	17.8	3.8	101	0.2	1.9	1	1	100	0	0	0
GU4_SS1	ESE	20	31.6	3.1	117	0.2	3.2	10	5	99	0	1	0
GU4_SS2	S	29	31.8	1.4	45	0.2	2.6	0	0	100	0	0	0
GU4_SS3	NE	30	38.4	0.0	39	0.2	2.4	0	0	100	0	0	0

Section 1: General correlations and patterns in the forest as a whole

To determine if associations between measured variables exist in the forest as a whole, Pearson correlation statistical tests were conducted for each normally distributed variable and for those normally distributed after transformation.

Number of trees and tree volume

There is a significant negative association between number of trees and tree volume ($r_p = -0.384$, $N = 130$, $P < 0.001$). This is exactly what we would expect in that there would be fewer trees of larger diameter and this would have similar total basal areas to larger numbers of smaller trees. For Cusuco plots, it is clear that the greater the number of trees, the smaller the tree volumes would be in what appears to be a density-regulated floral population (Fig 1).

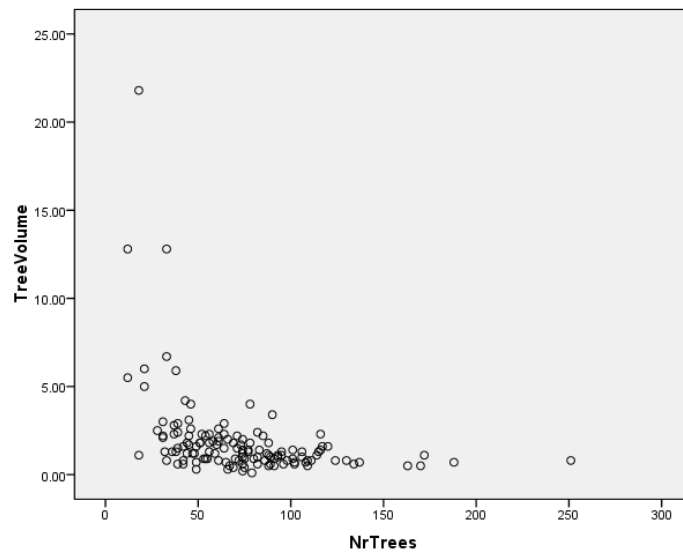


Figure 1. Correlation between number of trees and individual tree volume (m^3).

Average diameter at breast height (DBH) and total trees

There is a strong negative association between total trees and average DBH ($r_p = -0.527$, $N = 130$, $P < 0.001$). As the number of trees in a plot increases, the average size of those trees in the plot decreases (Fig 2).

Canopy and DBH

There is a weak positive relationship between canopy and average DBH of the plot ($r_p = +0.25$, $N = 130$, $P = 0.004$). There is a significant association between canopy height and diameter of individual trees (Fig 3).

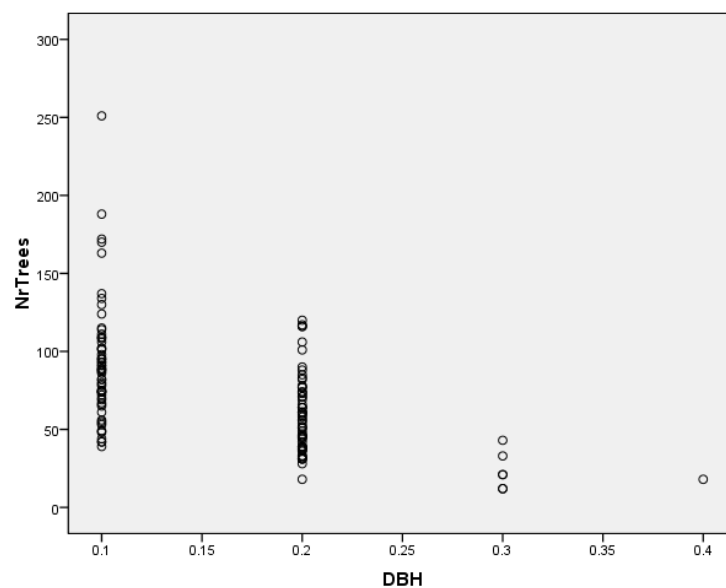


Figure 2. Correlation between mean individual diameter (m) at breast height of tree (DBH) and total number of trees in plot.

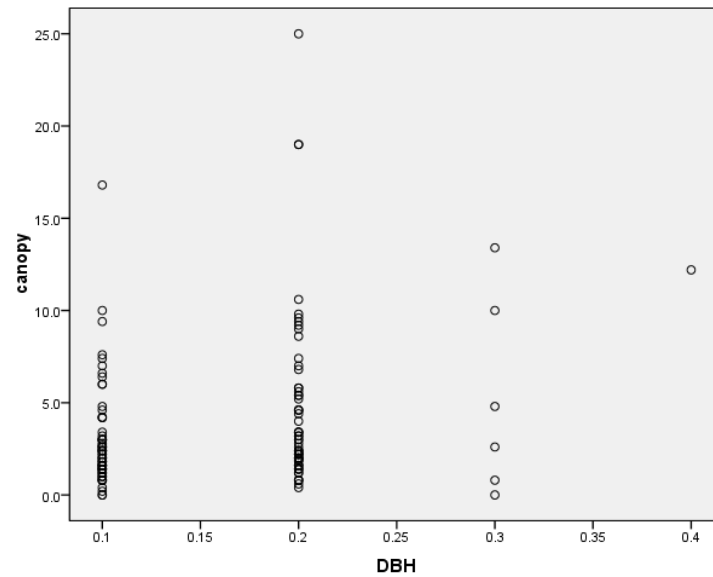


Figure 3. Correlation between mean individual diameter (m) at breast height of tree (DBH) and canopy score.

Canopy and total trees

There is a weak negative association between canopy score and total number of trees ($r_p = -0.203$, $N = 130$, $P = 0.020$). There are fewer numbers of trees in a plot where the canopies are scoring high and more trees where canopy scores are lower (Fig 4).

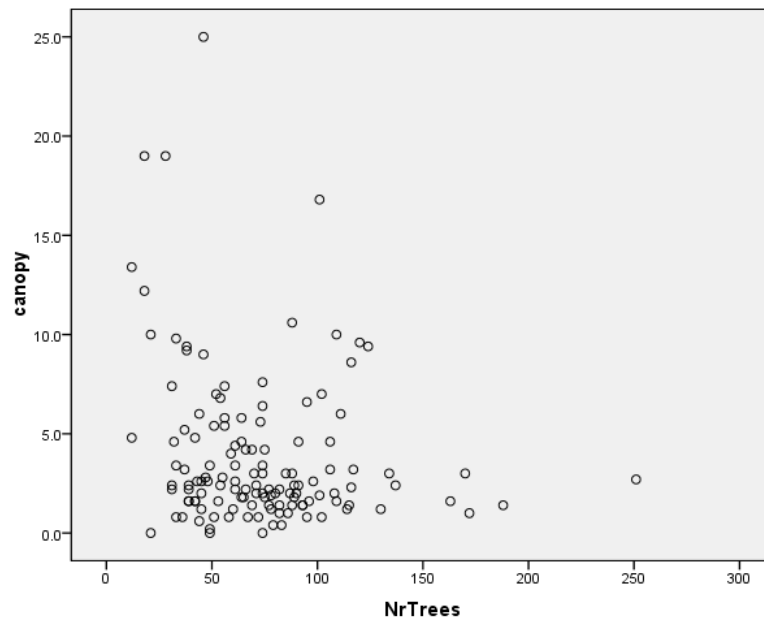


Figure 4. Correlation between number of trees and canopy score.

Canopy and total tree volume

There is a weak positive relationship between canopy and total basal tree volume of the plot ($r_p = +0.186$, $N = 130$, $P = 0.034$). This can be explained by the strong negative association highlighted above between tree frequency and tree volume (Fig 5).

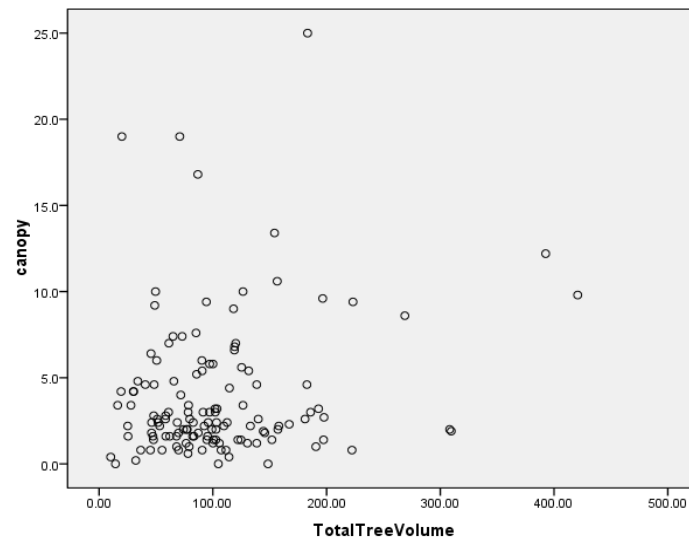


Figure 5. Correlation between total volume of trees in a plot (m^3) and plot canopy score.

Slope and total trees

There is no association between slope and total trees per plot ($r_p = +0.07$, $N = 130$, $P = 0.43$).

Slope and total tree volume

There is no association between slope and total volume of trees per plot ($r_p = +0.08$, $N = 130$, $P = 0.38$).

Slope and DBH

There is no association between slope and mean DBH ($r_p = +0.08$, $N = 130$, $P = 0.38$).

Elevation and total trees

There is a strong positive association between slope and total trees per plot ($r_p = +0.36$, $N = 130$, $P < 0.001$). The higher the elevation, the greater the number of trees per plot (Fig 6).

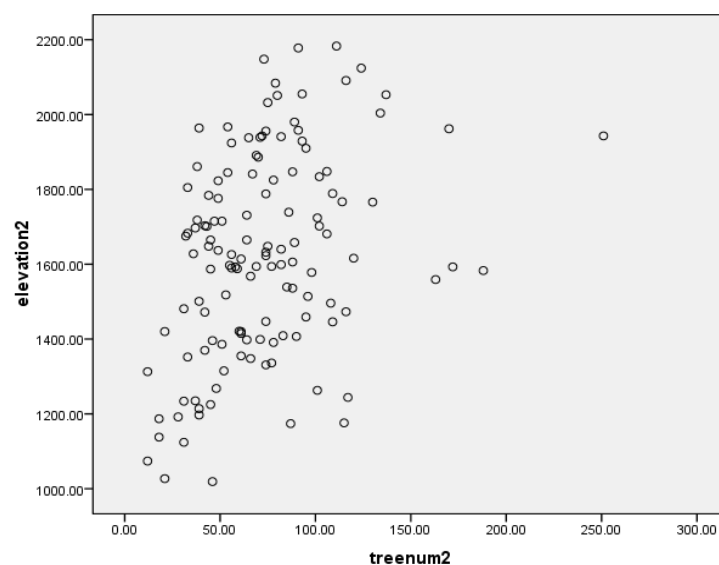


Figure 6. Correlation between total number of trees in a plot and elevation (m).

Elevation and total tree volume

There is a significant negative association between slope and total volume of trees per plot ($r_p = -0.19$, $N = 130$, $P < 0.05$). The higher the elevation, the lower the total volume of trees per plot (Fig 7).

Elevation and DBH

There is a strong negative association between slope and mean DBH ($r_p = -0.43$, $N = 130$, $P < 0.001$). The higher the elevation, the lower the mean DBH measured on trees (Fig 8).

Overall this suggests that as elevation increases and plots become increasingly higher in altitude, the tree volumes individually and collectively decline and there are more trees of smaller size.

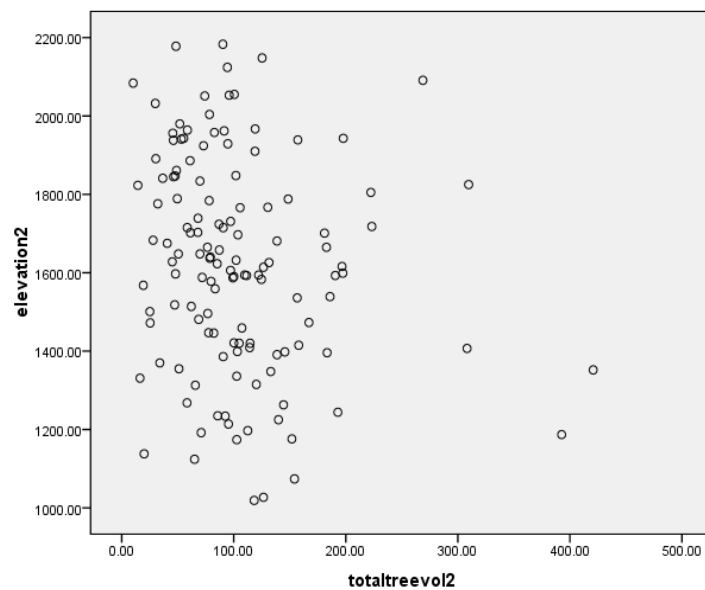


Figure 7. Correlation between total volume of trees (m^3) in a plot and elevation (m).

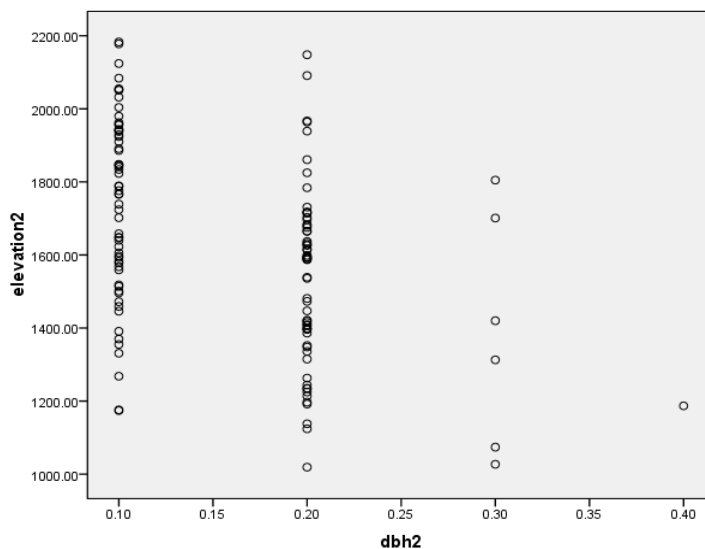


Figure 8. Correlation between mean individual diameter (m) at breast height of tree (DBH) and elevation (m).

Section 2: Vegetation categorical analysis

Cusuco plots are primarily dominated by broadleaf trees (and secondarily by ferns, palm and pine trees are much smaller components of the overall vegetation mosaic (Fig 9). However, pine and palm trees have similar DBH measures to ferns and so are still valuable carbon assets (Fig 10). Furthermore, ferns have a greater proportion of dead trees found compared to the other tree-types. It is evident that broadleaf trees are the fundamental tree type to Cusuco forests.

BROADLEAF TREES	N	Range	Maximum	Mean	Mean SE	Std Dev
Number of trees	130	144	147	49.7	2.1	24.1
% plot broadleaf	130	83	100	72.7	2.0	22.8
Mean DBH	130	30	37	17.4	0.4	4.8
% broadleaf dead	130	100	100	10.1	1.2	13.2

PINE TREES	N	Range	Maximum	Mean	Mean SE	Std Dev
Number of trees	130	25	25	1.0	0.3	3.5
% plot pine	130	83	83	2.6	0.9	10.4
Mean DBH	130	118	118	7.2	1.8	20.4
% pine dead	130	100	100	3.3	1.3	14.3

PALM TREES	N	Range	Maximum	Mean	Mean SE	Std Dev
Number of trees	130	39	39	3.9	0.6	6.9
% plot palm	130	53	53	4.7	0.7	7.7
Mean DBH	130	27	27	4.7	0.5	6.2
% palm dead	130	100	100	3.1	1.0	11.3

FERN TREES	N	Range	Maximum	Mean	Mean SE	Std Dev
Number of trees	130	111	111	18.4	2.2	24.9
% plot fern	130	71	71	19.9	1.9	21.5
Mean DBH	130	32	32	7.5	0.5	5.6
% fern dead	130	100	100	4.5	1.1	12.8

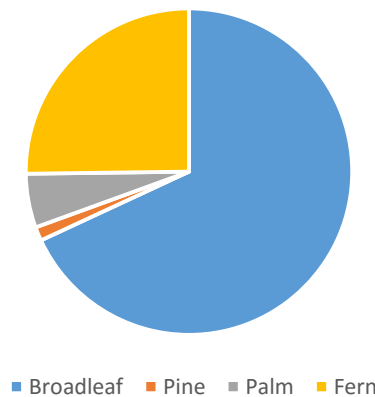


Figure 9. Percentage of each plot that contained broadleaf, pine, palm and fern vegetation.

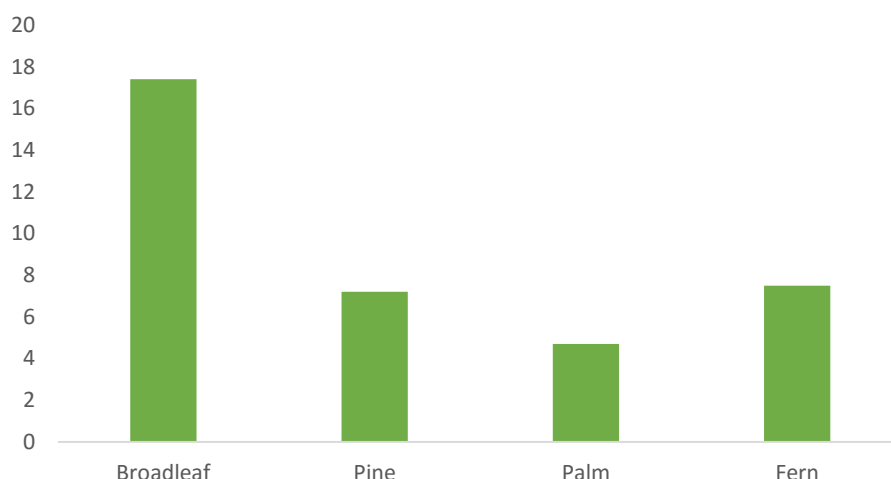


Figure 10. Mean individual diameter (m) at breast height (DBH) of trees in Cusuco plots.

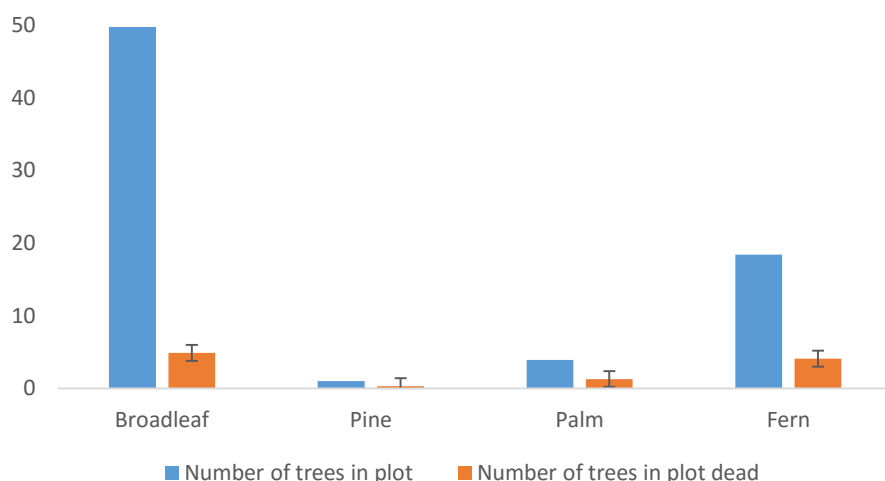


Figure 11. Mean number of different types of trees found in Cusuco plots compared to the number of those trees of each of those types found dead.

Section 3: Camp by Camp Analysis

Buenos Aires

Buenos Aires plots are situated between 999 and 1479 meters above sea level. Average elevation is 1255, 892 meters below the highest plot in the survey area, 2147 metres above sea level and 605 meters above the lowest elevated plot in the survey. Mean elevation is 237m below the mean of all survey plots, at 1487m. Mean DBH is 21cm, which is above the forest mean of 18.0cm (no camps mean DBH measures significantly differed from one other or differed to the rest of the survey area: $F = 0.70$, $df = 25$, $P = 0.65$). Mean tree volume for BA plots is 4.4m^3 , which is above the park average of 2.0m^3 (no camps mean tree volumes significantly differed from one other or differed to the rest of the survey area: $F = 2.42$, $df = 25$, $P = 0.66$). The average total trees for BA is 36, which is well below the park average of 82 trees per plot (BA tree numbers are significantly different to Cantiles: $F = 4.16$, $df = 25$, $P < 0.05$; this is interesting given BA has the highest elevation in contrast to Cantiles with the lowest elevation). BA plots average 5.7 cut stumps, which is close to the park average of 4.4. BA plots average 4.8 cut

saplings, which is also close to the park average of 4.2. The average number of saplings per plot is 2.8, below the park average of 4.2. The % of broadleaf trees in BA plots is 82, compared to 79% in the forest as a whole and 1% palm compared to 6% across the forest. There is a relatively large proportion of pine, 13%, compared to the 3% found across all plots; and a relatively low proportion of fern, 4%, compared to the 24% found across all plots.

	Elevation	DBH	Tree volume (m ³)	Tree number	Sapling number	Cut stumps	Cut saplings	broadleaf %	fern %	palm %	pine %
BA	1255.1	0.21	4.38	36.05	2.82	7.73	4.83	82.37	3.92	0.83	12.87
Cusuco	1592.7	0.18	2.00	81.90	4.01	4.41	4.15	79.25	23.61	6.13	2.55

Means for Buenos Aires and all Survey Sites

Base Camp

Base camp plots are situated between 1570 and 1734 metres above sea level and on average are 576m below the highest plot and 84 metres above the park average. DBH is 2cm below park average of 18cm. Tree volume is not significantly different to the park average ($t = 0.724$, $df = 24$, $P > 0.4$) nor is total trees per plot ($t = 0.777$, $df = 24$, $P > 0.05$). The average total trees for BC is 64, which is below the park average of 82 trees per plot (BC tree numbers are also significantly different to the Cantiles site: $F = 4.16$, $df = 25$, $P < 0.05$). The number of cut stumps found in base camp sites is half the park average but the number of cut saplings found exceeded the park average. In previous years, number of saplings found at base camp plots were very high but in 2015 they were in fact lower than the park's average which shows that recruitment and regeneration potential has in fact slowed down and this could be due to the relatively-closed canopy in BC sites. BC plots have a large proportion of broadleaf trees, but are below park average for all other tree types (fern, palm and pine).

	Elevation	DBH	Tree volume (m ³)	Tree number	Sapling number	Cut stumps	Cut saplings	broadleaf %	fern %	palm %	pine %
BC	1572.4	0.16	1.44	64.03	3.39	2.20	4.99	87.90	8.42	1.78	1.90
Cusuco	1592.7	0.18	2.00	81.90	4.01	4.41	4.15	79.25	23.61	6.13	2.55

Means for Base Camp and all Survey Sites

Cantiles

Cantiles is the highest camp with survey sites between 1836 and 2147 meters, 494 metres above the park average. DBH is above the Cusuco mean which is largely down to one specific survey site 'CA4' which proved to be an anomaly when compared to any other survey site in Cantiles with a mean DBH of 0.48m and obscenely high % measures of broadleaf (222) and fern (143). DBH tends to be lower at higher altitudes generally and this is true for Cantiles excluding 'CA4'. Trees tend to be under the mean volume size for the park at Cantiles but total tree number is way above the park means (tree number significantly differs for Cantiles compared to the rest of the park $F = 4.164$, $df = 25$, $P < 0.01$; specifically compared to Buenos Aires $P = 0.04$, Base camp $P = 0.34$ and Guanales $P = 0.04$) again, this association with tree number was expected at the highest altitude.

The number of cut stumps is only just above average despite one plot, CA4 containing a noticeably greater number of cut stumps. Number of cut saplings per plot was consistent across the transect and was below average for the park but this could be due to the high sapling numbers observed, particularly at the CA4 site, suggesting this area is particularly good for generating new trees. Cantiles plots have proportionally high abundance of tree fern, 69% of trees in these plots are ferns (44% if you exclude the seemingly anomalous site CA4) compared to the park average of 24%. There were very few pines recorded in the area with a mean around zero and again CA4 had a significantly greater percentage of palm (bringing the site mean to 10%) compared to the very low levels recorded at other sites in this camp (excluding CA4 the site mean drops to 2%).

	Elevation	DBH	Tree volume (m ³)	Tree number	Sapling number	Cut stumps	Cut saplings	broadleaf %	fern %	palm %	pine %
CA	1989.8	0.22	1.57	156.07	6.92	6.57	3.69	96.32	68.49	10.19	0.00
*exc CA4	2013.9	0.14	1.02	111.42	4.69	4.43	3.25	54.31	43.77	1.91	0.00
Cusuco	1592.7	0.18	2.00	81.90	4.01	4.41	4.15	79.25	23.61	6.13	2.55

Means for Cantiles and all Survey Sites, in addition to Cantiles excluding one anomalous site CA4

Cortecito

Cortecito survey sites span from 1203 metres above sea level to 1648. The mean elevation is 246 m above the parks mean elevation. DBH, tree volume and tree / sapling number are all similar but below the park mean. The noticeably higher number of cut stumps and saplings per plot compared to the park average shows large scale disturbance, and this has been shown previously to be related also to the canopy openness at this site. There are no pine trees found anywhere in the Cortecito plots and there are above average numbers of palm but below average numbers of fern.

	Elevation	DBH	Tree volume (m ³)	Tree number	Sapling number	Cut stumps	Cut saplings	broadleaf %	fern %	palm %	pine %
CO	1447.4	0.17	1.90	69.83	3.84	8.98	7.69	77.75	7.10	15.15	0.00
Cusuco	1592.7	0.18	2.00	81.90	4.01	4.41	4.15	79.25	23.61	6.13	2.55

Means for Cortecito and all Survey Sites

Capuca

Capuca survey sites are situated between 1675 and 2032 meters above sea level, on average 245 metres above the park average. Tree morphometrics are similar in Capuca plots to the overall park averages although individual tree volumes are around half the size of the average at ca 1m³. There are less cut stumps and saplings at this site. There is a high proportion of tree fern found in the area, 40%, with 4% palm, and 55% broadleaf. Next to no pine trees were recorded at <1%.



	Elevation	DBH	Tree volume (m ³)	Tree number	Sapling number	Cut stumps	Cut saplings	broadleaf %	fern %	palm %	pine %
CP	1839.2	0.14	1.03	77.78	2.85	3.08	2.55	54.94	40.50	4.05	0.51
Cusuco	1592.7	0.18	2.00	81.90	4.01	4.41	4.15	79.25	23.61	6.13	2.55

Means for Capuca and all Survey Sites

El Danto

El Danto survey sites are situated between 1549 and 1699 meters above sea level, on average 542 metres below the highest point and 118m above the average plot elevation. This site has a greater number of trees per plot compared to the park average but this has a trade off with the size of the tree, as DBH and tree volumes are below average for El Danto sites. There is a high proportion of tree fern found in the area, 30%, with 11% palm, and 58% broadleaf. Next to no pine trees were recorded at <1%.

	Elevation	DBH	Tree volume (m ³)	Tree number	Sapling number	Cut stumps	Cut saplings	broadleaf %	fern %	palm %	pine %
DA	1602.1	0.15	1.30	106.19	4.62	2.08	4.62	58.40	30.32	11.00	0.28
Cusuco	1592.7	0.18	2.00	81.90	4.01	4.41	4.15	79.25	23.61	6.13	2.55

Means for El Danto and all Survey Sites

Guanales

Guanales survey sites cover a large range of elevations, from 1190 to 1940 metres above sea level with an average of 184 metres below the park's average elevation. With the low elevation, the predicted associations are shown here by having less trees per plot than the park average, but these trees are of greater volume, although DBH measures are similar to the park area with only single centimetre differences. There are very few cut stumps in Guanales survey sites. Sapling count is very low, however there are a large numbers of trees in the 5-15cm DBH category, suggesting recruitment is high.

	Elevation	DBH	Tree volume (m ³)	Tree number	Sapling number	Cut stumps	Cut saplings	broadleaf %	fern %	palm %	pine %
GU	1409.0	0.17	2.16	66.38	3.72	0.79	1.36	91.48	4.07	3.40	1.06
Cusuco	1592.7	0.18	2.00	81.90	4.01	4.41	4.15	79.25	23.61	6.13	2.55

Means for Guanales and all Survey Sites

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ADDITIONAL INFORMATION	N	Range	Min	Max	Mean		Std. Dev	Skewness	
	Statistic	Statistic	Statistic	Statistic	Statistic	Std. Error	Statistic	Statistic	Std. Error
Slope	130	54	1	55	25.08	1.003	11.435	-.172	.212
SoilDensity	130	68.8	5.0	73.8	31.196	1.0458	11.9237	.634	.212
LeafLitter	130	124	10	134	49.91	2.043	23.297	1.182	.212
NrSaplings	130	21.8	.0	21.8	3.793	.2648	3.0189	2.587	.212
NrTrees	130	239	12	251	72.98	3.234	36.870	1.426	.212
TreeHeight	130	74.7	4.2	78.9	18.587	.7639	8.7102	3.171	.212
DBH	130	0	0	0	.16	.005	.062	.763	.212
TreeVolume	130	21.70	.10	21.80	1.9100	.22167	2.52740	5.220	.212
TotalTreeVolume	130	410.60	10.20	420.80	1.039E2	5.92030	67.50185	1.984	.212
percentDEAD	130	95.7	.0	95.7	8.998	.9404	10.7224	4.891	.212
canopy	130	25.0	.0	25.0	3.940	.3530	4.0254	2.488	.212
cutsaplings	130	35	0	35	3.93	.510	5.814	2.525	.212
cutstumps	130	55	0	55	3.95	.678	7.729	3.950	.212
NrBroadleaf	130	144	3	147	49.72	2.109	24.050	1.071	.212
percentBroadleaf	130	83	17	100	72.74	2.001	22.812	-.534	.212
broadleafDBH	130	30.00	7.00	37.00	17.4231	.41968	4.78510	.977	.212
percentBleafDEAD	130	100.00	.00	100.00	10.0708	1.15744	13.19683	4.791	.212
NrPine	130	25	0	25	1.02	.303	3.456	4.451	.212
percentPine	130	83	0	83	2.64	.909	10.363	5.527	.212
pineDBH	130	118.00	.00	118.00	7.2231	1.79319	20.44553	3.269	.212
percentpineDEAD	130	100.00	.00	100.00	3.3192	1.25108	14.26455	5.504	.212
NrPalm	130	39	0	39	3.88	.603	6.873	2.531	.212
percentPalm	130	53	0	53	4.74	.677	7.724	2.707	.212
palmDBH	130	27.00	.00	27.00	4.7154	.54167	6.17602	1.538	.212
percentpalmDBH	130	100.00	.00	100.00	3.0600	.98705	11.25409	6.045	.212
NrFern	130	111	0	111	18.37	2.182	24.879	1.697	.212
percentFern	130	71	0	71	19.88	1.887	21.510	.770	.212
fernDBH	130	32.00	.00	32.00	7.5385	.48853	5.57007	.416	.212
percentFernDEAD	130	100.00	.00	100.00	4.4846	1.11944	12.76358	5.078	.212

Amphibians and Chytrid – By Jim Labisko

A total of 507 anuran swabs (representing 11 species across 9 genera) were delivered to the lab during the 2015 field season (Table 1). Of these 367 were from the four target species of *Deullmanohyla soralia* (174), *Plectrohyla dasypus* (85), *P. exquisita* (51), and *Ptychohyla hypomykter* (57). Samples were processed as follows:

Step 1 – swab processing:

1. Each FTA card used was numbered in sequence (e.g. 001-2015)
2. Swab data transferred to FTA card (following 2015 sample naming protocol)
3. Swab introduced to FTA target and rolled to transfer biological material (DNA) to ensure as even as possible coverage on FTA target
4. FTA card left to dry
5. Data from FTA card (i.e. swab data) entered onto spreadsheet, including any additional notes¹
6. Dried FTA cards stored in plastic pouch/bag with desiccant pack until use

Step 2 – sample processing (according to the Whatman protocol [<http://tinyurl.com/zy6mseal>]):

1. 2-3 punches (medium punch) for each sample removed from FTA card
2. Place punches in 1.5 ml eppendorf tube labelled with sample reference
3. Add 200µL of FTA Purification Reagent to tube
4. Shake/flick the tube to aid mixing and washing
5. Incubate for 5 minutes at room temperature
6. Remove and discard all used FTA Purification Reagent (using vacuum pump)
7. Repeat steps 3-5 twice, for a total of 3 washes with FTA Purification Reagent
8. Add 200µL of TE-1 Buffer (10mM Tris-HCl, 0.1mM EDTA, pH 8.0).
9. Incubate for 5 minutes at room temperature.

¹ Many swabs were received dry. This may have been due to evaporation of preservation medium (ethanol) following a leak from the tube, or perhaps no ethanol being present in the tube. Some tube caps were pushed partially open due to the swab tip having been broken off too long for the cap to remain closed properly. Instructing the herpetologists to snap the swab after pulling it up slightly within the sample tube should lessen the number of dry samples.

10. Remove and discard all used TE-1 Buffer (using vacuum pump)
11. Repeat steps 7-9 once for a total of 2 washes with TE-1 Buffer.
12. Remove all liquid
13. Dry each sample tube in the heat block for 30 minutes (lid open) to ensure all the liquid has been removed/evaporated before performing PCR analysis

Step 3 – PCR prep:

Each dried sample transferred to a pre-labelled puReTaq Ready-To-Go™ PCR tube containing the freeze-dried reagents (in bead form) necessary for PCR².

Step 4 – Master mix (25 x1 µl reactions with primer dilutions of 10 µmol per µl):

1. x1 µl of forward primer (ITS-1: 5'-CCT TGA TAT AAT ACA GTG TGC CAT ATG TC-3')
2. x1 µl of reverse primer (5.8S: 5'-AGC CAA GAG ATC CGT TGT CAA A-3')
3. x23 µl H₂O

Step 5 – Hot-start PCR assay (performed using methods adapted from Boyle et al. 2004). Positive and negative controls were used in each run. Cycling conditions were saved on each PCR machine as CHY2015):

1. Initial denature at 93°C for 10 min
2. Denature at 93°C for 45 sec
3. Annealing at 65°C for 45 sec
4. Extension at 72°C for 1 min
5. Steps 2-4 cycled x30
6. Final extension of 72°C for 10 min.
7. Holding at 10°C.

² Magnesium chloride (MgCl₂) is contained within the dehydrated PCR bead. More MgCl₂ can be added according to the reaction volume; details here: <http://tinyurl.com/j5z8mqy>

Step 6 – Gel preparation:

1. 0.6g agarose
2. 50ml TE
3. 7.5ml gel-red

Step 7 – Gel electrophoresis:

1. 5µl buffer added to each sample
2. 15µl PCR product per well (leaving 15µl for a second run if necessary)
3. 10µl ladder
4. Gel run at ca. 160v/75 ma for 20-25 minutes

We did have some issues. Initially there were no controls available, so the first samples processed to check everything was working as well as we could, were ‘flown blind’ until a decent enough positive was found. When we had a few good positive samples/signals, I ran a PCR of stronger/weaker samples and various solutions without much success. However, we got easily replicated positives by using 2µl of product from an initial PCR (protocol as detailed above), and repeating. From this double-PCR, 100µl of product was dropped onto FTA cards for a control stock (sometimes being technical doesn’t work as well as brute force – cheers Steve G!). For the eagle-eyed, our positives are of unknown quantity as normally run with a *Bd* qPCR (DNA Standards of 100, 10, 1 and 0.1 *Bd* DNA genomic equivalents) but for the presence-absence work that isn’t an issue.

The other problems were fairly standard bearing in mind we’re running PCRs 1500 m up a mountain, in a cloud forest, with equipment that’s been in storage for a year, and multiple opportunities for contamination (we had a bat flying around in the office on more than one occasion). There were a few bits of kit and reagents that were used for (I think) the first time last year and although it took time, we eventually got there. I imagine that this season should settle much quicker. Due to the concerns about contamination, we got through a lot of tips but again, things should go smoother this year with luck, so protocols may be possible to revise in order to reduce use of consumables.

In the end we processed 239 samples (55 samples run twice, 184 samples ran once) (Table 2, 3). For consensus runs (and primarily for the benefit of the dissertation students), results were interpreted as: positive + positive = positive; positive + negative = inconclusive; positive + inconclusive = positive. Single runs were interpreted as was. Positive samples from consensus runs numbered 5, with 12 from single runs. Negative samples from consensus runs numbered 48, with 154 from single runs. The remainder (consensus = 2; single = 18) were inconclusive.

Just over 7% of swabs proved positive (consensus + single run). Of the four target species, the most frequently recorded carrier of *Bd* zoospores (note that to my knowledge no frogs were found in any state of distress or poor health during 2015) appears to be *D. soralia* at 12%. However, bearing in mind their ecology (riparian treefrogs), this figure may be disguising

multiple sampling of the same individual(s) from certain transects as well as probable pseudoreplication. *C. rostralis* is a terrestrial species and showed 29% *Bd*-positive results. Being (presumably) further ranging, it is possible that fewer individuals were sampled multiple times, and the level of *Bd* detection more indicative (all things being equal) of our results. This species is assumed to reproduce by direct development, which can be a limiting factor in *Bd* transmission (Todd 2007) but our data could infer *C. rostralis* as a reservoir and vector of *Bd* to other amphibians. No positive results were obtained for *P. dasypus* in either the consensus or single runs, and only 1 sample was positive for *Pt. hypomykter* (single run). Interestingly (and this is the only comparison I've made), Kolby et al. (2010) found a ~40% prevalence of *Bd* in *D. soralia*, 78% in *P. dasypus*, ~58% in *Pt. hypomykter*, no positives from *P. exquisita*, and ~5% prevalence in *C. rostralis*.

Table 1. Number of anurans swabbed per camp during 2015. The four target species (*D. soralia*; *P. dasypus*, *P. exquisita*, *Pt. hypomykter*) are highlighted in grey rows.

species	Camp								Total
	Buenos Aires	Base Camp	Cantilles	Cortesito	Capuca	Danto	Guanales	Santo Tomas	
<i>Bromeliahyla bromeliacia</i>	-	4	-	-	-	-	-	-	4
<i>Craugastor laticeps</i>	-	-	-	-	-	-	1	-	1
<i>Craugastor rostralis</i>	3	14	-	2	-	-	75	1	95
<i>Craugastor</i> spp	-	-	-	-	-	-	-	4	4
<i>Deullmanohyla soralia</i>	5	87	4	40	-	9	27	2	174
<i>Hyalinobatrachium fleischmanni</i>	1	-	-	-	-	-	-	-	1
<i>Lithobates maculatus</i>	6	1	-	1	-	-	-	2	10
<i>Plectrohyla dasypus</i>	-	6	18	25	9	26	1	-	85
<i>Plectrohyla exquisita</i>	-	14	22	7	1	7	-	-	51
<i>Ptychohyla hypomykter</i>	3	24	6	6	-	10	8	-	57
<i>Rhinella marina</i>	3	-	-	-	-	-	-	-	3
<i>Smilisca baudinii</i>	17	-	-	-	-	-	1	-	18
Anuran spp	-	4	-	-	-	-	-	-	4
Total	38	153	50	82	10	52	113	9	507

Table 2. Results of chytrid screening for swabbed anurans following x2 runs (initial consensus). Results indicated as positive (+), negative (-), inconclusive (?). N = no data for this group.

Camp Result	Base Camp			Cortesito			Danto			Total		
	+	-	?	+	-	?	+	-	?	+	-	?
<i>Deullmanohyla soralia</i>	1	5	0	0	9	0	1	2	0	2	16	0
<i>Plectrohyla dasypus</i>	0	5	0	0	8	0	0	3	0	0	16	0
<i>Plectrohyla exquisita</i>	2	2	1	0	3	1	1	2	0	3	7	2
<i>Ptychohyla hypomykter</i>	0	5	0	0	4	0	N	N	N	0	9	0
Total	3	17	1	0	24	1	2	7	0	5	48	2

Table 3. Results of chytrid screening for swabbed anurans based on a single run (no consensus). Results indicated as per Table 2.

Camp Result	Base Camp			Cantilles			Cortesito			Danto			Guanales			Total		
	+	-	?	+	-	?	+	-	?	+	-	?	+	-	?	+	-	?
<i>Craugastor rostralis</i>	1	2	0	N	N	N	N	N	N	N	N	N	4	8	2	5	10	2
<i>Deullmanohyla soralia</i>	3	10	5	0	4	0	1	12	0	1	5	0	1	13	0	6	44	5
<i>Plectrohyla dasypus</i>	N	N	N	0	16	1	0	11	1	0	13	2	0	1	0	0	38	4
<i>Plectrohyla exquisita</i>	0	7	1	0	14	1	0	3	0	0	4	0	N	N	N	0	28	2
<i>Ptychohyla hypomykter</i>	1	11	3	0	5	0	0	2	0	0	9	1	0	7	1	1	34	5
Total	5	30	9	0	39	2	1	28	1	1	31	3	5	29	3	12	154	18

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Avifauna- By Samuel Jones

1. Point Counts

The standard transect-based survey sites were surveyed throughout the season at all camps excepting the now largely disbanded Santo Thomas. As a general rule, specific survey sites on some transects (e.g. DA-SS5/6) that are largely removed from protocols by other teams remain surveyed for avifauna due to the minimal extra effort required. A large ornithological team, coupled with a constant presence across all camps throughout the season lead to an exceptional volume of data collected during point count surveys, particularly, 7,039 independent detections (21% of all PC data collected over 10yrs!) of at least 91 species.

Minimum sampling requirements of three replicates were completed on all transects (including reverse replicates to account for temporal sampling bias) and in many cases considerably exceeded. Also of particular note was the excellent feedback received from students/helpers from point counts. These results serve as a testament to the hard work, aptitude and excellent spirit of the whole team (often working together on surveys where applicable) throughout the season. While this yielded substantial quantities of data, however, this intensity of sampling was possibly unnecessary and has the potential to cause confounding disturbance levels from foot traffic on certain transects.

In future seasons we may need to tailor some work schedules per camp in order to direct field activities to achieve the most valuable data-spread for methods per camp by including and developing some newer survey methods as illuminated below. The inclusion of formal nocturnal playback surveys may be a particularly useful way of addressing this to collect quantitative information on some of the poorest known avifauna of the park. Naturally there were many un-identified detections in the recorded data but as many of these as possible were identified post-hoc where team members had consistently coded unidentified records.

Table 1. provides a simple breakdown of all species making up $\geq 1\%$ of all detections during all surveys. These form the basis of our indicator species primarily used as proxies for assessing community health to control for year on year staff turnover and unavoidable observer differences.

Table 1. Most frequently recorded species (in descending order)

Vernacular	Binomial	% of records (to 1 d.p)
Slate-coloured Solitaire	<i>Myadestes unicolor</i>	10.5%
Common Bush-Tanager	<i>Chlorospingus flavopectus</i>	10.4%
Grey-breasted Wood-Wren	<i>Henicorhina leucophrys</i>	6.7%
Black-headed Nightingale- Thrush	<i>Catharus mexicanus</i>	6.5%
Chestnut-capped Brush-Finch	<i>Arremon brunneinucha</i>	4.6%
Black Thrush	<i>Turdus infuscatus</i>	4.1%
Yellowish Flycatcher	<i>Empidonax flavescens</i>	3.9%
Slate-throated Whitestart	<i>Myioborus miniatus</i>	3.1%
Spectacled Foliage-gleaner	<i>Anabacerthia variegaticeps</i>	3%
Spotted Woodcreeper	<i>Xiphorhynchus erythropygius</i>	2.9%
Highland Guan	<i>Penelopina nigra</i>	2.1%
Collared Trogon	<i>Trogon collaris</i>	1.7%
Emerald Toucanet	<i>Aulacorhynchus prasinus</i>	1.7%
Azure-hooded Jay	<i>Cyanolyca cucullata</i>	1.5%
Brown-capped Vireo	<i>Vireo leucophrys</i>	1.5%
Olivaceous Woodcreeper	<i>Sittasomus griseicapillus</i>	1.5%
Flame-coloured Tanager	<i>Piranga bidentata</i>	1.4%
Resplendent Quetzal	<i>Pharomachrus mocinno</i>	1.4%
White-winged Dove	<i>Zenaida asiatica</i>	1.4%
Blue-crowned Chlorophonia	<i>Chlorophonia occipitalis</i>	1.3%
Keel-billed Toucan	<i>Ramphastos sulphuratus</i>	1.3%
White-faced Quail-Dove	<i>Zenytgon albifacies</i>	1%
Spotted Wood-Quail	<i>Odontophorus guttatus</i>	1%

2. Mist-netting

The 2015 season marked the fourth season undertaking more structured and standardised mist-netting since its initiation in 2012, modelled on well-established TMAPS³ and CES⁴ survey schemes. This is in order to better understand the basic demographics, longevity, survivorship/recruitment and moult/breeding phenology in under-storey residents, of which

³ Tropical Monitoring Avian Productivity and Survivorship (developed in the United States)

⁴ Constant Effort Sites (used by the British Trust for Ornithology)

most resident species lack almost any quantitative study. Data collected from this are generally of high quality but in previous seasons there remain frustrating inaccuracies from poor recording of data and occasionally poor understanding of the methods involved by some ornithological field workers. A focus for the 2015 season in recruiting team members with qualified and independent experience working with birds in the hand (e.g. BTO⁵ licensing) went a long way to address this. Further, revisions of training material for the Wolfe-Ryder-Pyle tropical ageing codes used and more concise data sheets also helped this. Core constant effort sites are now operated at Base Camp, Guanales, Cantiles, Cortecito and El Danto, with mist-netting at other camps solely for demonstration purposes. With the opening of Capuca for the 2015 season however, we opened up a new constant effort site, although this will be disbanded in future seasons due to logistical constraints. Minimum effort requirements were met at all sites with the exception of El Danto, where only 5 (of 6) days banding effort was undertaken. This was due to unforeseen logistical issues but is unlikely to pose many issues analytically.

A total of 424 captures were made throughout the season comprising of 403 unique individuals and including 78 recaptures across 42 species. A summary breakdown of captures is presented in **Table 2** below.

Table 2. Summary table of all mist-net captures across all camps in 2015 season

Vernacular	Binomial	Total captures (recaptured individuals)
Green-throated Mountain-gem	<i>Lampornis viridipallens</i>	64 (2*)
Black-headed Nightingale-thrush	<i>Catharus mexicanus</i>	42 (14)
Chestnut-capped Brush-finch	<i>Arremon brunneinucha</i>	40(17)
Slate-throated Whitestart	<i>Myioborus miniatus</i>	32(7)
Common Bush-Tanager	<i>Chlorospingus flavopectus</i>	29(7)
Violet Sabrewing	<i>Campylopterus hemileucurus</i>	21
Slate-coloured Solitaire	<i>Myadestes unicolor</i>	19(3)
Ochre-bellied Flycatcher	<i>Mionectes oleagineus</i>	16(3)
Stripe-tailed Hummingbird	<i>Eupherusa eximia</i>	15
Red-capped Manakin	<i>Ceratopipra mentalis</i>	15(4)
Spectacled Foliage-gleaner	<i>Anabacerthia variegaticeps</i>	14(5)

⁵ British Trust for Ornithology

Vernacular	Binomial	Total captures (recaptured individuals)
Magnificent Hummingbird	<i>Eugenes fulgens</i>	9(1*)
Yellowish Flycatcher	<i>Empidonax flavescens</i>	8
Grey-breasted Wood-wren	<i>Henicorhina leucophrys</i>	7(4)
Northern Nightingale Wren	<i>Microcerculus philomela</i>	6(2)
White-faced Quail-dove	<i>Zenytgon albifacies</i>	5
Stub-tailed Spadebill	<i>Platyrrinchus cancrominus</i>	5(1)
Olivaceous Woodcreeper	<i>Sittasomus griseicapillus</i>	5(2)
Spotted Woodcreeper	<i>Xiphorhynchus erythropygius</i>	5(2)
Mayan Ant-thrush	<i>Formicarius moniliger</i>	4(2)
Louisiana Waterthrush	<i>Parkesia motacilla</i>	4
Long-billed Hermit	<i>Phaethornis longirostris</i>	4
Ruddy-capped Nightingale-thrush	<i>Catharus frantzii</i>	3(1)
Ruddy Woodcreeper	<i>Dendrocincla homochroa</i>	3
Azure-crowned Hummingbird	<i>Amazilia cyanocephala</i>	2
Bananaquit	<i>Coereba flaveola</i>	2
Green Violetear	<i>Colibri thalassinus</i>	2(1*)
Azure-hooded Jay	<i>Cyanolyca cucullata</i>	2
Slaty Antwren	<i>Myrmotherula schisticolor</i>	2
Eye-ringed Flatbill	<i>Rhynchocyclus brevirostris</i>	2
Tawny-throated Leaf Tosser	<i>Sclerurus mexicanus</i>	2
Emerald-chinned Hummingbird	<i>Abeillia abeillei</i>	1
White-breasted Hawk	<i>Accipiter striatus</i> [chionogaster]	1
Emerald Toucanet	<i>Aulacorhynchus prasinus</i>	1
Buff-throated Foliage-gleaner	<i>Automolus ochrolaemus</i>	1
Ruddy Foliage-gleaner	<i>Automolus rubiginosus</i>	1
Golden-crowned Warbler	<i>Basileuterus culicivorus</i>	1
Grace's Warbler	<i>Dendroica graciae</i>	1
Barred Forest-Falcon	<i>Micrastur ruficollis</i>	1
[Southern] House Wren	<i>Troglodytes aedon</i>	1
Collared Trogon	<i>Trogon collaris</i>	1
Black Thrush	<i>Turdus infuscatus</i>	1

Recurrent recaptures of breeding condition birds in the same nets serves to evidence that birds almost certainly retain year round territories and are generally long-lived.

Table 3. Selected capture histories for some species- FCF age-codes are immature birds (not juveniles), DCB are adults.

Species	Capture Date	Camp	Ring #	Age	Sex	Net #
Black-headed Nightingale-thrush <i>Catharus mexicanus</i>	16/06/2012	Base Camp	Y1/HN-B144	DCB	F	9
	02/07/2012			DCB		9
	14/06/2013			DCB		6
	11/07/2015			DCB		8
	25/07/2015			DCB		9
Time since 1 st capture- 3yrs 1 month 9 days						
Black-headed Nightingale-thrush <i>Catharus mexicanus</i>	16/06/2012	Base Camp	Y2/HN-B170	FCF	M	9
	19/06/2012			FCF		9
	28/06/2012			FCF		8
	14/07/2013			UCU		3
	13/06/2015			DCB		8
	15/06/2015			DCB		8
	25/06/2015			DCB		7
	11/07/2015			DCB		9
	23/07/2015			DCB		8
	25/07/2015			DCB		9
Time since 1 st capture- 3yrs 1 month 9 days						
Black-headed Nightingale-thrush <i>Catharus mexicanus</i>	22/06/2012	Guanales	Y10/HN-B143	DCB	M	?
	25/06/2012			DCB		5
	14/07/2012			DCB		2
	22/06/2014			DCB		5
	06/07/2014			DCB		5
	31/07/2015			DCB		6
Time since 1 st capture- 3yrs 1 month 9 days						
Chestnut-capped Brush-finch <i>Arremon brunneinucha</i>	22/06/2012	Guanales	B2/HN-C310	FCF	M	9
	21/06/2013			UCU		10
	03/08/2015			DCB		4
Time since 1 st capture- 3yrs 1 month 12 days						
Common Bush-tanager <i>Chlorospingus flavopectus</i>	06/07/2012	Cantiles	G47/HN-AB015	FAJ	M	4
	10/07/2014			FAJ		2
	21/06/2015			FAJ		2
Time since 1 st capture- 2yrs 11 months 15 days						

Before meaningful demographic information and trends can be extracted from this we require at least one more year of data, however, a large and novel set of data has been collected on morphometrics and ageing/moult patterns thus far.

Extensive feather samples were collected from captured birds for isotopic analysis for food web structure adding to initial samples collections in 2014. As part of this, a smaller project on Hummingbird spp. niche breadth is being undertaken. For further details on this, see the summary of mammalian research 2015.

3. Opportunistic surveys

Historically, opportunistic records have been recorded ad-hoc and very sparsely, leading to an unrepresentative and largely uninformative dataset except for documenting occasional occurrence of less frequently recorded species. This season, new methods were employed to maximise opportunistic surveys and reporting effort using simple but well-established methods employed by large citizen science birding schemes, BirdTrack and eBird. These involve recreational birding but simply defining effort (start and end times) with complete lists of all species seen and heard during the time at a given location. These offer strong predictive power of relative abundance (when accounting for location/altitude) by % occurrence of species lists.

This offers an exciting site-specific dataset that will become increasingly valuable with input, of which some analysis is being undertake currently (see project outputs below). To this end, opportunistic nocturnal surveys were also undertaken, particularly focusing on playback for Middle-American Screech-Owl *Megascops guatemalae*, known only from one previous record in the park. The results of this confirmed their presence at six camps and it was found to be relatively common including at significantly higher altitudes than have been reported for the species elsewhere. Results such as this illustrate the misrepresentation of some species abundance when specifically targeted.

This resulted in 2872 records of 171 species, a large number of which were not documented in any other methods. A focus on quantitatively using recreational birding encouraged a very motivated team to bird in the field outside of scheduled survey protocols and was certainly a factor in the finding of 2 new species for the park, these being the Green-breasted Mango *Anthracothorax prevostii* and Purple-crowned Fairy *Heliothryx barroti* respectively. Further, numerous other rare species such as Lovely Cotinga *Cotinga amabilis* and Keel-billed Motmot *Electron carinatum* were recorded as a result of increased opportunistic survey efforts.

Finally, all bird records from camera-trapping in 2014 and 2015 have been identified and compiled for their use in analyses for both camera-trap and ornithological work, these datasets are relatively small, but provide particularly interesting records of species such as Great Curassow *Crax rubra* and Slaty-breasted (Boucard's) Tinamou *Crypturellus boucardi* that are very infrequently recorded otherwise.

4. Project outputs

i) Projects

This season the ornithological team included and supported two projects from Kathleen Farley (Rutgers University, New Jersey) and Adam Milligan (Edinburgh Napier University), respectively, both graduate students. While working as ornithologists in the field, both students used this and past seasons data. Kathleen's project is still underway and is assessing the efficacy of time-per-method comparisons to compile as complete as possible a species list under tight time constraints, to inform a 'roadmap' of rapid assessment methodologies for avian cloud forest communities. Adam's project was submitted in December (2015) entitled 'Integrating open access phylogeny data in the rapid assessment of biodiversity: The application of non-neutral phylogenetic indices in identifying patterns of avian diversity in Mesoamerican cloud forest, Cusuco National Park, Honduras'.

ii) Publications

The following manuscript is in press following recent revisions-

Martin, T, Rodrigues, F., Simcox, W. Dickson, I., van Dort, J., Reyes, E. & Jones, S.E.I. A review of notable range and altitudinal records from Parque Nacional Cusuco. *Cotinga*

Several other manuscripts are in preparation as outlined below.

5. Analysis of current data

An analysis of all point count data from 2006-2015 is currently being undertaken and being led by Monte Neate-Clegg, a member of the ornithology team from this year and myself. Early results indicate that while species richness at the community level and for montane specific species has not changed, there has been a significant change over time of the montane community evenness (Shannon diversity index). Further, both general and montane avifaunal communities (e.g. **Fig 1**) appear to show some gradually upslope shift, becoming more akin to the community of a lower elevational band. This analysis is currently being fine-tuned with the inclusion of more abiotic variables to greater understand how various factors are interacting and possibly even mediating climate effects. This is currently in the early stages of preparation for journal submission once analyses have been fine-tuned.

2015 was also very successful for an ongoing preparation of a manuscript to provide a 'digitised' and unambiguous inventory of the national park to publish along with density data. Large numbers of sound recordings from the park have and are continually being uploaded to the avian internet sound archive xeno-canto in the following collection (<http://www.xeno-canto.org/set/406>) with the same being undertaken for photographs on Internet Bird Collection (<http://ibc.lynxeds.com/locality/neotropical/honduras/cusuco-national-park>).

Finally, several short natural history notes are close to submission documenting new aspects of natural history of various cloud forest birds, such as breeding behaviour of Violet Sabrewings *Campylopterus hemileucurus* and new prey species of the White-breasted Hawk *Accipiter striatus* [*chionogaster*].

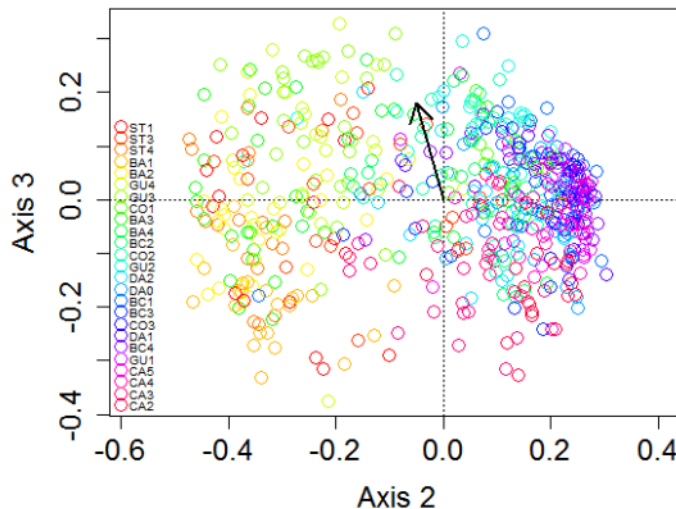


Fig 1. Community composition shifts in specifically montane species indicating a slight shift in montane bird communities towards that of a lower elevational band and also community evenness. Particularly with altitude shifts this is slightly less than that may be expected and with the inclusion of more abiotic factors we hope to illuminate this further.

Bats- By Dr Kevina Vulinec

The primary bat team (Kevina Vulinec, Aniko Kurali, Pamela Medina, Juan Carlos, Tom Davey) spent 83 mist-net nights capturing bats between 10 June 2015-1 Aug 2015. 41 species were collected, some of these rare in previous collections (Table 1). We collected a total of 326 individuals; the most common species were *Sturnira ludovici* (69 individuals) and *Artibeus jamacensis* (62 individuals). Other species were collected 23 times or less, mostly numbering in the single digits. We captured the greatest number of bats at Base Camp (95), followed closely by Buenos Aires (93). The total number of individuals was less than collected for previous years; this result may be due to the number of days with rain, the number of bat team volunteers was smaller than previous years, or that the Santo Tomas site was not surveyed this year (e.g. 143 bats were captured in 2014 at this site). If no bats were caught, that result does not mean that there are no bats or few bats in a site, but that mist-nets were not effective at capturing bats at a particular location (Macswiney et al. 2008). In the case of bats, absence of data does not connote absence of bats or necessarily even a low abundance.

Preliminary Acoustic Results

We (primarily K. Vulinec and T. Davey) recorded bats for 20 recorder-nights and a total of 5372 passes were identified as bat calls. These have not been identified completely yet, but around the main camping area of Base Camp, we could identify many calls by *Pteronotus parnellii*, *Pteronotus davyi*, and *Molossus rufus*, insectivorous bats rarely caught in mist nets (Table 1). Some highlights of the survey include the first capture of *Lasiurus ega* (Fig. 1) since 2010, the first *Eptesicus fuscus* since 2012 and the first capture of this species at Base Camp, and the first record of *Tonatia saurophila* at Cusuco.

Lights and Bats Study

We collected a total of 5013 bat calls (mean per night = 263.8) from around the light trap while it was either on or off. The light trap study revealed that there was no significant difference in the number of bat passes between nights when the light was on or off (Wald Chi-square = 0.063, df = 1, P = 0.802) (Table 3; Fig. 2). We identified six species (an example in Fig. 3), mostly insectivorous bats, and expect with closer scrutiny to identify more species (Table 4). This result is good news for those times bat recording and insect collecting overlap in space and time. From these results, we conclude that light traps, or smaller lights like torches, do not significantly affect bat activity.

Table 1. The numbers of each species mist-netted at each camp.

Species	Base Camp	Buenos Aires	Capuca	El Cortecito	El Danto	Guanales	Grand Total
<i>Anoura geoffroyi</i>			1				1
<i>Artibeus aztecus</i>		3				7	10
<i>Artibeus jamaicensis</i>	2	24				36	62
<i>Artibeus lituratus</i>		4					4
<i>Artibeus phaeotis</i>	1	4				3	8
<i>Artibeus toltecus</i>	10	6	1	2		4	23
<i>Artibeus watsoni</i>	1	7		1			9
<i>Bauerus dubiaquercus</i>		4	2			3	9
<i>Carollia brevicauda</i>		1					1
<i>Carollia sowelli</i>	2	9	1	3		5	20
<i>Centurio senex</i>		3	4			2	9
<i>Chiroderma salvini</i>		1				1	2
<i>Choeroniscus godmani</i>						3	3
<i>Chrotopterus auritus</i>		1				4	5
<i>Desmodus rotundus</i>	2	5			1	1	9
<i>Diphylla ecaudata</i>	2						2
<i>Enchisthenes hartii</i>		1		3		1	5
<i>Eptesicus furinalis</i>	5						5
<i>Eptesicus fuscus</i>	1						1
<i>Glossophaga commissarisi</i>	1						1
<i>Glossophaga leachii</i>		1				1	2
<i>Glossophaga soricina</i>		1					1
<i>Hylonycteris underwoodi</i>		1	2	1	2	1	7
<i>Lasiurus ega</i>	1						1
<i>Lonchophylla mordax</i>	1	3	1	5		5	15
<i>Lophostoma silvicolium</i>		1					1
<i>Micronycteris microtis</i>		1					1
<i>Micronycteris schmidtorum</i>					1		1
<i>Myotis albescens</i>			1				1
<i>Myotis keaysi</i>	13		2	1	3	1	20
<i>Myotis sp.</i>				1			1
<i>Phyllostomus hastatus</i>		1					1
<i>Platyrrhinus helleri</i>		1					1
<i>Pteronotus davyi</i>		1					1
<i>Pteronotus parnellii</i>				2			2
<i>Sturnira lilium</i>	1	3	1				5
<i>Sturnira ludovici</i>	50	5	4	6	2	2	69
<i>Trachops cirrhosus</i>					1	1	2
<i>Tonatia saurophila</i>						1	1



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<i>Unknown sp.</i>	1						1
<i>Vampyrodes caraccioli</i>	1	1				1	3
Grand Total	95	93	20	25	10	83	326

Table 2. Total counts of individual bats over last 4 years.

YEAR	# INDIVIDUALS CAPTURED
2010 Count	385
2011 Count	624
2012 Count	582
2013 Count	565
2014 Count	552

Table 3. The number of bat calls recorded nightly from around the mercury vapor insect light trap.

Date	Call Count	Light
6/7/2015	263	OFF
7/7/2015	149	OFF
14/7/2015	353	OFF
20/7/2015	423	OFF
23/7/2015	33	OFF
28/7/2015	141	OFF
1/7/2015	226	ON
2/7/2015	225	ON
5/7/2015	228	ON
11/7/2015	337	ON
12/7/2015	71	ON
13/7/2015	123	ON
15/7/2015	273	ON
16/7/2015	447	ON
17/7/2015	302	ON
18/7/2015	336	ON
19/7/2015	355	ON
21/7/2015	603	ON
22/7/2015	125	ON

Table 4. Species identified from recorded calls at mercury vapor lamp study.

Species	Number of calls
<i>Eptesicus furinalis</i>	1
<i>Eptesicus fuscus</i>	1910
<i>Lasiurus ega</i>	1467
<i>Molossus rufus</i>	2
<i>Myotis keaysi</i>	112
<i>Pternotus davyi</i>	496
Unknown	1025
Total	5013



Figure 1. *Lasiurus ega* from Road transect.

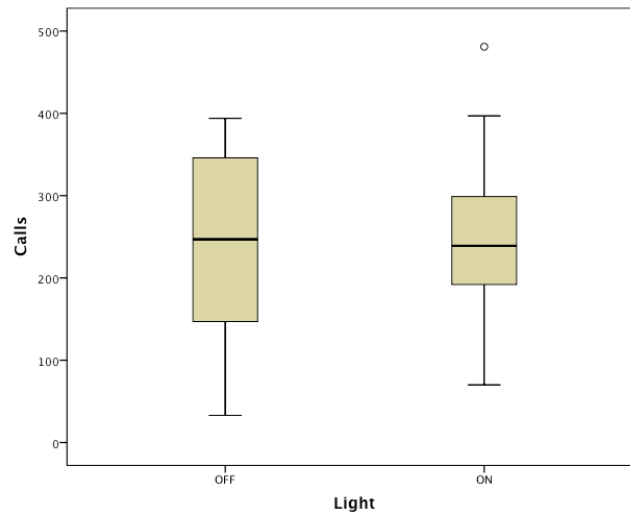


Figure 2. The difference between bat calls/night during times when the insect mercury vapor lamp was on versus when it was off. Boxes are the 25th quartile to the 75th quartile; the central line is the median, whiskers represent the range of the data and small circles are outliers.

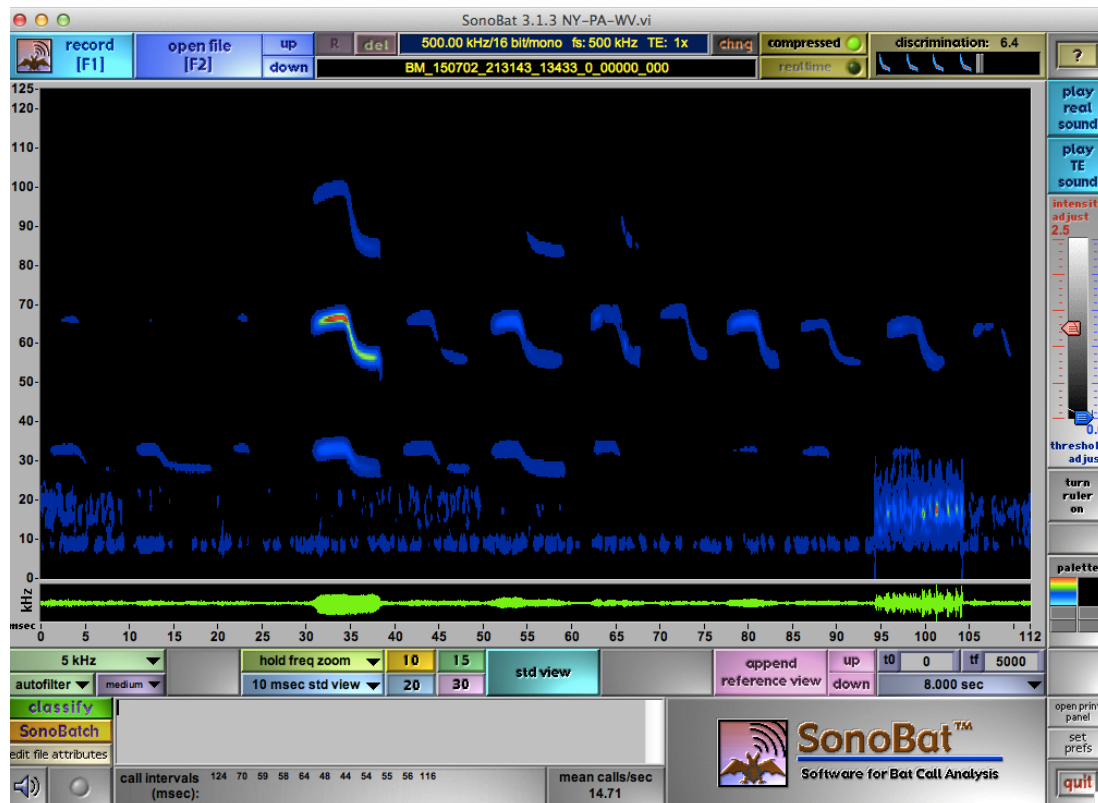


Figure 3. Sonobat sonograph of the insectivorous bat *Pternotus davyi* recorded during the insect light trap study. X-axis is time in msec; Y-axis is frequency in kHz. Katydid call can be seen in lower right of screen.

Future Analyses

The species of bats collected in mist-nets and those identified from calls will be analysed using occupancy models with habitat, vegetation clutter, altitude, human impacts, and occurrence of predators as predictor variables. In addition, correspondence analysis, multivariate analyses of site-by-species abundance tables, or methods of spatial pattern analysis will be used to examine microhabitat and spatial variable effects on communities of bats (Dray *et al.* 2012). In addition, trait-based community analyses would be appropriate given the differences between fruit, pollen, and insect-eating bats and their habitat requirements (Nichols *et al.* 2012; Mouillot *et al.* 2016).

Recommendations

We recommend the inclusion of the road to Buenos Aires as a permanent transect. The wide road serves as a corridor and we collected more bats (and several unusual species) at that transect than any other. It was also a good spot for students, as we almost always caught bats to show the students and demonstrate our methods, discuss bat biology, and encourage them to become involved in bat conservation.

Acoustic monitoring should be conducted at every site during the mist-netting timeframes to record calls of bats that are usually not caught in nets (mostly insectivorous bats). Given recent technology and the many recording devices on the market, I suggest 5 (or more) Wildlife Acoustics SM3BAT units or SM4BAT-FS units if possible. I make this recommendation based on cost, sturdiness, and the quality of recordings. In addition, studies have shown that the best analysis software program is Sonobat™ for species identification and analysis of behavior.

It is clear that different sites within the park vary considerable in their species compositions (Table 1). Analyses of community structure at each site over time (2006-2015) should begin as soon as possible, as changes to the buffer zone of the Park are occurring rapidly.

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Mammals- By Dr Neil Reid

a) Small mammals 2015

Twelve wire Freya traps were placed in pairs approx. 10m apart (i.e. six pairs) in each of three microhabitats (riverine, riparian or terrestrial) at each camp. All traps were left in-situ for 5 consecutive days being checked each morning. The riverine traps targeted an unknown water mouse (*Rheomys* spp.) and were placed within streams on flat rocks, logs or on sandy river banks at water level and were baited with fresh crab. All remaining traps were baited with a mix of peanut butter, oats and syrup. The riparian traps targeted the Mexican deer mouse (*Peromyscus mexicanus*) and were placed, not at water level, but within 3m of a stream bank. The terrestrial traps targeted Desmarest's spiny pocket mouse (*Heteromys desmarestianus*) and were placed >150m from the nearest stream. Results are expressed in mean numbers caught per trap night to account for survey effort. A total of 139 individuals of five species were caught (Table 1).

Table 1 Small mammal trapping results for each camp during summer 2015.

Camp	Mexican deer mouse	Desmarest's spiny pocket mouse	Water mouse <i>Rheomy</i>	Slender harvest mouse <i>Reithrodontomy</i>	Alston's singing mouse	Total
Base	17	10	3	2	2	34
Cantilles	18	2	1			21
El	12	20	1	5		38
Capuca	3	7				10
El Danto	4	7	2	2	1	15
Guanale	11	10				21
Santo	n/a	n/a	n/a	n/a	n/a	n/a
Total	65	56	7	9	3	139

Small mammal survey protocols have varied over the years (2012-2014) to test varying hypotheses and to trial different baits and trap placements. 2015 was the first year in which a standardised trapping protocol was designed and implemented at all camps to form the basis of future monitoring to enable comparisons between camps and years. As a first attempt to create a standardised index of abundance by which to assess temporal trends in populations, data from previous years were subsampled to retrospectively create comparable subsets i.e. data were restricted to riparian and terrestrial trap lines baited with peanut butter, oats and syrup mix only adjusted for trapping effort. This approach suggested, in line with previous work, that small mammal abundance is greatest in close proximity to waterways (principally driven by the abundance of the Mexican deer mouse) and lowest in terrestrial environments (principally driven by Desmarest's spiny pocket mouse) with a positive temporal trend (Fig. 1). During 2015, small mammal abundance was greatest at El Corecito and lowest at Capuca (Fig. 2)

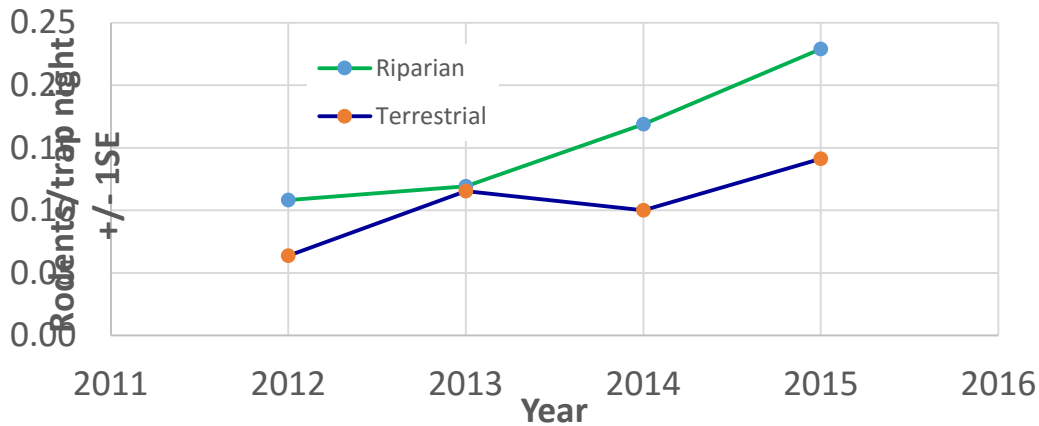


Fig. 1 Temporal trends in small mammal abundance (all species aggregated) between 2012 and 2015 (note that trends within camps may be different and this is the overall mean trend). Small mammal populations elsewhere can exhibit between-year variation with some showing distinct multiannual cyclicity due to climatic forcing. As Cusuco National Park is likely to be affected by the El Niño Southern Oscillation (ENSO), peaking in winter 2015/16, it will be necessary to continue standardised small mammal monitoring over the next few years to establish if such cyclicity exists in this system.

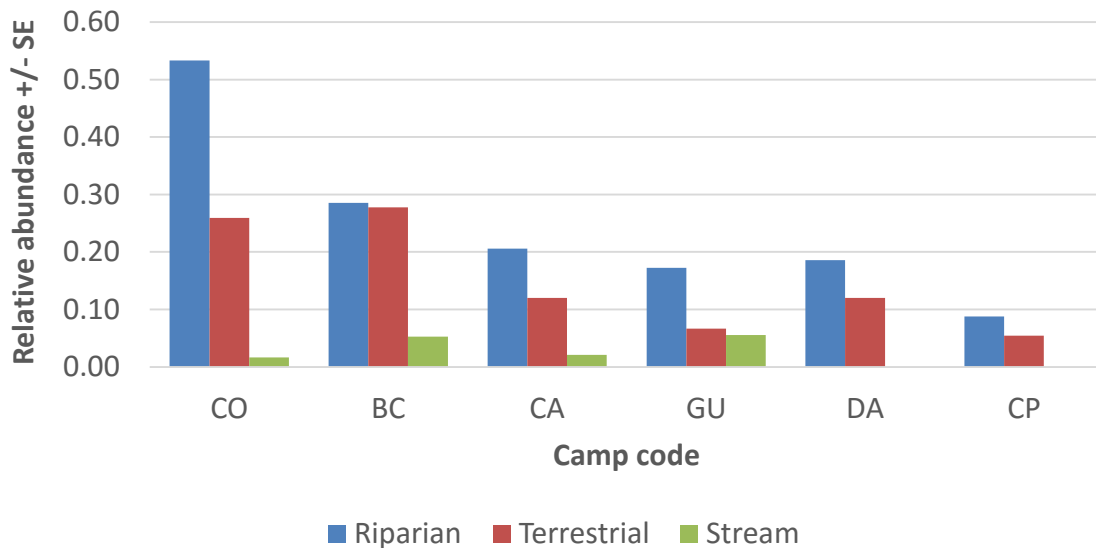


Fig. 2 Small mammal abundance during 2015 was greatest at El Corecito and lowest at Capuca with abundances consistently lowest in the streams, highest along the riparian corridor and intermediate in the terrestrial microhabitat.

b) Large mammals 2015

i) Tracks & signs

All transects at all camps were surveyed for field tracks and signs of large mammals during 2015 consistent with previous years. The large mammal team always attempt to be the first, or one of the first survey teams, to survey each transect when each camp opens in an attempt to minimise disturbance. A total 130 field signs were identified belonging to 10 species including 29 tracks or signs of the endangered Baird's tapir (*Tapirus bairdii*). Five hunting platforms were found; 3 at Base Camp and 1 at each of Capuca and El Cortecito (Table 2).

Table 2 Large mammal tracks and signs identified at each camp during summer 2015.

Common name	Scientific name	Base	Cantiles	Capuca	Cortecito	Danto	Guanales	Total
Baird's tapir	<i>Tapirus bairdii</i>	1	11	13		3	1	29
Deer, likely Red brocket	<i>Likely</i> <i>Mazama americana</i>	2			3	12	15	32
Collared peccary	<i>Pecari tajacu</i>	1				8	4	13
Cat, likely margay	<i>Likely</i> <i>Leopardus wiedii</i>	0	1	1	0	0	2	4
Howler monkey	<i>Alouatta palliata</i>				2	2		4
White-nosed coati	<i>Nasua narica</i>	2	1	1	1	2	4	11
Paca	<i>Cuniculus paca</i>	3	0	1	2	3	3	12
Nine-banded Virginia opossum	<i>Dasypus</i> <i>Didelphis</i>	1		2	7	8	2	20
Kinkajou	<i>Potos flavus</i>			1	1	2	1	4
Total		10	13	19	16	40	32	130
Hunting platforms		3		1	1			5

ii) Camera trapping

A total of 28 camera traps were deployed at 129 locations in triplets (<20m, 150m and 300m perpendicular to the transect) at all camps for an average of 3 days duration each. A total of 175 detections were made of 16 species including the endangered Baird's tapir (*Tapirus bairdii*; Fig. 3). This is the second year of widespread camera trap deployment. During 2014, cameras were set for a prolonged period i.e. 3 weeks each at few location (n=42) but in 2015 the same number of cameras were set for a shorter period i.e. 3 days and moved regularly to more locations (n=129) to test whether long or short deployments maximised detections per unit effort. For all taxa, detections were higher in 2015 and significantly so for medium

and small sized mammals (Fig. 4). Thus, future surveys should adopt a methodology of greater numbers of cameras placed for shorter periods i.e. moved regularly.



Fig. 3 A Baird's tapir (*Tapirus bairdii*) detected 166m from Transect 1 at El Danto in July 2015.

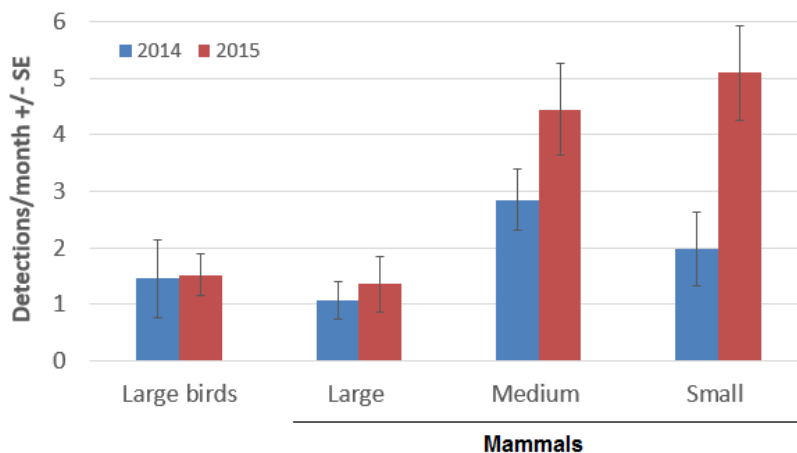


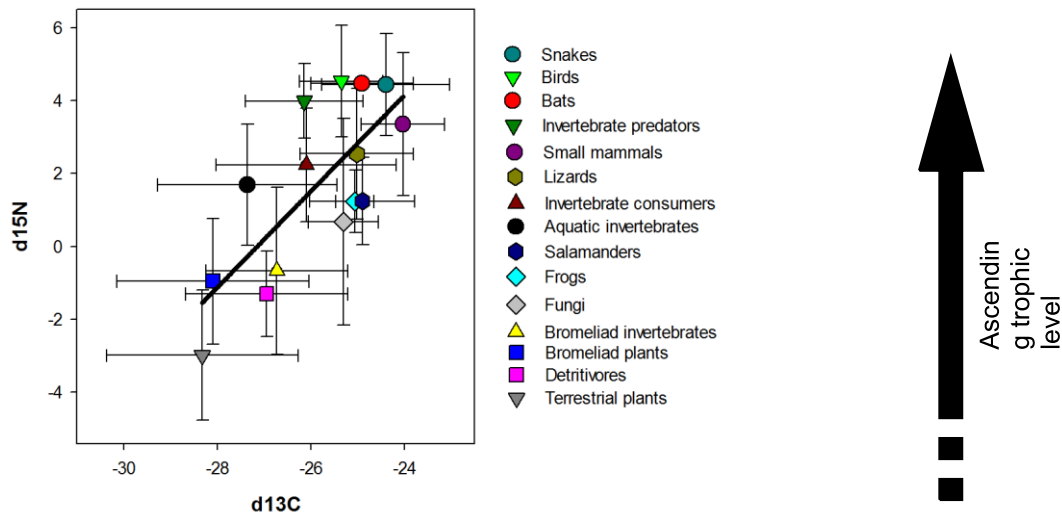
Fig. 4 Differences in detection rates for mammals (large, medium and small sized) and large bird species between 2014 (lower number of independent locations $n=42$ but longer deployment i.e. ~3 weeks) compared to 2015 (larger numbers of independent locations $n=129$ but shorter deployment i.e. ~3 days).

c) Stable Isotope Analysis 2015

A total of 612 samples have been taken and analysed for stable isotopes spanning all taxa for a food web based analysis. Analysis of focal taxa e.g. snakes needs to be completed but the data are available to dissertation students for specific projects. Otherwise, additional sampling builds a database of samples for future analysis once suitable sample sizes within-taxa are achieved ($n>15$). Analysis of snake diet and hummingbird niche breath are being conducted as an MSc and BSc project in 2015/16 (results pending).



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Invertebrates- By Thomas Creedy

Dung beetles – methods and preliminary findings

The standard survey network of sites was sampled using 4x dung baited pitfall traps left for between 3 days and a week, previous data having shown no significant difference in catches within that time period. Certain areas of disturbance were also sampled, resulting in a total of 152 locations surveyed. These consisted of 8 disturbance sites on the west side of the park and 10 sample sites from Santo Tomas that are not or no longer considered part of the core monitoring, resulting in 134 core monitoring sites including the 20 sites in the new camp of Capuca. Of these 152 locations, 99.3% (151) were sampled the planned minimum of four times (once for Santo Tomas), with an average of 5.8 samples per site, as many as 8 or 9 in some east side camps. A total of 829 samples were collected, the most ever collected in a single season. This was due to having a large enough team that all camps could be staffed for the entire time they were open, and although this will provide us with excellent data it is probably an unnecessary intensity of sampling. For this reason, in Base Camp and Guanales camps the standard traps were closed after eight and five collections respectively, and a novel system of live trapping was piloted. This consisted of six sites in each camp set with live traps of varying design, checked every 24 hours. This reduced the number of dung beetles killed, the number of samples needing identifying, and hopefully provided some interesting data on dung beetle population sizes.

All samples were sorted (dung beetles separated from bycatch) and identified to species or morphospecies before the end of the season. The hard work and dedication of this year's staff was overwhelmingly the major contributor to this achievement. Identification was carried out using the OpWall-funded Creedy and Mann 2011 identification guide. A total of 18,787 *Scarabaeinae* dung beetles were found, an average of 22.8 per sample. 24 of the 40

species known to exist in Cusuco National park were found, although this is likely to rise to 26 once a few tricky-to-resolve species pairs are ID'd in the UK. Nonetheless, this is relatively lower than in previous years: this may partly be because Santo Tomas, an area of high diversity due to the heterogeneity of the disturbed forest, was sampled much less. However, across the park there seems to be a reduction in the number of smaller species collected: for the first time, no individuals of the genus *Uroxys*, exclusively >5mm beetles, were collected, and several of the smaller *Canthidium* species were not found. Only one individual of *Cryptocanthos sp.nov.*, and no individuals of *Copris sp.nov.* – two new species to science found only in Cusuco – were found this season. *Cryptocanthos* is very small, and the *Copris* is found mostly in Santo Tomas and the west side of the park.

Light trapping – methods and preliminary findings

Each camp was sampled for jewel scarabs and moths using a 125W MV positioned in front of and above two white sheets. Light traps were run between 19.30 and 21.30 unless rained off. The time of arrival and species of each jewel scarab that arrived at the trap was recorded and the individuals marked with unique codes and released. Sphingid and Saturniid moths were collected from traps, as were all beetles. This standard protocol was carried out at all camps, in one or two locations at each camp. If a jewel scarab arriving at the light trap was marked, this was recorded in the data.

Data is still coming in and being validated, but approximately 130 light traps were run during the season across the park, including opportunistically in Santo Tomas and Buenos Aires outside of the standard protocol. Of the about 110 traps run in forest camps, the distribution was uneven – Cantiles, Danto and Cortecito only ran between 12 and 14 traps, whereas almost 36 were run in Base Camp. This is clearly due to variation in opening times for these camps; in general, traps were run about every other night in every camp.

A total of roughly 200 *Chrysina spp.* jewel scarabs will have been recorded over the season, with every known species recorded at least once, including three individuals of the putative new species. Occurrence of the species was unsurprisingly uneven, *C. karschi* being highly prevalent and *C. pastori* and *C. strasseni* being relatively rare. These numbers are substantially higher than in previous years, although this will be due to the greater number of higher strength light traps. Taking effort into account, it's estimated that the numbers will still be higher, but not as substantially. Only five recaptures took place, all in Base Camp. The very low number of recaptures means this data is not suitable for mark-recapture analysis – statistics would suggest a very high population size, but there is not sufficient replication for this to be valid. That is not to say the population sizes are not high, it is likely they are, but the trapping did not sufficiently sample the jewel scarabs to show this. Unlike in previous years, the marking system was relatively resilient, so we do not expect this to be the reason for the low number of recognised recaptures.

Approx. 650 moths of the families *Sphingidae* and *Saturniidae* were collected of 59 species. Of these, approximately 20 are thought to be new to the park, and 9 may be new species to

science. Specimens are highly dominated by a few species, with only 10 species having been found more than 10 times. In general, this has been a substantially improved season for moth collection, based on both improved light trapping conditions, and specimen storage and on-site taxonomy.

Orchid bees

A small-scale study looking at the effect of bait concentration on the attraction of orchid bees to traps was conducted as a dissertation project. This was very successful, with over 3,400 orchid bees of 15 species. 36 locations were sampled with one of two baits at 6 concentrations in a randomised order. Clear community differences were found between the two baits, as expected from previous years' sampling, and a general response of increased attractiveness with bait concentration was found, although with differences between species that will need further statistical analysis to tease apart.

Opiliones

Ad-hoc collection of opiliones was carried out by Brittany Damron, resulting in the collection of six species. These will be used for genetic and morphometric analysis.

Canopy communities

Trees from four camps were sampled using canopy fogging, four trees each of two species, *Liquidambar styraciflua* and *Pinus sp.* Approximately 250 community samples were collected.

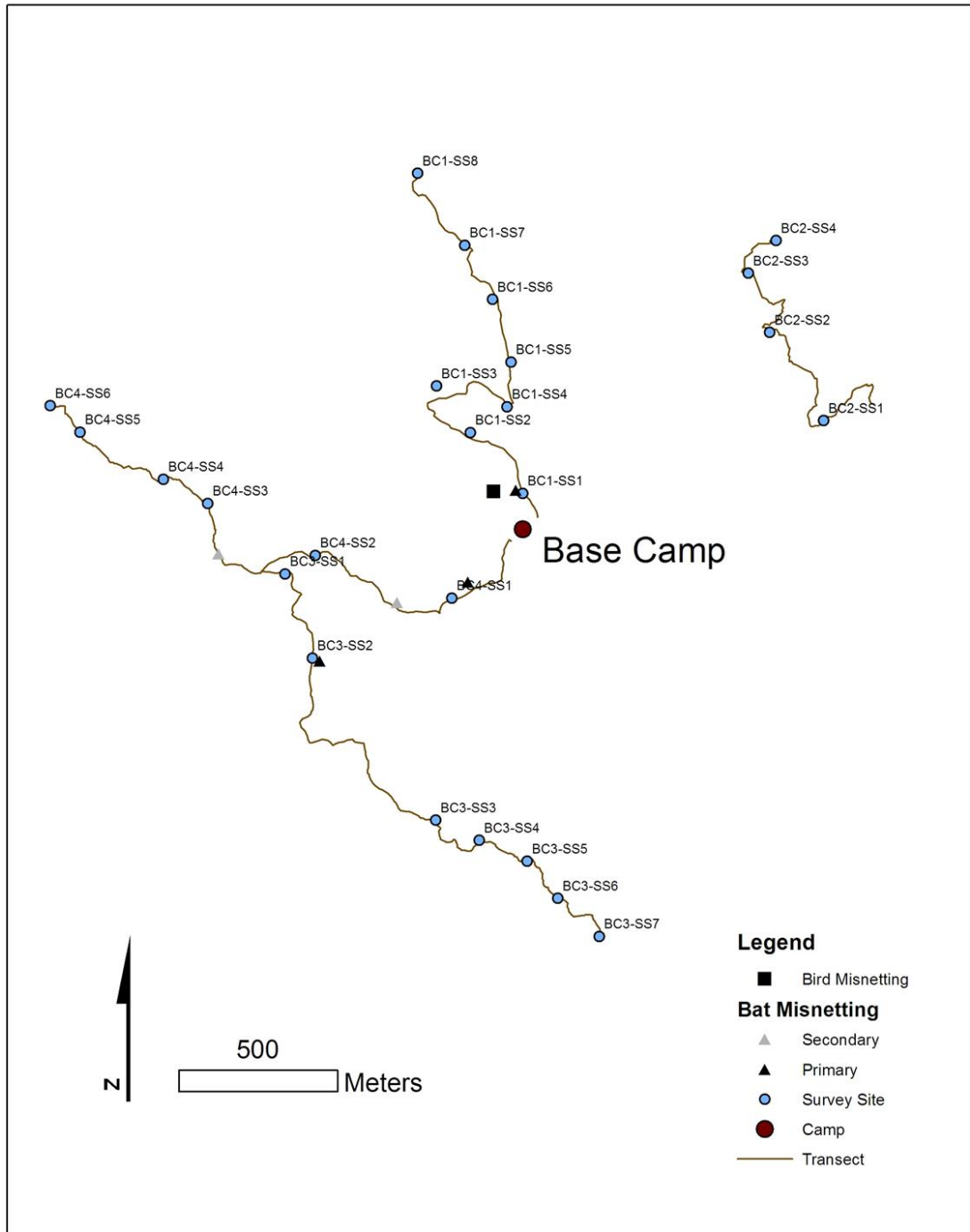
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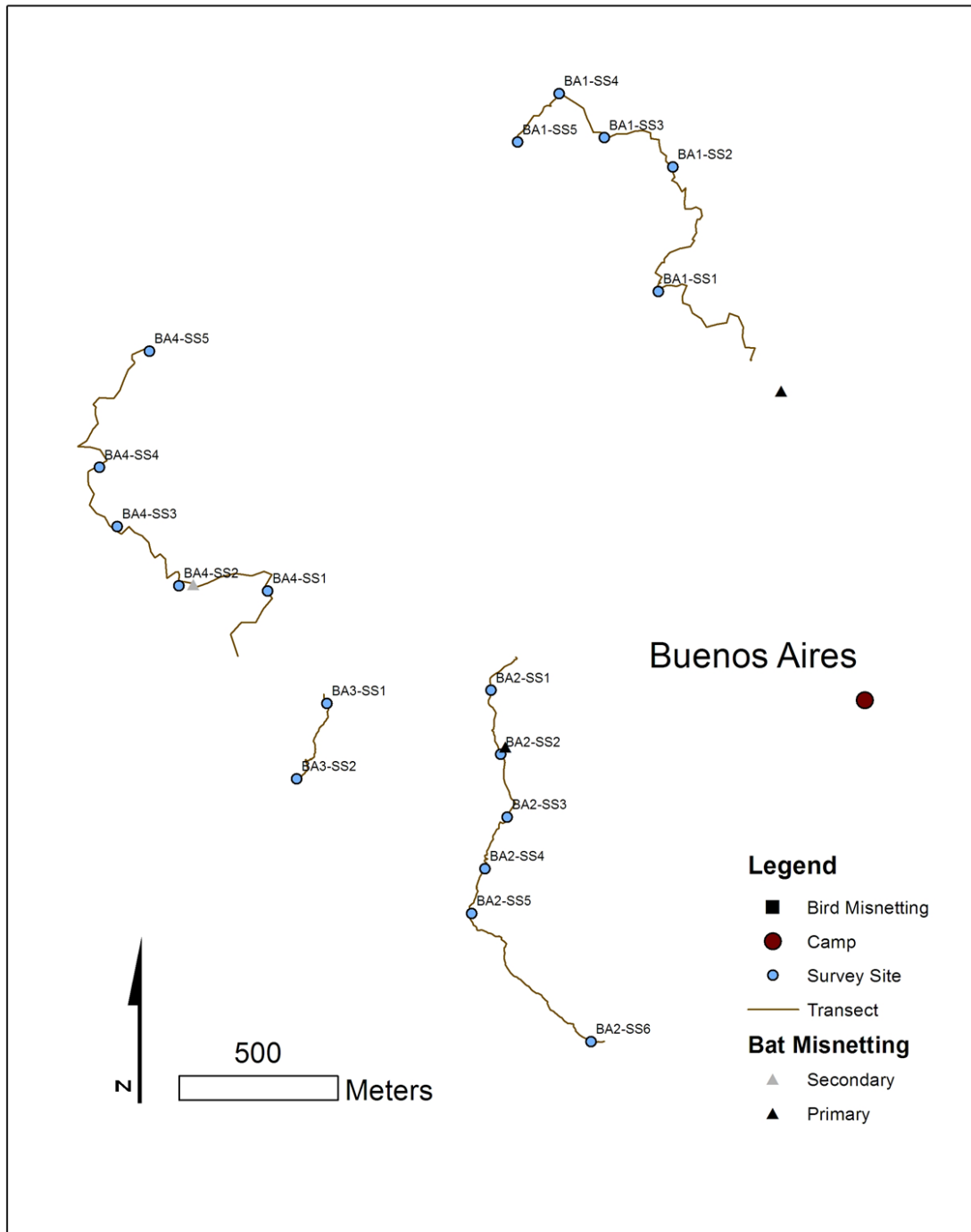
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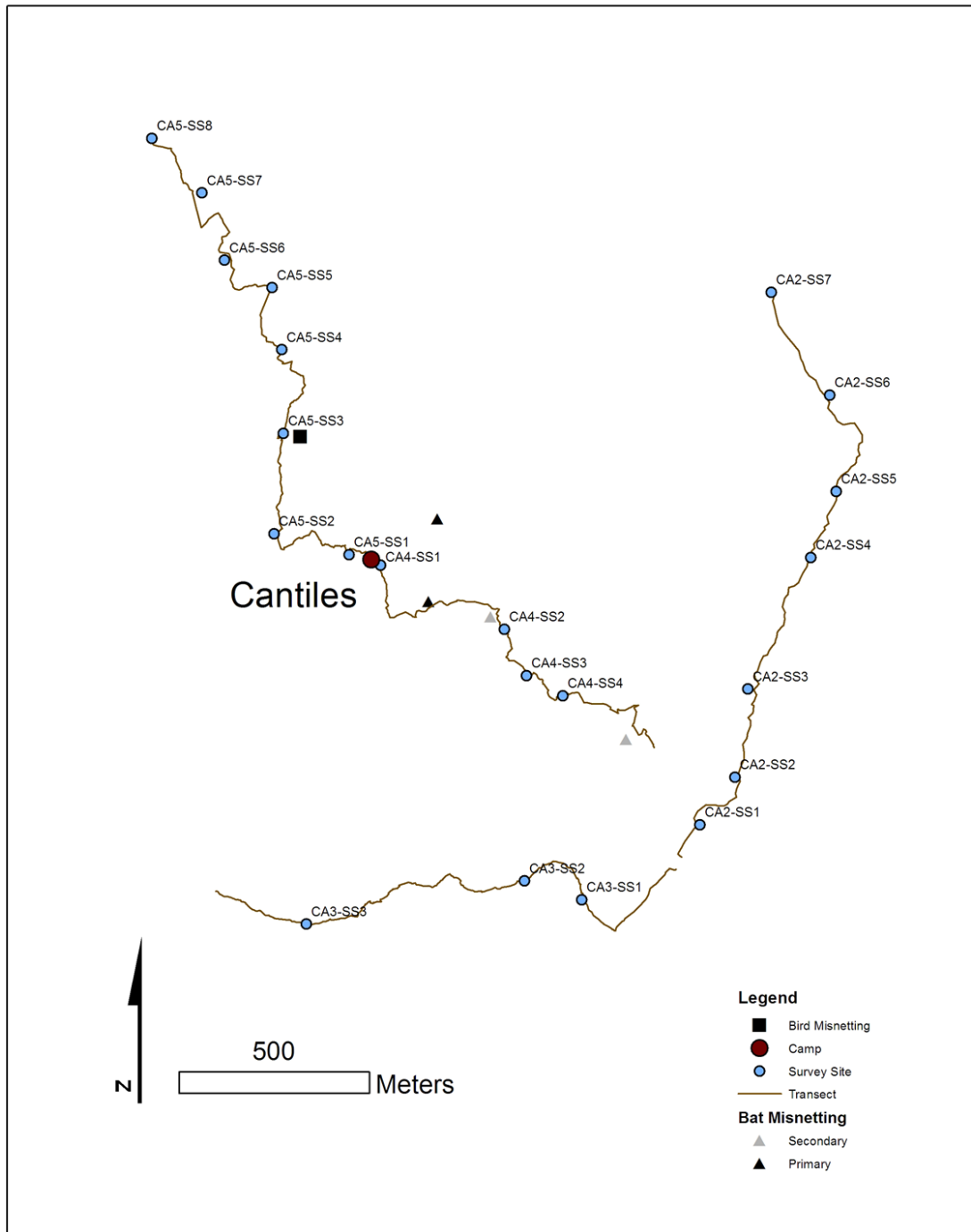
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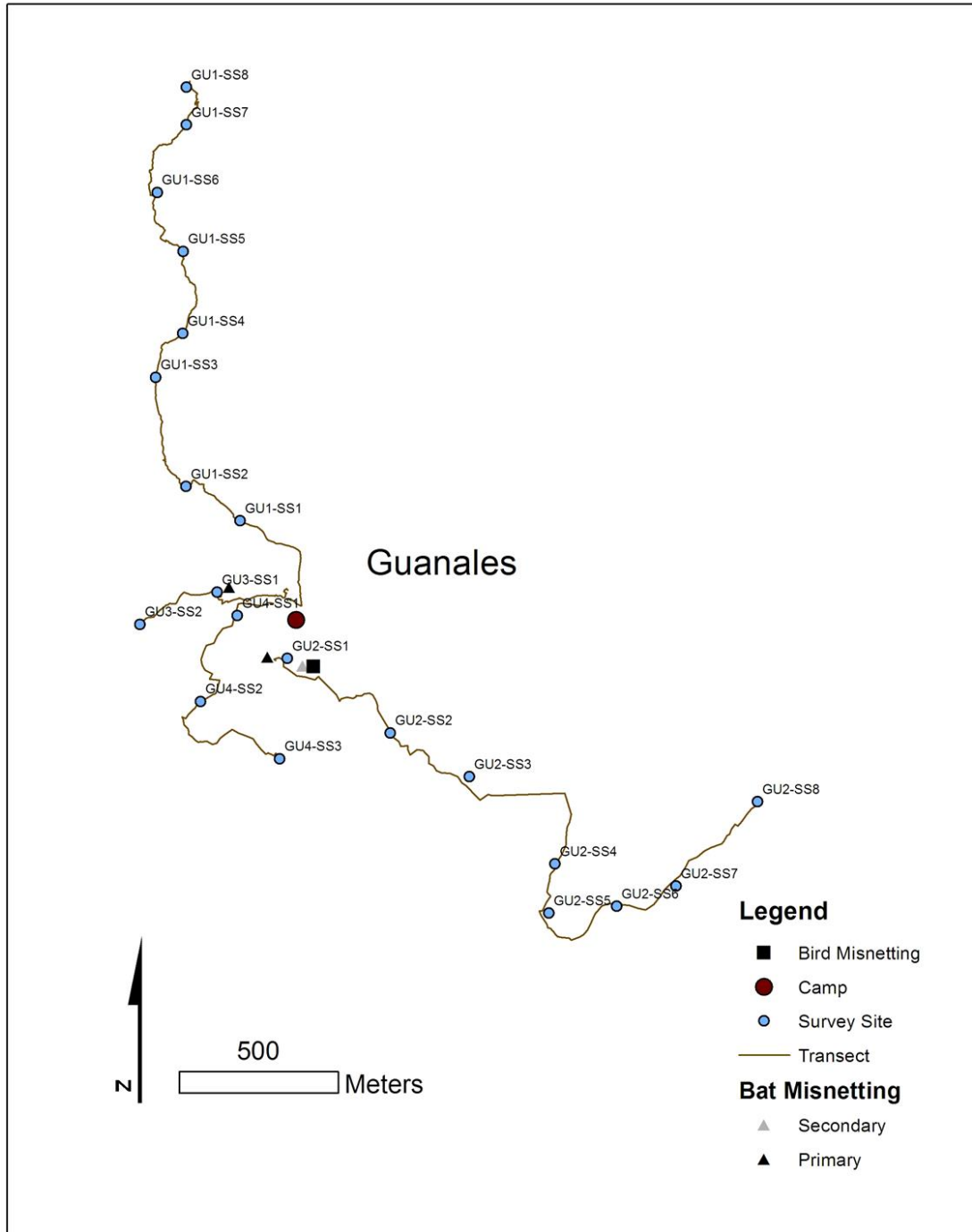
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Appendix 1. Maps of camp transect networks and survey site locations











Operation
Wallacea
Conservation research through academic partnerships

