

Operation Wallacea Cusuco National Park, Honduras 2016 & 2017: End of Season Report

Winter 2017

This end of season report is submitted as a review of the summer 2016-2017 seasons and the research activities of the Operation Wallacea research teams in Cusuco National Park over the course of the two summers. 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. A more detailed analysis of the herpetofauana dataset, coving the period 2007-2017, is provided, as this information was absent from more recent reports.

Any questions relating to this document or Operation Wallacea's research activities in Cusuco National Park should be directed to the Senior Scientist Dr Danielle Gilroy.

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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 2016 and 2017 field seasons and provide a complete report of the data collected and analysed from these seasons. We present ways forward for our research in the coming summer of 2018.

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 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' (Buenos Aires and Santo Tomas) and six within the core area of CNP (Base Camp, Guanales, Cantiles, El Danto, El Cortecito and



Capuca). 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. 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. In both the 2016 and 2017 seasons, Capuca was not open and so is not included on Figure 1.



Figure 1 Map of Cusuco National Park Buffer Zone (outer green area) and Core Zone (inner whitw area), showing Operation Wallacea camps (red circles) and transect network (lines)

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 Microsoft 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. 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, although trained herpetologists will capture non-venomous species (after careful visual identification) in order to collect additional morphometric data Venomous snakes are only processed



by the dedicated venomous snake handling team (specialist staff trained in the safe handling practices of venomous snake species), however, coral snakes of the genus *Micrurus*, are never handled by the team for any reason other than to be safely removed from camp or off of trails in close proximity to people. Remaining amphibians and reptiles will be captured, whenever possible, to collect data on sex, weight, snout-vent length (SVL) and to photograph the specimen for later confirmation of the identification. Photographs will be 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 will be added to the day transects by opportunistic surveys both during the day as well as during the night. Additional time will be 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 will be recorded for each specimen as in the daytime survey. Total search time for each survey session will be 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 will be installed near each camp, wherever possible, and checked daily each morning over the 8-week survey period. This method produces records for fossorial species not recorded from other surveys. In some cases, live traps will be used instead, which replace the killing fluid with 1/3 of a cup of soil. A funnel is placed at the top of the trap. These may be used instead of standard traps when the minimum sampling has been reached, to reduce the impact of sampling, or in other small studies located in and around camps.

4.1.4 Population density surveys

For a select group of species (*Plectrohyla exquisita, Plectrohyla dasypus, and Duellmanohyla soralia*) relative abundance is estimated based on capture-recapture data. A selected river/stream track (of about 200m) in each camp will be surveyed three-four times at night during the season to estimate population densities. All animals encountered will be caught and photographed (back, side and close-up of head) so that individuals may be recognised from their unique patterns and markings. From the photo data collected during these surveys, we hope that a population estimate for that area may be calculated in the future. 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 will be 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 by coupling the population/demographic fluctuations with 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 understory species, including those that are inconspicuous or seldom vocal and thus often



missed in point counts. The use of mist nets also 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 several 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). This is why we have identified a list of bird species that are particularly good indicators of health for the forest ecosystem, whether it is from their behavior, diet, social activity or IUCN status (Table 1).

Common name	Scientific name
Common Bush-Tanager	Chlorospingus ophthalmicus
Slate-colored Solitaire	Myadestes unicolor
Grey-breasted Wood-Wren	Henicorhina leucophrys
Black-headed Nightingale Thrush	Catharus mexicanus
Slate-throated Redstart	Myioborus miniatus
Yellowish Flycatcher	Empidonax flavescens
Chestnut-capped BrushFinch	Arremon brunneinucha
Spectacled Foliage-gleaner	Anabacerthia variegaticeps
Spotted Woodcreeper	Xiphorhynchus erythropygius
Highland Guan	Penelopina nigra
Emerald Toucanet	Aulacorhynchus prasinus
Collared Trogon	Trogon collaris
Keel-billed Toucan	Ramphastos sulphuratos
Brown-capped Vireo	Vireo leucophrys
White-winged Dove	Zenaida asiatica
Resplendent Quetzal	Pharomachrus mocinno
Ochre-bellied Flycatcher	Mionectes oleagineus
Olivaceous Woodcreeper	Sittasomus griseicapillus
Flame-coloured Tanager	Piranga bidentata
White-breasted Wood-Wren	Henicorhina leucosticta
Green-throated Mountain-Gem	Lampornis viridipallens
White-faced Quail Dove	Geotrygon albifacies
Nightingale Wren	Microcerculus philomela
White-throated Thrush	Turdus assimilis
Blue-crowned Motmot	Momotus momota
Black Thrush	Turdus infuscatus
White-crowned Parrot	Pionus senilis
Golden-crowned Warbler	Basileuterus culicivorus
Azure-hooded Jay	Cyanolyca cucullata
Blue-crowned Chlorophonia	Chlorophonia occipitalis

Table 1 Proposed bird indicator species for CNP



Variation between observers can be substantial in this type of survey, dependent upon experience and ability. The initial week at Basecamp will be spent training members of the bird team, where protocols for bird banding/mist netting and ageing/sexing neotropical bird species in the hand will be discussed and practised. Subsequently, the team will be 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 teams will work simultaneously in two camps, using ten 12-meter mist nets per camp. Each station must receive 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 will be operated for 6 hours after opening time (dawn). This will make a total of 36 hours (360 net hours per week).

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 will be 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 commencement of 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 fulfill the objectives of the protocol and monitor the population trends of the avifauna with a variety of different team members, several indicator species have been identified that are potential cloud forest indicators specifically for CNP. These species have been selected based on their representation across avian guilds, depth of robust historic data and their ability to be readily and distinctively detected in the field visibly and audibly.

4.2.2 Bird banding

Bird banding will be performed at permanent banding stations in each camp. Nets will be checked at least once every 40 minutes, dependent on climatic conditions. Captured birds will be extracted and placed in individual cotton bags while waiting to be processed. Birds will be banded with uniquely-numbered aluminium rings (size according to species). Important morphometric, condition and breeding status data will be 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 age0. 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 will be released close to the net site but far enough away to avoid their immediate re-capture. Abundance and community composition will be 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 will be noted on the provided bird banding data sheets. Furthermore, separate data will be 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 will be checked after each session for minor mistakes and entered as promptly as possible in the Base Camp system.

4.2.3 Avian physiology

Aspects of the physiological drivers of 'species replacements'- where related species replace each other at different elevation- are under examination, particularly focussing on Nightingale-thrushes (Catharus sp.).

- Avian metabolic rates. To measure avian metabolic rates, open-flow respirometry will be i) employed by using metabolic chambers. The chamber itself is simply a sealed container whereby an organism is 'roosted' with the input and output air measured to assess oxygen consumption (converted to energy consumption in kilojoules). Two measures of avian metabolism are planned. Firstly, basal (resting) metabolic rates will be measured in naturally resting birds- this method requires the retention of birds overnight as they must be operating in a complete resting state (roosting). Secondly, thermo-neutral zones (the upper and lower temperature limits at which basal metabolism increases) will be measured by experimentally manipulating the temperature within the chamber within a range (*ca.* 10-30°C). Birds will be captured by targeted mist-netting in the late afternoon, then roosted overnight in the chambers, before being released at the catching location the following morning. Birds showing signs of breeding condition or nest-tending (brood patches or bill swipes) will not be measured, and released on capture. Birds in chambers are routinely checked throughout the procedure to ensure the pumps are working correctly. Metabolic rates will be measured on 2-4 species. depending on time. Samples for this method are typically low (c10 per species) owing to low intraspecific variation in metabolic rates. This method is invasive, but is not harmful to birds and is used extensively by physiological ecologists. On a smaller portion of birds, a pilot study will be undertaken on the physiological costs of singing. These experiments will be undertaken in a very short period <30 mins and involve playing conspecific playback to birds in chambers so that the energetic costs of song bouts can be measured. This pilot study will be undertaken on birds before they are 'roosted' for the evening.
- ii) *Blood physiology.* Haematocrit (% of red-blood cells per unit blood volume) and Corticosterone (a hormone widely measured for physiological stress) will be measured in at least 4 species in relation to their elevational range limits. Birds will again be captured by targeted mist-netting at a variety of elevations and blood samples taken from the alar vein in



the wing. Three to four measures of 40ul will be taken and then birds released at the site of capture. Blood will be stored in ethanol in Eppendorf tubes and require exporting out of country, although some of the measures and analysis is planned on site.

All methods pertaining to avian physiology have also been granted ethical permissions by Royal Holloway University of London research welfare committee.

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 $75m^2$. Two permanent mist netting sites will be used per camp, each one as close to the main survey site as possible. Each mist netting location will be marked and the GPS location recorded. Mist netting will be 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 will be 36 hours or 2,700m².

The nets will be checked every 15 to 20 minutes during the first 3 hours of sampling and every 30 minutes for the last three. All the bats will be extracted from the nets following standardized protocols to minimize the stress and will be kept in a capture bags for 30mins, maximum. This time will vary depending on the size of the bat and the sex; pregnant females will be measured and released. Bats will be weighed, sexed, and the length of the forearm, feet and leg will be measured. We will also be taking fungal samples from individuals with fungal infections with plans to export these tissue samples for genetic analyses. Any ectoparasites will also be sampled but analysed within Cusuco National Park along with any available faecal samples during the mist-netting survey that are observed.

An additional study was included for the 2017 season only, which focused on *Sturnira horundrensis*. This is a common and widespread American species of fruit-eating bat from the Phyllostomidae family, highly abundant within Cusuco National Park and an ideal model to implement novel genetic analysis on the bats of Cusuco. We collected 25 tissue samples from 25 caught individuals (1 sample per 1 individual caught) via a wing-puncture protocol established by the American Museum of Natural History which has no detrimental effect upon the bat. It involved a 3mm sterilised biopsy punch on the wing membrane, away from any large blood vessels, and extracted a small piece of tissue stored in alcohol preserving solution.

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 species-specific signs. Surveys focus on Baird's Tapir, but evidence of the presence of any large mammals will be recorded. Digital images and GPS locations of tracks, spoor, and scat are recorded. Survey teams will walk each transect as soon as each camp opens to



ensure they are the first team to encounter fresh tracks. Multi-season, multi-species analysis of large mammal detections will aid our understanding of the impacts of hunting and human encroachment, 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

Camera traps (Bushnell Trophy CAM HD) will be placed along the transects associated with each of the camps. Cameras will be put out at the start of the season and left set up *in situ* for two weeks before collection. Cameras are placed in triplets; one <20m, one $\sim150m$ and one $\sim300m$ perpendicular to the transect to examine variation in detectability as a function of human disturbance. 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 will be 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 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 ca 25g of fresh horse or mule dung, wrapped in muslin or similar fabric and tied to form a ball. Excess string from tving 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 the contents of the trap are collected. Some scarabs are <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. 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, Saturnidae, Noctuidae and Notodontidae)

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, longhorns and click beetles. Any relevant environmental conditions should be recorded in the logbook.

5. Additional biodiversity surveys in Cusuco National Park

5.1 Small mammals

Sherman small mammal traps will be used to survey the small mammal communities in CNP. Relative abundance of species sat each camp is recorded. 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.

5.2 Dragonflies (Odonata)

Dragonflies and (day) butterflies are collected whenever encountered on the transects and along the rivers with a hand net. GPS coordinates for every animal are collected. 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 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.



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 many 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-liter 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.

7.2 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.

In 2014 we performed the first survey to determine whether Ranavirus is affecting the amphibians in CNP and was found to be present in the park. Amphibian ranaviruses (genus *lridovirus*) 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. Although a low number of samples were found positive, we aim to collect additional samples to substantiate the presence of ranavirus in CNP.



All species of amphibians will be swabbed whenever encountered 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 will be 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. Metamorphs will not be swabbed. Swab buds will be broken off and stored in 2 mL microcentrifuge tubes containing 1 mL of 70% ethanol as a preservative. Samples will later be analyzed by molecular analysis (PCR) to detect the presence of *B. dendrobatidis* DNA and to determine the infection status of each amphibian sampled. Swabs will be collected across a range of different species and habitats.

For the detection of ranavirus, amphibians will be 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 will be broken off in 2ml cryovial tubes and stored for subsequent PCR analysis. A fresh pair of Nitrile gloves will be worn each time an amphibian is sampled for either *B. dendrobatidis* or ranavirus, to prevent any risk of cross infection. Any amphibian found dead will be preserved for subsequent histological examination to investigate the cause of death.

7.3 Trophic ecology and population genetics of snakes of Cusuco National Park

Snakes will be 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 will be captured and secured using appropriate techniques (snake hooks/tongs and clear plastic handling tubes will always be used for venomous species). Snakes will be measured (SVL and tail), weighed, sexed and photographed. Up to three ventral scale clips will be taken using a pair of sharp scissors and stored in ethanol in a 1.5ml plastic Eppendorf tube. Scales will be retained as tissue samples for genetic and stable isotope analysis. Tissues samples for genetic analysis will be stored at Cornwall College Newquay, UK 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 will be AT Cornwall College Newquay for planned stable isotope research (once sufficient tissue samples have been obtained) to 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.

7.4 Spatial ecology of the Honduran Emerald Palm Viper Bothriechis marchi and Wilson Pit Viper (Cerrophidion wilsoni)

This year we aim to launch a pilot study into the use of radio-telemetry methods to study the spatial ecology of *Bothriechis marchi* and *Cerrophidion wilsoni*. Radio transmitters will be attached externally to the skin of adult snakes using methods in line with Nash and Griffiths (2016). This method will be tested for suitability in an arboreal (*B. marchi*) and terrestrial (*C. wilsoni*) snake. Based on the findings of this pilot study a funding application will be submitted to expand this work in future years to get a much better picture of how these snakes are using their spatial environment.



7.5 Freshwater ecology monitoring in Cusuco National Park

Arguably the most important aspect of cloud forests is their unusual hydrological features and role in protecting water resources and quality in headwater streams. Net precipitation (rain through fall) in cloud forests is significantly subsidised by fog interception. This in combination with lower solar radiation and a generally wet canopy (both of which have a role in reducing evapotranspiration) increases the water budget of the catchment and together with the moderating effect of natural forest on waterways results in a remarkably reliable and clean water resource. Cusuco is no exception and is the major water source for several urban areas including San Pedro Sula. The protection of the water resource was the greatest driving factor in the designation of Cusuco as a national park. However, the freshwater habitats of the park are under threat from deforestation and pollution inputs from agriculture. Little monitoring occurs as no biomonitoring tools exist due to lack of information on the biological communities and their responses to pollutants.

This study builds on previous sampling regimes carried out in 2009 and 2010 and experimental work conducted in 2011 and 2016 by experimentally examining the response of freshwater macroinvertebrates, key indicators of water quality, to commonly occurring local pollutants to refine potential biomonitoring tools and to protect water quality and associated biodiversity. We employed a streamside mesocosm approach to 1) calculate response thresholds for the various pollutants including sediment and nutrients, 2) identify effects of other pressures such as local fertilisers and acidification 3) test effects of combinations of pressures which are likely to co-occur such as an increase in temperature and sedimentation with forest

clearance. We conducted instream sampling using a standard kick sampling methodology in order to improve information on the structure and composition of the aquatic macroinvertebrate community as well as employing light trapping to collect adult specimens. Further we attempted to raise nymphal specimens of select groups to adulthood to improve taxonomic information. All specimens will be preserved in ethanol and returned to University College Dublin for identification and analysis.

8. 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)



9. Reported results for 2016 and 2017

9.1 Amphibians and Chytrid by Dr Danielle Gilroy and Chris Phipps

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/zy6msea]):
 - 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-HCI, 0.1mM EDTA, pH 8.0).

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.



- 9. Incubate for 5 minutes at room temperature.
- 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-GoTM PCR tube containing the freezedried reagents (in bead form) necessary for PCR².

Step 4 – Master mix (25 x1 μ l reactions with primer dilutions of 10 μ mol per μ l):

- 1. x1 μ l of forward primer (ITS-1: 5'-CCT TGA TAT AAT ACA GTG TGC CAT ATG TC-3')
- 2. x1 μ l of reverse primer (5.8S: 5'-AGC CAA GAG ATC CGT TGT CAA A-3')
- 3. x23 μl H₂0

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.

Step 6 – Gel preparation:

² Magnesium chloride (MgCl₂) is contained within the dehydrated PCR bead. More MgCl₂ can be added according to the reaction volume; details here: <u>http://tinyurl.com/j5z8mgy</u>



- 2. 50ml TE
- 3. 7.5ml gel-red
- Step 7 Gel electrophoresis:
 - 1. 5μ l buffer added to each sample
 - 2. 15μ I PCR product per well (leaving 15μ I for a second run if necessary)
 - 3. 10µl ladder
 - 4. Gel run at ca. 160v/75 ma for 20-25 minutes

Results

A total of 493 samples of the four focal species (Deullmanohyla soralia - 169, Plectrohyla dasypus - 158, P. exquisita - 100, and Ptychohyla hypomykter – 66) were processed over the 2016 and 2017 field seasons (Table 2).

Table .	2 Infection	prevalence	of four	focal	species
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Species	(n) Processed	Samples	(n) Samples positive	% Prevalence
Deullmanohyla soralia	169		34	20
Plectrohyla dasypus	158		35	22
Plectrohyla exquisita	100		14	14
Ptychohyla hypomykter	66		9	13

One of the biggest issues faced by the DNA lab in the 2017 field season was a persistent low level of contamination. Most likely caused by contaminated pipettes. This was resolved by soaking the contaminated instruments in a bleach solution, and testing them on negative control samples until we could be sure the contamination had been cleared. In future field seasons preventative measures should be taken to avoid these types for contamination, for example using filter pipette tips and aliquotting reagents wherever possible.

9.2 Herpetofauna Research Report 2017 by Dr Steve Green and Tom Brown

1.1 Introduction

The last formal update on the Operation Wallacea (Opwall) herpetofauna monitoring programme in Cusuco National Park (CNP) was in 2012 (Green et al., 2012). At the time, the total number of herpetofauna species recorded in CNP stood at 82 and it looked as though species accumulation curves had likely plateaued due



to the already extensive monitoring completed by the Opwall team. This already extensive herpetofauna dataset contributed to the ranking of CNP as the 25th most irreplaceable protected area for threatened amphibian conservation (Le Saout et al., 2013). However, recent efforts of the Opwall team have continued to shine a light on the incredible diversity of CNP, as well as documenting the significant threats facing this small, but highly valuable national park. Several peer-reviewed journal articles have been published by the herpetofauna team since the previous report (e.g. Kolby, Ramirez, Berger, Griffin, et al., 2015; Kolby, Ramirez, Berger, Richards-Hrdlicka, et al., 2015; D'Souza et al., 2016; Blooi et al., 2017; Solis, Taylor and Lopez-Paredes, 2017), as well as numerous natural history notes documenting previously unknown aspects of diet and behaviour (e.g. Solis and Brown, 2016; Clause and Brown, 2017). In addition, a comprehensive field guide to the herpetofauna of CNP has been produced and field tested over the past two field seasons, with the intention of publication and distribution in 2018 (Brown and Arrivillaga, in prep). Here we briefly summarise the most important results from the team's monitoring programme and look for trends in the overall status of herpetofauna diversity within the park.

1.2.1 Monitoring effort

A considerable amount of effort has been made to monitor the standardised transect network across all research camps within the park. Figure 2 shows the total number of transects completed per year and Table 3 provides a detailed breakdown of the number of transect surveys completed within each research camp per year since 2007. A marked increase in the number of transects completed in the years from 2013 – 2017 is due to an important change in the way nocturnal river/stream surveys were conducted and recorded.

Missing data in Table 3 are due to some research camps not being surveyed in all years. Notably, a new research camp named Capuca was established on the east of the park in the 2015 field season, but was discontinued the following year due to logistical difficulties and the lack of a nearby permanent stream water source. Official monitoring of Santo Tomas on the northwest of the park was discontinued after 2013 due to the significant deforestation that has occurred in this area of the park. Although there is significant value in continuing to monitor areas of the park undergoing rapid habitat loss, sadly, it has not been possible to justify sending paying volunteers to monitor such degraded sites. However, such circumstances present a prime research opportunity in evaluating the effects of anthropogenic disturbance on the herpetofaunal diversity. Undoubtedly, such a project would prove to be of great importance in understanding the impact deforestation has on biodiversity and abundance within the core zone of the park.



Figure 2 Total number of herpetofauna transect surveys completed in Cusuco National Park (2007 – 2017). Note that river surveys were only recorded as opportunistic surveys until standardised river transects were established in 2013, thus, explaining the noticeable increases in transect survey effort from 2013 to present

Table 3. The total number of transects surveyed within each research camp per year. Note that river surveys were
only recorded as opportunistic surveys until standardised river transects were established in 2013, thus, explaining
the noticeable increases in transect survey effort from 2013 to present. Missing values are due to research camps
not being surveyed in that year.

CAMP	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	TOTAL
BASE CAMP	7	3	9	18	24	20	54	55	69	55	48	362
BUENOS AIRES	5	12	7	9		9	37	7	34	16	18	154
CANTILES	16	10	12		27	14	25	27	44	29	31	235
CAPUCA									40			40
EL CORTECITO	7	4	4	5	20	11	24	21	22	23	15	156
EL DANTO	24	12	3	5	15	4	19	29	20	19	20	170
GUANALES	17	4	11	4	38	28	47	33	49	41	42	314
SANTO TOMAS	19	15	14	3	19	7	23	1				101
GRAND TOTAL	95	60	60	44	143	93	229	173	278	183	174	1532

The variable monitoring effort across camps is a product of the logistical constraints of running a very large and complex volunteer field programme. Ideally, survey effort should be approximately evenly dis-tributed across all sites, yet unfortunately this is not always possible in such a challenging location. When analysing the data and interpreting results, this unequal survey effort should be considered and taken into account.



Furthermore, this proves slightly more problematic when survey effort is broken down further to the number of times each transect route has been completed, with it being apparent that the survey teams have not managed to complete the advised four repeats of each transect per research camp in all years. The reasons for this are complex, and often outside the control of the research teams (i.e. poor weather conditions), however, some lessons can be learned, and future work schedules are to be adjusted to ensure minimum transect repeats are achieved in all camps each year. Factors contributing to the failure to complete the required number of transect repeats in El Cortecito and El Danto camps include the relatively short camp opening period (three weeks), alongside conflict with other research teams to access transects before they become disturbed by other people walking the transect route. Ad-ditionally, these camps have suffered substantial increases in deforestation, habitat loss and general dis-turbance throughout the transect network; which is tragic considering their importance for many threat-ened species populations.

1.2.2 Introduction of river transects

Green et al. (2012) identified the need to improve quantification of survey effort of night river and stream surveys for amphibians, which had previously been recorded as opportunistic species encounters. As the vast majority of amphibian encounters occurred during stream and river surveys, and because this search effort was going unreported, the decision was made to establish river transects, consisting of approximately 200 m of stream/river closest to each research camp. These new river-transects were introduced to the survey protocol in 2013 and have been an important modification to the monitoring design, resulting in dramatic increases of streamside amphibian encounters. River transects are conducted in the same way as terrestrial transects, with start and end time and distance travelled being recorded. However, the inclusion of these river transects within the standard transect database must be considered when analysing the overall transect data, as outlined in section 1.6.

1.3 Species Counts and Accumulation Curves

Species counts and accumulation curves were created from all species records (opportunistic and transect data) to quantify overall species richness. Only records where full species identification had been confirmed were included (i.e. genus name sp. removed from species record data). Several specimens are awaiting genetic analysis to confirm taxonomic status.

1.3.1 Amphibians

The number of positively identified amphibian species in CNP has increased from 26 to 28 since the last herpetofauna report (Figure 3), with Bolitoglossa mexicana and Ecnomiohyla salvaje being added to the list.



Figure 3 Amphibian species accumulation curve for Cusuco National Park, Honduras

1.3.2 Reptiles

The number of positively identified reptile species in CNP has increased from 62 to 72 since the last herpetofauna report (Figure 4), with Lampropeltis triangulum hondurensis, Coniophanes imperialis, Holcosus undulates, Norops yorensis, Geophis fulvoguttatus, Scolecophis atrocinctus, Hemidactylus frenatus, Tantillita lintoni, Amastridium sapperi and Hydromorphus concolor being added to the list.



Figure 4 Reptile species accumulation curve for Cusuco National Park, Honduras

The rate of new species encounters for reptiles suggests that despite over a decade of surveying CNP, new species are likely to continue to be discovered. Whether this is a reflection of the highly cryptic nature of some reptile species or the impacts of habitat and climate change (or both) is yet to be investigated.



Consequently, many critical questions concerning the conservation of the parks unique herpetofauna remain unanswered.

1.4 Species Diversity

A total of 100 species of herpetofauna have been detected in CNP, consisting of 9 salamanders, 19 anurans, 45 snakes and 27 lizards.

1.4.1 Salamander diversity

Nine species of salamander are known to occur in CNP, five of which are classified as critically endangered, one endangered and one near threatened by the IUCN Red List of Threatened Species (IUCN, 2017). Two of these species (C. nasalis & N. brodiei) are co-endemic between CNP and another site (Sierra de Caral) in closely neighboring Guatemala, whilst another two (B. diaphora & O. tomasi) are specifically endemic to CNP in Honduras (Figure 5).

1.4.2 Anuran diversity

Nineteen species of anuran are known from CNP, five of which are classified as critically endangered, four endangered and four near threatened by the IUCN Red List of Threatened Species (IUCN, 2017). Five species are endemic to Honduras and, of these, three are endemic to CNP (Figure 6).



*Figure 5 Total number of recorded encounters of salamander species in CNP across all years (2007–2017) (*endemic to Honduras, + endemic to Cusuco National Park).*



*Figure 6 Total number of recorded encounters of anuran species in CNP across all years (2007-2017) (*endemic to Honduras, + endemic to Cusuco National Park).*

1.4.3 Snake diversity

An incredible 45 snake species have been detected in CNP, with the ground-dwelling Wilson's pit viper (Cerrophidion wilsoni) being by far the most commonly recorded species. However, more than half of these species have been detected fewer than ten times across the entire study period (2007-2017), with eight species having been detected on just one single occasion. Five species are endemic to Honduras, with three of those being endemic to CNP (Figure 7).

1.4.4 Lizard diversity

Twenty seven species of lizard are now known from CNP. Three species are endemic to Honduras, one of those being endemic to CNP (Figure 8).





Figure 7 All snake species records within Cusuco National Park (2007–2017). Actual data vlaues for number of times each species has been recorded are displayed above each bar (*endemic to Honduras, + endemic to Cusuco National Park).





Figure 8 All lizard species records within Cusuco National Park (2007-2017). Actual data vlaues for number of times each species has been recorded are displayed above each bar (*endemic to Honduras, + endemic to Cusuco National Park).



1.5 Distribution of herpetofauna diversity across CNP

To evaluate the eveness of herpetofauana diversity across all reasearch camps in CNP, species richness, Shannon-Weiner diversity index, and Simpson's index of diversity (1-D) were calculated for each camp using all species records for the entire study period.

1.5.1 Species Richness

Species richness was variable between camps (Figure 9), with highest scores being observed in the two lowest altitude camps, Buenos Aires and Santo Tomas. Lowest species richness was observed in Capuca camp, however, this result should be treated with caution as this research camp was only surveyed for a single field season (2015) within the total survey period.



Figure 9 Species richness calculated for each research camp within Cusuco National Park using all species records collected 2007-2017. Note that Capuca camp was only surveyd for a single field season in 2015 which likely contributes to the lower species richness score for this camp.

1.5.2 Biodiversity indices

Because the relative abundance of all species detected within camps is variable, it is also important to take this into account when comparing herpetofaunal diversity between camps. Shannon-Weiner diversity index and Simpson's index of diversity (1-D) were calculated for each camp (Figure 10). Simpson's index of diversity showed there to be a fairly even score across all research camps, wherease Shannon-Weiner index displayed the sample pattern as species richness scores, with highest diversity found in Buenos Aires and Santo Tomas. Interestingly, despite only having been surveyed for a single field season, Capuca camp had comparable diversity scores to the other research camps, suggesting the low species richness score for this camp is simply due survey time being insufficient to have detected the rare/difficult to detect species at this camp.

The results are inkeeping with a pattern of higher species richness in lower altitude and edge habitats, however, the majority of endemic and threatened species are found within the higher altitude camps with the core zone of the park and this must also be taken into account when taking management decisions.





Figure 10 Shannon-Weiner diversity index and Simpson's index of diversity (1–D) calculated for each research camp within Cusuco National Park using all species records collected 2007–2017.

1.6 Change in Diversity over time

Species richness and Shannon-Weiner diversity index scores of each research camp were calculated independently for each survey year. Mean species richness and mean Shannon-Weiner diversity index scores were then calculated per year using the scores of all camps surveyed in that year. Annual mean values (+/-SE) were then plotted and linear regression performed to test for any trend in species richness (Figure 11) and Shannon-Weiner diversity index (Figure 12). The results of the linear regression were not statistically significant for either species richness (DF = 1, F= 3.75 P = 0.085) or Shannon-Weiner diversity index (DF = 1, F = 0.001, P = 0.97), suggesting there has been no change in herpetofauna diversity during the study period. However, it is acknowledged that this analysis would not necessarily detect changes in species communities over time and this should be investigated in greater detail.





Figure 11 Mean species richness (mean of species richness scores of all research camps) per year (+/-SE). Linear regression was not statistically significant DF = 1, F = 3.75 P = 0.085. There has been no overall trend in herpetofauna species richness in Cusuco National Park across the study period (2007-2017).



Figure 12 Mean Shannon-Weiner diversity index score (mean of all research camps) per year (+/-SE). Linear regression was not statistically significant DF = 1, F = 0.001, P = 0.97. There has been no overall trend in herpetofauna diversity in Cusuco National Park across the study period (2007-2017).

1.7 Change in Relative Abundance

In assessing detectable change in relative abundance over the study period, only data from the transect database were analysed, as survey effort cannot be accounted for in the opportunistic data. In this analysis, the number of transects completed per camp/per year was used as a basic measure of survey effort. In reality, this is a somewhat unsatisfactory and crude measure of survey effort, as it does not take into account



variable length of different transects, the fact that the entire transect length is not always surveyed on every occasion, and variable amount of time spent walking the same transects. Prior to the 2012 field season only the start time and not the end time of transects surveys was recorded. This was identified as a significant problem for quantifying survey effort. In 2015 it was decided that the total distance completed (if terminating the transect early and not reaching the finish point) should also be recorded. Thus, improvements have been made to the transect survey protocol, however, these measures of survey effort are not available for all survey years. Here we report relative abundance as simply the number of detections per research camp divided by the number of transect occasions. However, a more robust analysis of survey effort should be performed to gain a more detailed picture of changes in relative abundance over time.

It is also extremely important that the introduction of river transects into the 'transect data' database are acknowledged as a potential source of data analysis error if combined with standard terrestrial transect data. If a linear regression is performed on relative abundance (number of herpetofauna detections divided by the total number of transect occasions in a given year) and survey year, when all herpetofauna and all transect data (terrestrial and river transects) are included, a significant regression is apparent (ANOVA, df = 1, F = 9.44, P = 0.015) (Figure 13). However, this is an artefact of amphibian encounter rates being much higher on river transects than on terrestrial transects, thus, resulting in a perceived greater relative abundance after 2013 when the river transect data. For this reason, here we analyse terrestrial transect data for the entire study period (2007-2017) and river transect data (2013-2017) separately.



Figure 13 Total number of encounters (all herpetofauna) per year divided by the total number of transect surveys completed in that year (terrestrial and rive transects combined). A significant regression between relative encounter rate and survey year is apparent (ANOVA, DF = 1, F = 9.44, P = 0.015), however, this is only because of the relatively higher encounter rate of amphibians on river surveys between 2013-2017 and cannot, therefore, be interpreted as a true increase in relative herpetofauna abundance over this period.

1.7.1 Change in Relative Abundance

The total number of all herpetofauana encounter records from terrestrial transect surveys was caluclated for each year and then divided by the number of transect survey occasions completed within that year to give a relative measure of detection (considered here as a measure of relative abundance). A regression on relative detection and survey year was not statistically significant (DF = 1, F = 1.65, P = 0.234),



suggesting there has not been an overall significant change in detection rates of herpetofauna on terrestrial transects within the study period (Figure 14).



Figure 14 Relative abundance (number of detections divided by total transect occasions) of herpetofauna detected on terrestrial transects in Cusuco National Park. Linear regression was not significant (DF = 1, F = 1.65, P = 0.234) suggesting there has not been any significant trend in relative abundance of herpeofauna detections on terrestrial transects between 2007 and 2017.

1.7.2 Relative Abundance on River Transects

As for terrestrial transects, the total number of all herpetofauana encounter records from river transect surveys was caluclated for each year and then divided by the number of transect survey occasions completed within that year to give a relative measure of detection (considered here as a measure of relative abundance). A regression on relative detection and survey year was not statistically significant (DF=1, F=0.006, P=0.943), suggesting there has not been an overall significant change in detection rates of herpetofauna on river transects between 2013 and 2017 (Figure 15).





Figure 15 Relative abundance (number of detections divided by total transect occasions) of herpetofauna detected on river transects in Cusuco National Park. Linear regression was not significant (DF=1, F=0.006, P=0.943) suggesting there has not been any significant trend in relative abundance of herpeofauna detections on river transects between 2013-2017.

In summary, there does not appear to have been any detectable change in relative abundance of herpetofauna on either the terrestrial transect surveys (2007-2017) or river transects (2013-2017), suggesting that, overall, herpetofauna abundance remains relatively stable within CNP. However, this analysis does not incorporate the individual population trends of the species which compose these amphibian and reptile communties, with some species potentially declining while others increase. Determining the population trends of particular herpetofauna (specifically endemics or those listed as critically endangered) should be a focus of future analysis.

1.7.3 Differences in relative abundance between research camps

Although no significant trend in relative abundance over time was detected, relative abundance scores were not consistent across research camps, with Guanales and Santo Tomas appearing to have the greatest overall abundance of terrestrial transect detections (Figure 16), but Cortecito having by far the greatest relative abundance of herpetofauna detections for river transects (Figure 17). Cortecito is known for having high encounter rates of amphibians and snakes along the designated river transect, however, the relative abundance score may have also been inflated slightly by additional, intensive herpetofauna survey work taking place at this camp where river transects have been walked very slowly by teams of experienced herpetologists. It would be interesting, therefore, to look at this again but with a slightly more robust measure of survey effort to be completely confident in this apparent large difference.



Figure 16 Total number of herpetofauna detections per research camp, divided by the total number of terrestrial transect survey occasions completed at that camp (2007–2017).



Figure 17 Total number of herpetofauna detections per research camp, divided by the total number of river transect survey occasions completed at that camp (2013-2017). The extremely high encounter rate along the Cortecito river (CORiver) is likely to be, at least in part, attributable to highly experienced herpetologists intensively surveying this camp for longer periods during the study period. That being said, these additional research efforts were specifically focused on this stretch of river in light of its exceptionally high amphibian abundance, notably being a hotspot for critically endangered endemics such as P. dasypus & P. exquisita.

1.8 Evaluation of evidence for species specific trends

It is important to consider that the patterns described thus far have been general patterns for herpetofauna within CNP and do not give detail on species specific distribution patterns or trends. Whilst it is beyond the scope of this report to consider each species in turn, here we provide data on the distribution patterns and temporal trends of four key species of tree frog: Plectrohyla exquisita, Plectrohyla dasypus, Duellmanohyla soralia and Ptychohyla hypomykter. These four species have been selected for the purpose of co-monitoring the prevalence of amphibian chytrid fungus, Batrachochytrium dendrobatidis (Bd), and population trends over time. Recent field results of Bd prevalence are presented elsewhere within this report



and a thorough analysis of Bd prevalence and amphibian population trends is being completed by the Operation Wallacea herpetofauna team. However, here we present relative abundance patterns for these four species on both a spatial (Figure 18) and temporal (Figure 19) scale within CNP Data presented here are for encounters on river transect surveys between 2013-2017.

It is clear that El Cortecito and El Danto camps are very important for the conservation of the two critically endangered and endemic spike-thumb frogs, P. exquisita and P. dasypus (Figure 18). Unfortunately, these camps are currently under severe pressure from illegal deforestation, with annual visits finding substantial areas of previously pristine cloud forest to be lost or significantly degraded comparatively. Evidently, the progressive encroachment of deforestation into the core zone must be halted, as habitat critical for a wealth of herpetofaunal diversity is being lost each year. Encouragingly, no clear statistically significant trend was found in relative abundance of any of the four species analysed here for the survey period (2013-2017) (Figure 19). Results of a linear regression on relative abundance of each species within CNP as a whole (total number of encounters divided by river transect occasions for all camps combined per year) were not significant (P. exquisita df = 1, F = 0.349, p = 0.614, P. dasypus df = 1, F = 0.003., p = 0.958, D. soralia df = 1, F = 0.874, p = 0.448, P. hypomykter df = 1, F = 1.641, p = 0.327). However, these results are preliminary and a more robust analysis that accurately quantifies and corrects for survey effort between years is necessary, before any firm conclusions can be drawn on the current population trends and conservation status of these species.

1.9 Summary of herpetofauna research team key messages

A vast amount of data has been amassed by the Operation Wallacea herpetofauna research team over the past eleven years, contributing to Cusuco National Park being ranked as one of the most valuable protected areas globally for threatened amphibian conservation (Le Saout et al., 2013). Field methods and data collection processes have been refined by the team during this time to improve the quality of the data, although further efforts are needed to ensure the minimum number of transect replicates are completed each year and that survey effort is always recorded in a consistent manner. Failures to accurately record survey effort during the early years of data collection reduce our ability to analyse populations trends across the entire study period, but more recent improvements to data collection and recording will allow for a more detailed analysis to be completed (although not possible within the scope of this report). Time is needed to carefully process the data to enable a more accurate measure of survey effort per transect within each research camp per year. Once this has been completed a more robust analysis can be performed to reassess the trends described here. Additionally, species specific distribution patterns and trends should be analysed, especially for all threatened and regionally endemic species.

Each year, the team returns to Cusuco National Park to find large new areas of illegal deforestation, even within the core zone of the national park. This deforestation severely threatens the biodiversity of the park and the ability of volunteer-based research programs to continue to operate. The extent of the problem has become so serious that several survey transects have been either partially or completely deforested and camps such as El Cortecito and El Danto, which were once located deep within the forest, now lie on the very edge of the deforestation frontline. It is essential that more is done to halt this disturbing trend, as ultimately, the long-term persistence of Cusuco's unique and globally significant biodiversity is increasingly jeopardised. Our current dataset suggests that there have been no significant declines in herpetofaunal diversity to date, and so we remain hopeful that it is not too late to recover from this situation. However, with Operation Wallacea's presence in the park being limited to 2 months of each year, the responsibility to protect this unique habitat and its species, in part, lies in the hands of its local people. We



strongly believe immediate conservation management approaches should be initiated which embrace the opportunity to apply creative solutions, educating and working with local communities to promote sustainable incomes, livelihoods and appreciation of this incredible natural resource. Whilst we recognise this is a complex and extensive challenge (and indeed one that shadows conservation efforts globally), it is one which certainly must be addressed in CNP sooner, rather than later.




Figure 18 Relative abundance of (a) Plectrohyla exquisita, (b) Plectrohyla dasypus, (c) Duellmanohyla soralia and (d) Ptychohyla hypomykter within river transects at each research camp in Cusuco National Park. Relative abundance calculated as the total n





Figure 19 Regression of relative abundance and survey year for (a) Plectrohyla exquisita, (b) Plectrohyla dasypus, (c) Duellmanohyla soralia and (d) Ptychohyla hypomykter. Relative abundance calculated as the total number of recorded encounters on river transect surveys (all research camps combined) divided by the total number of river transect occasions (2013-2017). All regressions were non-significant (P>0.05) suggesting there has been no obvious decline in abundance of these species in Cusuco National Park between 2013-2017, however, it should be noted that a more robust measure of survey effort may need to be applied for any such possible trend to be apparent. Note different scale on dependent axis between the four panels.



1. Point Counts

All standard transect-based survey sites were surveyed throughout both the 2016 and 2017 field seasons at all camps excepting the now largely disbanded Santo Thomas. In addition, the transects in Capuca camp (only opened for 2015 season) were also surveyed for additional work being undertaken on the elevational turnover of montane songbirds (see additional projects below). 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. Large ornithological teams, coupled with a constant presence across all camps throughout both the 2016/17 field seasons have lead to an exceptional volume of data collected during point count surveys. In total 12,187 (2016: 7,442 – 2017: 4,765) independent records were collected over the course of both field seasons (over 25% of all PC data collected in 12vrs of constant monitoring). The data volume was particularly large in the 2016 field season owing to a large volume of transect replication at some research camps for additional project work (see additional projects). In total, a minimum of 135 species were recorded on point count surveys over this two-year period (at least 100 in 2016 and 98 in 2017). Minimum sampling requirements of three replicates per survey point were completed on all transects each season (including reverse replicates to account for temporal sampling bias) and in most cases considerably exceeded. The quantity of data collected serves as a testament to the research teams working on the ground (often working together on surveys where applicable) over the past two field seasons. While such an intense survey effort has yielded substantial quantities of data, the intensity of sampling is possibly unnecessary and has the potential to cause confounding disturbance levels from foot traffic on certain transects. In future seasons it may be more profitable to invest time into other projects while still satisfying the core monitoring objectives, in order to achieve the most valuable data-spread for methods per camp. The inclusion of formal nocturnal playback surveys would be a particularly useful way to address a major knowledge-gap that exists in the lack of guantitative understanding of the status and distribution of nocturnal species – some of the poorest known avifauna of the park.

Naturally there are many un-identified detections in the data collected but as many of these as possible were identified post-hoc where team members had consistently coded unidentified records. A large bulk of these records also pertains to fly-through, unidentifiable hummingbird species. Table 4 below provides a simple breakdown of all species making up $\geq 1\%$ of all detections during all surveys over 2016 and 2017. 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 the unavoidable observer differences in collecting data on the whole avian community. Changes in volume of records in these two years should not be viewed necessarily as abundance changes as the summary provided is not controlled for effort at specific elevations, which contributes to the local abundance of certain species (e.g. higher Grey-breasted Wood-wren records are likely down to greater survey effort at higher elevations due to specific projects in 2016).



Table 4 Most frequently recorded species (in descending order) on point counts in 2016, 2017 and both years combined. One species, Oropendola Psarocolius wagleri, is left out because the volume of records relate to small incidences of very large flocks, rather

Vernacular	Binomial	2016	2017	Both years
		(% records)	(% records)	combined
Common Bush-Tanager	Chlorospingus	10.2 %	7.1%	9%
	flavopectus			
Slate-coloured Solitaire	Myadestes unicolor	8.4%	9.5%	8.9%
Grey-breasted Wood-	Henicorhina	7.2%	4%	5.9%
Wren	leucophrys			
Black-headed	Catharus mexicanus	6%	5.7%	5.9%
Nightingale-Thrush				
Chestnut-capped	Arremon	4%	4.2%	4.1%
Brush-Finch	brunneinucha			
Yellowish Flycatcher	Empidonax	4.3	3.2%	3.9%
	flavescens			
Slate-throated	Myioborus miniatus	3.8%	3.5%	3.7%
Whitestart	-			
Spectacled Foliage-	Anabacerthia	3.4%	3%	3.3%
gleaner	variegaticeps			
Spotted Woodcreeper	Xiphorhynchus	2.9%	2.7%	2.8%
	erythropygius			
Collared Trogon	Trogon collaris	2.1%	2.5%	2.2%
Highland Guan	Penelopina nigra	2.3%	1.7%	2.1%
Brown-capped Vireo	Vireo leucophrys	1.9%	2%	1.9%
White-faced Quail-Dove	Zenytrgon albifacies	2%	1.7%	1.9%
Emerald Toucanet	Aulacorhynchus	1.7%	1.9%	1.8%
	prasinus			
Flame-coloured	Piranga bidentata	1.8%	1.8%	1.8%
Tanager				
Olivaceous	Sittasomus	1.5%	1.7%	1.6%
Woodcreeper	griseicapillus			
Azure-hooded Jay	Cyanolyca cucullata	1.7%	1.4%	1.6%
Black Thrush	Turdus infuscatus	1.5%	1.6%	1.5%
Resplendent Quetzal	Pharomachrus	1.5%	1.3%	1.4%
	mocinno			
Keel-billed Toucan	Ramphastos	0.9%	2.1%	1.4%
	sulfuratus			
Lesson's Motmot	Momotus lessonii	1%	1.4%	1.1%
White-winged Dove	Zenaida asiatica	1.1%	1.1%	1.1%
Blue-crowned	Chlorophonia	1%	1.1%	1%
Chlorophonia	occipitalis			



The 2016/17 seasons marked the fifth and sixth seasons respectively undertaking structured and standardised mist-netting/banding protocols since its initiation in 2012. Using standardised effort and locations, this is modelled on well established TMAPS3 and CES4 survey schemes from Europe and North America. This protocol aims to better understand the basic demographics, longevity, survivorship/recruitment and moult/breeding phenology in resident cloud forest species, of which 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 in some of the data from poor recording of data and misunderstanding of the methods. Since 2015, a particular onus has been placed on recruiting team members with qualified and independent experience working with birds in the hand (e.g. BTO5 licencing) to independently lead mist-netting protocols at research camps which has gone a long way to address these occasional data quality issues. 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. Minimum effort requirements of six days banding at each sites (almost always separated by at least one day) were met at all sites in both seasons. A total of 665 captures were made across both field seasons (2016: 382 - 2017: 283). This comprised of 623 unique individuals, including 97 recaptures across 49 species (2016: 45 - 2017: 38). A summary breakdown of captures for both field seasons is presented in Table 5, below.

Vernacular	Binomial	2016	2017	Total captures (recaptured birds)
Green-throated Mountain-gem	Lampornis viridipallens	80 (3)	67 (3*)	147 (6)
Black-headed Nightingale- thrush	Catharus mexicanus	34 (18)	21 (11)	55 (29)
Violet Sabrewing	Campylopterus hemileucurus	30	17 (1*)	47 (1)
Chestnut-capped Brush-finch	Arremon brunneinucha	27 (14)	13 (4)	40 (18)
Stripe-tailed Hummingbird	Eupherusa eximia	15 (1)	24 (1)	39 (2)
Common Bush-Tanager	Chlorospingus flavopectus	18 (4)	14 (6)	32 (10)
Slate-coloured Solitaire	Myadestes unicolor	19 (4)	12 (3)	31 (7)
Slate-throated Whitestart	Myioborus miniatus	12 (7)	10 (4)	22 (11)
Yellowish Flycatcher	Empidonax flavescens	5	17 (4)	22 (4)
Grey-breasted Wood-wren	Henicorhina leucophrys	10 (1)	11 (2)	21 (3)
Green Violetear	Colibri thalassinus	16	4	20
Ochre-bellied Flycatcher	Mionectes oleagineus	9 (5)	10 (7)	19 (12)

Table 5 Summary table of all mist-net captures across all camps in 2016/17 field seasons.

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

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

⁵ British Trust for Ornithology



Red-capped Manakin	Ceratopipra mentalis	13 (2)	4 (1)	17 (3)
Ruddy-capped Nightingale-	Catharus frantzii	9 (5)	(4)	13 (9)
thrush				
Magnificent Hummingbird	Eugenes fulgens	9 (1)	3	12 (1)
Olivaceous Woodcreeper	Sittasomus griseicapillus	5 (2)	6 (4)	11 (6)
Tawny-throated Leaftosser	Sclerurus mexicanus	4	7 (2)	11 (2)
Spectacled Foliage-gleaner	Anabacerthia variegaticeps	6	5	11
Louisiana Waterthrush	Parkesia motacilla	5 (1)	4	9 (1)
Long-billed Hermit	Phaethornis longirostris	5	3	8
Spotted Woodcreeper	Xiphorhynchus erythropygius	5 (3)	2	7 (3)
Northern Nightingale Wren	Microcerculus philomela	5 (2)	2	7 (2)
White-throated Thrush	Turdus assimilis	1	6 (1)	7 (1)
Ruddy Woodcreeper	Dendrocincla homochroa	3 (1)	2	5 (1)
Emerald Toucanet	Aulacorhynchus prasinus	4	1	5
Blue-black Grosbeak	Cyanocompsa cyanoides	4	1	5
Ruddy Foliage-gleaner	Automolus rubiginosus	4 (2)	-	4 (2)
Stub-tailed Spadebill	Platyrinchus cancrominus	4 (1)	-	4 (1)
Mayan Ant-thrush	Formicarius moniliger	(1)	2 (1)	3 (2)
Golden-crowned Warbler	Basileuterus culicivorus	1	2 (1)	3 (1)
Emerald-chinned	Abeillia abeillei	3	-	3
Hummingbird				
Brown Violetear	Colibri delphinae	3	-	3
Azure-crowned Hummingbird	Amazilia cyanocephala	1	1	2
Bananaquit	Coereba flaveola	1	1	2
White-faced Quail-dove	Geotrygon albifacies	1	1	2
Tody Motmot	Hylomanes momotula	1	1	2
Black Thrush	Turdus infuscatus	1	1	2
White-bellied Emerald	Amazilia candida	-	1	1
White-naped Brush-finch	Atlapetes albinucha	1	-	1
Azure-hooded Jay	Cyanolyca cucullata	1	-	1
Black-banded Woodcreeper	Dendrocolaptes picumnus	-	1	1
Wedge-billed Woodcreeper	Glyphorynchus spirurus	-	1	1
Black-and-white Warbler	Mniotilta varia	1	-	1
Lesson's Motmot	Momota lessonii	1	-	1
Slaty Antwren	Myrmotherula schisticolor	-	1	1
Stripe-throated Hermit	Phaethornis striigularis	1	-	1
White-winged Tanager	Piranga leucoptera	1	-	1
Clay-coloured Thrush	Turdus grayi	1	-	1

Many species (excepting hummingbirds, where recaptured birds cannot be individually noted and are thus released unprocessed) show a high percentage of recaptures. This is particularly true of birds in breeding condition, serving to evidence the lengthy life histories of sedentary tropical birds. Typically, many of these species will retain/defend year-round territories and are generally long-lived (compared to similar temperate species). Table 6, below illustrates this in selected individuals with relatively long capture histories. Work is currently being undertaken to determine survival rates of selected species as



well as longevity records (see analysis of current data). The current constant-effort mist netting database stands at 2499 captures of 92 species over 6 consecutive years.

Table 6 Selected capture histories for some species- FCF age-codes are immature birds (\sim 1yr old), DCB are adults (>2years old, exact age beyond that unknown) UPB are adult-type birds in primary moult.

Species	1st & most recent catch date	Camp	Ring #	Age	Sex
Black-headed Nightingale-	7 independent dates between	Base	Y1/HN-	DCB on first	F
thrush Catharus mexicanus	16/06/2012 - 10/06/2017	Camp	B144	capture	
Time since 1st capture-					
4yrs, 11months, 25days					
Black-headed Nightingale-	13 independent dates	Base	Y2/HN-	FCF on first	М
thrush Catharus mexicanus	between	Camp	B170	capture	
	16/06/2012 - 1/07/2017				
Lime since 1st capture-					
5yrs U months 15 days	0 in dan an dant dataa hatuuran	Querelee			N 4
Black-neaded Nightingale-		Guanales	YIU/HIN-		IVI
Time since 1st centure	22/00/2012 - 20/01/2011		D143	capture	
5 Syrs 1 month 6 days					
Chestnut-canned Brush-	3 independent dates hetween	Cortecito	R33/HN_	DCB on first	F
finch Arremon brunneinucha	0.00000000000000000000000000000000000	001100110	C308	canture	•
Time since 1st capture-			0000	oupturo	
4vrs 0 months 13 days					
Olivaceaous Woodcreeper	4 independent dates between	Guanales	G16/HN-	UPB on first	М
Sittasomus griseicapillus	22/06/2012 - 30/07/2017		A006	capture	
Time since 1st capture-				-	
5yrs 1 month 8 days					
Slate-throated Whitestart	6 independent dates between	Cantiles	G109/H	DCB on first	F
Myioborus miniatus	24/06/2012 - 08/07/2017		N-A102	capture	
Time since 1st capture-					
5yrs 0 months 14 days					_
Grey-breasted Wood-wren	3 independent dates between	Cortecito	G38/HN-	DCB on first	F
Henicorhina leucophrys	16/07/2012 - 10/07/2017		AB411	capture	
Time since 1st capture-					
4yrs I I months 24 days	3 Independent dates between	Contiloo	VOCI		N 4
Common Bush Tanager	22/06/2012 - 25/06/2017	Cantiles	130/	DCB on first	IVI
Uniorosphingus havopectus			AB107	capture	
Avra O mantha 2 dava					
4yrs o months 3 days					

The dataset is now large enough, with enough recapture data for some species, from which to undertake some survival analyses. Additionally, a very large set of morphometric data, ageing data and more descriptive longevity data has also been collected.



3. Opportunistic surveys and overall park inventory

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. In recent seasons, new methods have been 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 greater input. To date this data set stands at in excess of 5500 records comprising over 200 species, a large number of which were not documented in any other methods. A focus on quantitatively using recreational birding has certainly been a factor in documenting a number of new and/or rare species in the park in the previous two field seasons such as Lovely Cotinga Cotinga amablis, Keel-billed Motmot Electron carinatum, Black-and-white Owl Strix nigrolineata, Black Hawk-Eagle Spizaetus tyrannus, Ornate Hawk-eagle Spizaetus ornatus, Grey-collared Becard Pachyramphus major, Rufous Mourner Rhytipterna holerythra, Double-toothed Kite Harpagus bidentatus and Brown Pelican Pelecanus occidentalis. Further, a recent effort has also been made to document nesting birds encountered during all field activities to provide some semi-quantitative information on the longevity of breeding seasons of resident species, this has yielded some already valuable discoveries, such as the nest of a Grey-collared Becard, a species of which the nest and nesting behaviour was only recently been described from Mexico.

The park inventory currently stands at c. 288 species. This inventory is being worked on currently to establish all fully verified records and some old, likely erroneous records. More surveys in winter would undoubtedly add numerous new species of Nearactic migrants that are poorly represented in the database from very limited field time. Finally, all bird records from camera-trapping have been identified and compiled (although the 2017 data still need sorting) 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 Currasow Crax rubra and Slaty-breasted (Boucard's) Tinamou Crypturellus boucardi that are very infrequently recorded otherwise.

4. Additional projects- Assessing the behavioural, physiological and ecological drivers in the elevational range segregation of montane songbirds.

Since 2016, Operation Wallacea has provided the logistical and in-kind field support for my PhD research investigating aspects of the behaviour and physiology of cloud forest songbirds. In particular, my research focuses on the ecological and physiological determinants of elevational range segregation in closely related species, focussing primarily on the lower elevation Black-headed Nightingale-thrush and higher elevation Ruddy-capped Nightingale-thrush (Figure 20). To date, this has been broken down into three distinct elements, outlined below.



Figure 20 Schematics of the elevational range occupation of selected study transects of the two Nightingale-thrush species in CNP, where (dependent on the specific slope) C.frantzii 'replaces' C.mexicanus at ~1850masl. On the left image white-black represents high-low altitude

Assays on territorial aggression between species. A key theory underpinning elevational range segregation between related species is interspecific aggression. Interspecific aggression can be either symmetric (where both species are similarly aggressive towards one another) or asymmetric (where one is dominant over the other). Relevant to elevation range segregation, this occurs where both species meet at the edges of their elevational distributions forming 'contact zones'. In investigating this experimentally through reciprocal playback experiments, I found an asymmetric interaction where the lower elevation Black-headed Nightingale-thrush was behaviourally dominant over the higher elevation Ruddy-capped Nightingale-thrush, but the strength of this interaction declined rapidly with distance from the contact zone This indicates this interaction is not inherent, but learned due to context.

Investigating how ecotones influence elevation range segregation. A second key theory as to the driving causes of elevational range segregation is that of ecotones (habitat preferences/specialisms and microclimates). To interrogate this further, I surveyed birds along an extensive series of elevational transects in closed canopy forest in Base Camp, Capuca, Guanales and Cantiles, augmented by data collected along the same transects by the bird team. Along each of these gradients, microclimate attributes were collected using teams of temperature loggers, as well as habitat attributes already collected at survey locations by the habitat team. Initial results of this indicate that habitat differences are clearly different between species and work an analysis coupling these ecotone qualities with the results of behavioural aggression assays is currently underway.

Physiological tolerance and lower critical limits. A final key theory driving elevational ranges of species on tropical mountains is that of physiological tolerance to temperature regimes. Because of the different microclimates present at different elevations on a mountain in the tropics, theory has predicted that a

given species should evolve a tolerance to the specific microclimate in the elevation it occupies. Elevations (and subsequently microclimates) outside of the elevational range occupied should thus represent physiological barriers. To test this between the Nightingale-thrushes, I used an open flow respirometry setup to measure metabolic rates in relation to manipulated ambient temperature in a controlled temperature chamber. Particularly, a higher elevation species should be expected to have lower metabolic rates at lower temperatures than a species occupying a lower elevation, because of the cooler temperatures an organism will experience with higher elevations. Data for this is currently being analysed.



The data from these three key components are currently being analysed and will be combined in order to compile as complete a picture as possible to empirically assess the key determinants of elevational range segregation. To date, no studies have investigated these theories in such fine scale empirical detail. Additional work for my PhD is also ongoing on the inter and intra-specific differences in blood physiology (haemoglobin content), body condition indices and levels of territorial aggression related to aspects of individual physiology.

5. Outputs

i) Projects

In 2016/17, the ornithological team included and supported two projects from Isobel Godfrey (University of Oxford) and Matt Little (Edinburgh University), respectively, for their undergraduate dissertation. While working as ornithologists in the field, both students used this and past seasons data.

Isobel's project investigated the guild specific responses in avian community change compared to land use/disturbance, recently receiving a 1st for her excellent thesis. Matt's project is currently ongoing and is investigating the role of temperature regimes on predicting elevational ranges of certain cloud forest 'indicator' species and avian guilds. Matt's project also aims to make a comparison between a temperate and tropical latitude mountains (Cusuco and a field site in Canada).

ii) Publications

The following manuscript is currently in review-

Neate-Clegg, M.H.C., Jones, S.E.I, Burdekin, O., Jocque, M., Sekercioglu, C.H. Elevational changes in the avian community of a Mesoamerican cloud-forest park. Biotropica

The following manuscript was recently published-

Martin. T, Rodrigues, F., Simcox, W. Dickson, I., van Dort, J., Reyes, E. & Jones, S.E.I. (2016) A review of notable range and altitudinal records from Parque Nacional Cusuco. Cotinga. 38: 32-39

iii) Analysis of current data

The current focus on Cusuco ornithology is to finalise the park inventory and undertake a full taxonomic update of the databases. A review of records is currently underway with the aim of publishing an extensive quantified inventory of the birds of Cusuco. How exactly this will take shape is currently in discussion, but may take form in the shape of a long monograph including status (in CNP), local distributions, longevity records and survival rates as well as an inventory of digitised, unambiguous records. The advantage of a monograph is that it would subsequently be made into a short book on the parks avifauna, available for future visitors, field teams and perhaps most importantly, translated into Spanish for Honduran audiences both local to CNP or otherwise.

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*, nest predation by Strong-billed Woodcreepers *Xiphocolaptes promeropirhynchus* and new prey species of the White-breasted Hawk *Accipiter striatus* [*chionogaster*].



Small mammal trapping

i. Small mammals 2016

Ten Sherman traps were placed 10m apart starting at 100m from the start of three transects in each camp (with the exception of Cantiles where CA2 and CA3 were not surveyed). All traps were left in-situ for 4 consecutive days and checked each morning. Bait consisted of peanut butter, honey and oats mix, placed in each trap and only replaced during the study period if an animal had entered the trap. A total of 75 individuals of five species were caught (Table 7). During 2016, small mammal abundance was greatest at Cantiles and lowest at Cortecito. Freya traps were not used in 2016, a number of repeats were carried out but these are not included in Figure 1. Additionally, one specimen of an unknown *Rheomys* water mouse was opportunistically collected (having been found dead at a river) and was exported to Dr Neil Reid at Queen's University Belfast to aid in the description of this species although, unlike in previous years, there were no targeted surveys for this species.

Camp	Heteromys desmarestianus	Peromyscus mexicanus	Marmosa mexicana	Scotinomys teguina	Tylomys watsoni	Grand Total
Base Camp	4	15	3			22
Cantiles	9	14				23
Cortecito	3				1	4
Danto	4	11				15
Guanales	2	8		1		11
Grand Total	22	48	3	1	1	75

Table 7 Small mammal species caught and identified at each camp in 2016

ii. Small mammals 2017

Ten Sherman traps were placed 10m apart starting at 100m from the start of three transects in each camp (with the exception of Cantiles where CA2 and CA3 were not surveyed) and with the same bait as the previous year. A total of 62 individuals of four species were caught with small mammal abundance greatest at Cantiles and Iowest at Danto (Table 8).

Table 8 Small mammal species caught and identified at each camp in 2017

Camp	Heteromys desmarestianus	Peromyscus mexicanus	Scotinomys teguina	Didelphis marsupialis	Grand Total
Base Camp	9	8		1	18
Cantiles	12	7			19
Cortecito	5		1		6
Danto	5				5
Guanales	7	7			14
Grand Total	38	22	1	1	62

Small mammal survey protocols have varied over the years (2012-2015) to test varying hypotheses and to trial different baits and trap placements. 2016 and 2017 were the first years in which a standardised trapping



protocol were implemented at all camps across multiple field seasons to form the basis of future monitoring to enable comparisons between camps and years. This was a second 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 terrestrial trap lines baited with peanut butter, oats and syrup mix only adjusted for trapping effort (Figure 21). Preliminary statistical analysis suggests a significant increase in the average number of small mammals caught per trap night from 2016-2017 (t value = 3.05, P<0.05) but there was no significant difference between the average numbers trapped from 2012-2016 (r²=0.384, 3df, P=0.158). This approach additionally ensures that all camps can be surveyed without need to rely on more difficult to come by bait (i.e. no need for cat food or tuna) and ensures that all future data collected are useful for comparison.



Figure 21 Average number of rodents caught per trap night across each year under 'terrestrial' trapping protocols.

b) Large mammal tracks and signs

i. Tracks & signs 2016

All transects at all camps were surveyed for field tracks and signs of large mammals during 2016 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, however CA4 was impossible to survey due to its use as the main entrance point into camp. A total 97 field signs were identified (Table 3) belonging to 14 species with the greatest number found at Cortecito and the fewest at Base Camp (once numbers were corrected for effort i.e. only three transects were observed at Cantiles), this is logical as the transects at Base Camp are more regularly frequented, making it more difficult to assess the transect undisturbed by footfall. The total number of records was lower than 2015 (130) but the number recorded in the following year (2017) once again increased (Table 9).



Table 9 Large mammal tracks and signs identified at each camp during summer 2016

Species	Base Camp	Cantiles	Cortecito	Danto	Guanales	Total
Cuniculus paca	1		2	2	5	10
Dasypus novemcinctus			15	6		21
Didelphis virginiana	1				1	2
Leopardus wiedii		1			1	2
Mazama temama	3	2	3	3	5	16
Conepatus semistriatus					2	2
Nasua narica	2	2	4	3	10	21
Orthogeomys sp.			1			1
Pecari tajacu	1		3	2		6
Potos flavus	2		2	1		5
Tapirus bairdii	1	4				5
Alouatta palliata	1					1
Panthera onca				4		4
Leopardus pardalis				1		1
Grand Total	12	9	30	22	24	97

ii. Tracks & signs 2017

All transects at all camps were surveyed for field tracks and signs of large mammals during 2017 consistent with previous years. Once again, CA4 was impossible to survey due to its use as the main entrance point into camp. A total 149 field signs were identified belonging to 14 species including jaguar tracks (*Panthera onca;* Table 10) which was captured on camera trap last year by Panthera within Cusuco. Most tracks and signs were detected in Danto and fewest at Cantiles.

Species	Base Camp	Cantiles	Cortecito	Danto	Guanales	Total
Alouatta palliata	3		1	5	1	10
Bassariscus sumichrasti				1		1
Cuniculus paca	5		9	11	1	26
Dasypus novemcinctus	2	5	13	13	5	38
Orthogeomys sp.					1	1
Leopardus wiedii		1			1	2
Mazama americana	2		1	4	1	8
Nasua narica	15	5	4	6	8	38
Odocoileus virginianus			1	9	2	12
Pecari tajacu		1		4		5
Conepatus semistriatus					1	1
Panthera onca				1		1
Potos flavus	2					2
Tapirus bairdii		3		1		4
Grand Total	29	15	29	55	21	149

Table 10 Large mammal tracks and signs identified at each camp during summer 2017



- a) Large mammal camera trapping
- i. Camera trapping 2016

A total of 28 camera traps were deployed at 94 locations throughout the 8 week field season, however one camera was stolen from BC3 and a further two cameras triggered too frequently to produce useful data. Cameras were left out for an average of 2.93 days which, coupled with the requirement for 1/3 of cameras to be 300m from transect, 1/3 to be 150m from transect and 1/3 to be 20m from transect, was a very labour intensive process. This was necessary to be consistent with the previous year's data collection and maximise the number of locations surveyed. Nine species of interest were detected with highest number of detections at Base Camp and lowest at Guanales (not including species of squirrels and small rodents).

ii. Camera trapping 2017

For 2017, the period for which cameras were left in the field was increased to an average of 15.8 days which was made possible by the use of additional 32 cameras although the survey design of two sets of three cameras per transect remained (although in some areas of severe deforestation such as in Cortecito, this was not possible). Cameras were placed at 95 locations, six of these locations were along the route know as La Torre in light of difficulties with camera placement at Cortecito; here a striped hog-nosed skunk (*Conepatus semistriatus*) was captured there creating the first physical record of the species in Cusuco. A total of five cameras and one SD card were stolen. Ten species of interest were detected with the highest capture rate at Cantiles and lowest at Base camp (taking into account trapping effort as La Torre was surveyed less). There were fewer detections per month (accounting for survey effort) in 2017 than in previous years (Figure 22), although due to many confounding factors it is not suitable to compare directly between years at this time.



Figure 22



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. A total of 113 locations were surveyed, slightly fewer than in previous years due to ending research in the extra disturbance sites on the west side. Compared with previous years, sampling effort was most similar to 2016, compared with a much greater sampling intensity in 2013-2015. Like 2016, sampling was reduced this season partly because camps were not open for as long, and partly as an active decision to reduce uneccesary identification workload. Baseline levels of community data have already been reached, and year-on-year surveying is now undertaken for the purpose of monitoring, which does not require as large a sampling effort. In particular, Base Camp and Guanales hosted a dissertation student project exploring the effects of methodological variation, rather than the much higher numbers of core samples undertaken in previous years. A total of 542 samples were collected, of which 404 used the standardised sampling protocol (the remainder being part of the dissertation student project). Considering only these 404, 107 of the 113 sample locations (95%) were sampled the planned minimum of 3 times, with an average of 3.6 samples per site. Of the 6 under-sampled locations, 5 belonged to a single transect that could not be completed sufficiently in the available camp opening time (BA4), and 1 is likely a missing sample.

The vast majority of samples were sorted (dung beetles separated from bycatch) and identified to species or morphospecies before the end of the season. The team is to be commended for this effort, as this took place without me being on site and with no returners on staff this year. Identification was carried out using the OpWall-funded Creedy and Mann 2011 identification guide. Data was recorded using excel spreadsheets and, new for this year, an ODK form. This ODK form provided data that was much less error-prone, but was reportedly slow to use. We aim to work to improve this for future seasons, as the hand-entered excel data contained many small errors that required substantial work to correct.

A total of 16,367 *Scarabaeinae* dung beetles were identified, of which 11,671 were collected from the 404 standardised samples. Approximately 28 of the 40 species know to exist in Cusuco National Park appear to have been found, although this is likely to rise to 30+ once a few tricky-to-resolve species groups are ID'd in the UK. This is comparable to 2016. The species which are absent are generally those associated with the disturbed habitat surrounding Santo Tomas, which was not sampled this year.

Although the majority of samples were processed in the field, substantial work remains to be carried out in the UK. The field datasheets needed to be compiled, checked for errors and validated (checking that values and IDs were reasonable, and double-checking if not). While we attempted to do this during the season, the internet connection was too poor to stay sufficiently in sync, and while some validation managed to take place the majority had to be done back in the UK. Furthermore, as mentioned the species that are hard to separate have to be done in the UK as the skills and equipment simply aren't present in the field. Last year we established a system for doing this during the season, but again this did not work because the internet connection on site is not suitable for the field team to stay in sync. Furthermore, the species that are hard to separate have to be identified in the UK as the skills and equipment simply weren't present in the field.

Based on the identification data as it currently stands, across all 542 samples, 72 will require reidentification for validation purposes at the OUMNH as they comprise species that were not identified fully or that are hard for non-experts to ID accurately.



Habitat and forest structure data for Cusuco National Park were collected at 114 survey sites between June 3^{rd} and August 3^{rd} , 2017. For detailed descriptions of the methods used please refer to the protocol document. Number of trees present, mean tree girth at breast height (GBH), mean leaf litter depth and soil density, mean canopy openness score (0 = fully closed, 25 = full open), sapling count per m², number of stumps and cut saplings, along with elevation, aspect, and slope data were calculated for each survey site (Table 11).

Table 11 Site by site analysis of habitat and forest structure along transects at Base Camp (BC) and at each satellite camp (BA = Buenos Aires, CA = Cantiles, CO = Cortecito, DA = Danto, GU = Guanales).

						Leaf	Soil				
Survey	Flevation		Slone	Tree	GBH	denth	Density	Canopy	Sanlings		Cut
Site	(m)	Aspect	(°)	count	(cm)	(mm)	(mm)	Score	per m ²	Stumps	Saplings
BC1 SS1	1599	E	3	81	56.7	56.0	41.0	1.8	0.65	12	7
BC1 SS2	1588	S	15	84	46.0	30.2	52.0	2.0	0.45	0	0
BC1 SS3	1626	NW	27	32	78.4	59.2	51.4	5.0	1.20	0	4
BC1_SS4	1587	Ν	32	156	34.1	45.6	34.2	1.2	1.25	4	0
BC1_SS5	1597	W	15	99	42.5	50.0	35.6	2.8	0.85	0	2
BC1_SS6	1623	W	32	57	57.6	53.0	35.0	1.2	0.70	0	0
BC1_SS7	1640	SW	40	85	38.0	44.8	46.6	1.6	2.50	0	0
BC1_SS8	1697	Ν	25	48	72.0	49.0	45.4	1.0	0.70	2	0
BC2_SS1	1447	Ν	29	55	54.2	65.3	27.6	1.4	0.75	4	7
BC2_SS2	1386	NW	9	52	54.3	25.8	23.4	5.6	0.40	3	7
BC2_SS3	1421	-	27	58	62.6	50.8	60.2	2.0	0.70	4	0
BC2_SS4	1446	S	15	104	44.8	87.8	25.6	1.6	1.05	1	10
BC3_SS1	1658	SW	14	64	34.2	31.0	18.6	3.6	0.55	8	13
BC3_SS2	1665	E	32	35	84.1	86.4	37.0	9.0	0.25	1	3
BC3_SS3	1648	W	9	43	67.0	29.4	36.8	8.8	1.05	3	1
BC3_SS4	1590	W	40	70	47.2	47.6	22.2	6.2	3.20	4	25
BC3_SS5	1518	Ν	20	65	47.8	131.4	41.2	1.0	0.80	3	9
BC3_SS6	1459	W	20	123	39.1	124.8	30.0	6.6	2.00	0	6
BC3_SS7	1399	NW	18	70	44.3	36.0	30.0	4.2	0.50	0	5
BC4_SS1	1614	SW	4	84	47.3	39.6	28.0	2.0	0.45	0	4
BC4_SS2	1648	W	8	68	41.7	37.6	31.2	2.4	2.00	8	8
BC4_SS3	1683	NW	37	29	53.3	36.0	24.0	1.2	0.30	1	3
BC4_SS4	1703	Ν	4	34	61.3	50.0	37.6	4.6	1.00	2	8
BC4_SS5	1715	NE	2	65	57.1	47.2	31.0	0.8	0.35	1	1



<u> </u>		Valla	acea	Ĺ							
Survey Site	Elevation (m)	Aspect	Slope (°)	Tree	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m ²	Stumps	Cut Saplings
BC4 SS6	1731	N	12	75	45.9	94.0	49.0	1.8	0.20	0	0
BA1 SS1	1027	SW	2.5	25	120.7	34.0	40.6	8.8	0.30	11	1
BA1 SS2	1074	-	8	11	166.0	8.6	21.8	23.8	0.00	6	8
BA1 SS3	1138	-	-	4	83.5	4.6	22.0	11.2	0.00	3	6
BA1 SS4	1187	SE	31	6	160.7	43.6	16.6	16.6	0.00	3	0
BA1 SS5	1192	SW	19	10	133.3	32.0	15.6	17.6	0.00	16	1
BA2_SS1	1313	SW	22	13	115.5	26.2	33.2	6.6	0.25	3	18
BA2_SS2	1235	-	-	38	73.6	56.2	-	6.2	0.20	2	6
BA2_SS3	1214	NE	38	31	46.1	54.2	76.0	4.2	0.50	0	9
BA2_SS5	1124	W	24	44	46.4	48.2	58.6	0.6	1.00	0	0
BA2_SS6	1019	S	20	40	67.8	57.2	42.2	12.8	0.50	4	1
BA3_SS1	1370	W	16	83	38.4	58.8	34.8	5.8	1.45	5	18
BA3_SS2	1268	-	-	45	54.0	42.6	51.2	6.0	0.45	0	8
BA4_SS1	1352	SW	25	30	63.6	88.8	68.6	7.6	1.95	0	18
BA4_SS2	1348	NW	30	113	47.6	57.6	28.0	1.4	1.10	20	14
BA4_SS3	1409	W	20	73	48.2	32.0	28.8	1.0	0.90	0	1
BA4_SS4	1420	NE	24	39	82.9	41.6	34.6	2.0	1.40	0	0
BA4_SS5	1481	W	22	43	70.8	35.4	36.0	4.4	3.00	2	0
CA2_SS1	2055	Ν	24	123	43.5	55.4	38.2	2.6	0.70	0	1
CA2_SS3	2091	NE	35	91	44.0	62.4	41.6	3.2	0.40	0	0
CA2_SS4	2124	E	35	93	46.1	35.7	45.9	2.6	0.10	1	0
CA2_SS5	2148	NE	28	71	55.8	56.6	31.6	4.8	0.60	2	2
CA2_SS6	2178	NE	18	77	54.4	66.0	38.0	3.0	0.00	3	0
CA2_SS7	2183	W	16	89	49.7	62.0	32.0	4.2	0.45	0	4
CA3_SS1	2051	W	30	77	42.3	78.0	85.0	2.4	1.25	1	0
CA3_SS2	2053	SE	45	91	48.6	77.0	46.6	4.4	1.40	0	0
CA3_SS3	1962	S	24	146	37.4	37.2	31.0	2.6	1.10	4	3
CA4_SS1	1847	E	26	81	37.0	35.2	30.8	0.8	0.15	13	11
CA4_SS2	1924	NE	40	61	55.9	87.0	39.6	5.0	0.70	3	5
CA4_SS3	1943	NE	38	48	39.1	56.6	44.6	3.8	1.40	3	1
CA4_SS4	1956	NE	35	114	38.6	27.6	31.6	1.6	0.70	0	5
CA5_SS1	1825	W	18	25	62.6	37.4	21.2	3.0	1.65	2	11
CA5_SS2	1891	E	37	68	41.1	45.0	14.0	1.4	1.60	0	0
CA5_SS3	1943	E	26	99	37.4	75.0	49.4	2.6	0.35	0	2
CA5_SS4	2004	SE	22	122	43.3	30.0	32.0	2.0	1.25	0	0
CA5_SS5	1967	SE	31	42	63.5	25.4	28.8	3.2	1.65	2	9
CA5_SS6	1910	SW	35	76	41.7	32.0	32.0	1.6	0.30	2	0
CA5_SS7	1841	N	42	63	39.8	36.0	39.0	1.8	0.15	3	0
CA5_SS8	1789	NE	35	74	43.9	58.6	34.8	2.8	0.50	1	7
CO1_SS1	1396	NW	20	29	66.2	0.0	30.4	25.0	0.00	65	200
CO1_SS2	1391	NE	24	25	49.3	0.0	21.6	25.0	0.00	56	200



<u> </u>		Valla	acea	l							
Survey Site	Elevation (m)	Aspect	Slope	Tree	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m ²	Stumps	Cut Saplings
CO1 SS3	1331	NE	16	39	63.7	13.4	28.6	25.0	0.00	79	200
C01 SS4	1176	N	37	63	59.1	34.6	26.0	2.2	1.30	6	18
CO1 SS5	1174	Ν	15	79	49.0	71.4	35.4	4.2	2.00	7	12
CO2 SS1	1398	E	22	69	58.0	39.2	48.4	3.2	0.70	2	2
CO2 SS2	1407	Ν	30	52	73.6	28.4	59.8	25.0	0.00	56	66
CO2 SS3	1472	S	24	51	60.4	45.8	33.0	1.4	0.65	12	7
CO3_SS1	1539	E	37	46	62.3	52.2	50.8	2.2	0.15	1	8
CO3_SS2	1587	S	23	47	58.5	63.6	48.0	5.4	1.00	3	10
CO3_SS3	1637	E	8	50	54.9	22.6	14.2	6.4	0.95	0	6
CO3_SS4	1681	S	10	91	44.3	33.0	29.8	3.8	0.70	11	7
CO3_SS5	1665	SW	28	49	74.2	52.0	29.6	1.8	0.65	2	2
CO3_SS6	1628	SE	35	34	59.4	63.6	36.2	0.8	1.40	0	1
DA0_SS2	1578	W	22	104	37.3	69.0	42.0	1.8	0.45	4	1
DA0_SS3	1594	W	15	116	45.0	63.0	39.0	3.2	1.50	1	2
DA0_SS4	1593	W	30	76	59.1	73.0	35.0	2.0	1.10	1	0
DA0_SS5	1598	W	18	128	44.9	62.0	64.0	2.4	0.55	0	0
DA0_SS6	1603	Ν	29	77	40.8	46.0	52.6	1.6	0.40	1	0
DA1_SS1	1559	S	11	89	47.5	71.8	55.2	2.8	0.70	11	0
DA1_SS2	1606	E	20	95	40.1	58.0	59.0	5.4	0.50	8	6
DA1_SS3	1701	S	27	46	72.3	70.0	70.0	3.0	0.75	8	0
DA1_SS4	1724	S	9	71	55.1	51.0	32.2	5.2	1.60	3	0
DA1_SS5	1715	Ν	16	137	37.7	41.0	25.4	2.0	0.75	11	14
DA1_SS6	1593	Ν	28	37	57.9	31.0	35.0	2.6	0.95	0	1
DA2_SS1	1583	Ν	26	114	48.7	86.4	55.4	2.2	0.55	1	0
DA2_SS2	1616	N	5	93	54.4	26.4	24.6	5.4	1.15	0	2
DA2_SS3	1536	NW	30	66	52.9	38.8	39.4	3.2	0.80	3	9
DA4_SS1	1633	NE	15	81	41.1	32.0	39.0	1.0	0.30	0	1
DA4_SS2	-	NE	22	40	55.2	43.2	55.0	18.4	1.20	11	16
GU1_SS1	1415	N	25	58	65.6	38.8	28.6	1.8	0.80	3	4
GU1_SS2	14/3	NW	20	64	54.3	92.0	40.0	1.8	0.40	2	8
GU1_SS3	1632	NE	29	55	51.5	48.8	28.6	2.4	2.00	0	0
	1/18	-	-	44	58.0	65.2	38.8	4.2	2.25	0	0
	1805		15	31	57.7	50.4	55.8	2.8	0.80	0	2
	1845	SE	40	4/	37.1	49.0	53.Z	1.0	0.45	U 1	5
	1941		20 4E	70 45	30.5	07.Z	29.0	2.0	0.05		0
	1904		40	40	43.0	02.Z	33.0 53.0	1.2	0.20	১ 1	4
	1000	SE E	20	00	40.0 52.6	00 0	02.2 22.0	0.4 0.2	0.20	0	0
002_002	1000	с СС	30 25	00 51	55.0	00.2	32.0 20.0	0.2 2.0	0.60	0	0
002_000 CII2 CC1	1/20		25	17	53.9	40.4 66.0	20.0 76.0	2.0 0.6	0.00	U 1	0
002_004	1420		33	47	50.7	12 Q	10.0 23 G	0.0 0.2	3.55	0	0
<u>uuz_</u> 000	1001	JE	33	40	50.7	43.0	۷۵.0	U.0	2.00	U	U



Survey	Elevation		Slope	Tree	GBH	Leaf Litter depth	Soil Density	Canopy	Saplings		Cut
Site	(m)	Aspect	(°)	count	(cm)	(mm)	(mm)	Score	per m ²	Stumps	Saplings
GU2_SS6	1496	NW	27	99	40.3	75.6	36.2	1.0	1.65	0	1
GU2_SS7	1514	SE	6	98	41.6	99.6	36.8	2.6	0.50	0	0
GU2_SS8	1594	Ν	18	69	43.1	116.0	69.0	1.8	0.30	3	14
GU3_SS1	1234	NW	22	44	62.2	35.4	37.4	2.2	0.20	2	5
GU3_SS2	1263	E	27	55	57.0	61.0	34.2	1.6	1.00	1	2
GU4_SS1	1244	W	8	40	85.3	37.0	35.4	0.4	0.45	3	8
GU4_SS2	1225	SE	41	67	50.7	42.4	23.8	1.8	0.50	0	0
GU4 SS3	1197	W	28	42	61.5	75.0	45.0	2.8	1.90	0	5

Camp by camp analysis

Base Camp

i) Comparison with all Cusuco survey sites

Base Camp survey sites are situated between 1386m and 1731m above sea level and on average are approximately only 6m above the average elevation for all sites surveyed across the Park. There was no significant difference for tree count, GBH, leaf litter depth, soil density, or sapling count per m² between Base Camp sites and all Cusuco survey sites. However, canopy openness score (t=4.548, df=124, P>0.05), stump count (t=4.007, df=24, P>0.05), and cut sapling count (t=4.512, df=24, P>0.05) were found to be significantly lower at Base Camp sites than at all sites. These trends indicate that there is a lower level of human disturbance at Base Camp sites than across Cusuco in general.

	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m²	Stumps	Cut saplings
BC	1587.5	69.4	48.7	58.0	35.8	3.2	0.95	2.4	4.9
Cusuco	1581.8	65.0	49.9	51.8	38.1	4.4	0.84	4.9	10.0

Mean values for Base Camp survey sites and all survey sites in 2017

Base Camp sites have a slightly higher percentage of broadleaf trees than Cusuco as a whole, along with corresponding lower percentages of ferns and palms. The percentage of dead trees found at Base Camp sites is about average for the Park.

	%	%	%	%	%
	Broadleaf	Fern	Palm	Pine	Dead
BC	83.2	13.6	1.1	2.0	12.0
Cusuco	74.9	19.5	3.6	1.8	12.7

Tree percentage breakdown for Base Camp and all survey sites in 2017

ii) Comparison with 2016

In Base Camp sites leaf litter depth (t=5.497, df=124, P>0.05) and cut sapling count (t=2.491, df=24, P>0.05) were both found to have significantly increased from 2016 to 2017. All other variables were not significantly changed.



					Soil				
	Elevation	Tree	GBH	Leaf Litter	Density	Canopy	Saplings		Cut
	(m)	count	(cm)	depth (mm)	(mm)	Score	per m ²	Stumps	saplings
BC_2016	1587.5	67.2	49.9	33.3	31.8	2.7	1.21	1.6	1.8
BC_2017	1587.5	69.4	48.7	58.0	35.8	3.2	0.95	2.4	4.9

Mean values for Base Camp in 2016 and 2017

A higher percentage cover of tree ferns was found in 2017 compared to in 2016, with decreases in the cover of broadleaf and palm trees. The percentage of trees found to be dead in survey sites increased by around a third from 2016 to 2017.

	% Broadleaf	% Fern	% Palm	% Pine	% Dead
BC_2016	85.7	9.5	2.1	2.7	9.2
BC_2017	83.2	13.6	1.1	2.0	12.0

Tree percentage breakdown for Base Camp in 2016 and 2017

Buenos Aires

i) Comparison with all Cusuco survey sites

Buenos Aires survey sites are situated between 1019m and 1481m above sea level and on average are approximately 340m below the average elevation for all sites surveyed across the Park. No significant difference between values for soil density, sapling count per m^2 , or number of stumps was found between Buenos Aires sites and all Cusuco sites. However, Buenos Aires sites had a significantly lower tree count (t=3.804, df=16, P>0.05), leaf litter depth (t=3.268, df=84, P>0.05) and cut sapling count (t=2.154, df=16, P>0.05) than all Cusuco sites. And additionally, GBH (t=5.628, df=647, P>0.05) and canopy openness (t=4.603, df=84, P>0.05) were significantly higher than for all survey sites. These trends are most likely explained by the high levels of deforestation and forest degradation at Buenos Aires sites which, especially in the case of shade grown coffee plantations, leaves only high GBH trees standing and reduces overall canopy coverage.

	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m²	Stumps	Cut saplings
BA	1245.4	38.1	62.7	42.2	38.0	8.0	0.76	4.4	6.4
Cusuco	1581.8	65.0	49.9	51.8	38.1	4.4	0.84	4.9	10.0

Mean values for Buenos Aires survey sites and all survey sites in 2017

Buenos Aires sites had a much higher percentage of pine trees compared to Cusuco overall, with a corresponding much lower percentage of fern and palm trees. The percentage of dead trees found in Buenos Aires sites was slightly lower than the Park average, however this is misleading as Buenos Aires sites have suffered the heaviest historic deforestation in the Park (evidenced now by the significantly lower tree count).

	%	%	%	%	%
	Broadleaf	Fern	Palm	Pine	Dead
BA	77.2	9.9	0.3	11.7	10.0
Cusuco	74.9	19.5	3.6	1.8	12.7

Tree percentage breakdown for Buenos Aires and all survey sites in 2017



Sites in Buenos Aires were found to have significantly higher counts of cut saplings (t=2.594, df=16, P>0.05) in 2017 than in 2016. No other variables were found to have significantly changed between the two years.

						Soil				
	Elevation	Tree	GBH	Leaf	Litter	Density	Canopy	Saplings		Cut
	(m)	count	(cm)	depth (mm)	(mm)	Score	per m ²	Stumps	saplings
BA_2016 ⁺	1240.1	39.2	60.7	55.2		33.3	9.9	0.64	2.4	1.8
BA_2017	1245.4	38.1	62.7	42.2		38.0	8.0	0.76	4.4	6.4
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Mean values for Buenos Aires in 2016 and 2017

Palm tree cover was found to be much lower in 2017 than in 2016. Similar to Base Camp, the percentage of dead trees in Buenos Aires sites was found to have increased by around a third over the year.

	%	%	%	%	
	Broadleaf	Fern	Palm	Pine	% Dead
BA_2016 ⁺	78.8	8.9	1.8	10.5	7.4
BA_2017	77.2	9.9	0.3	11.7	10.0

Tree percentage breakdown for Buenos Aires in 2016 and 2017 t one additional site was surveyed in 2016

<u>Cantiles</u>

i) Comparison with all Cusuco survey sites

Cantiles survey sites are situated between 1789m and 2183m above sea level and on average are approximately 400m higher than the average elevation for all sites surveyed across the Park. Cantiles sites showed no significant difference in leaf litter depth, soil density depth, or sapling count per m² when compared to all survey sites in Cusuco. However, tree count (t=2.798, df=20, P>0.05) was found to be significantly higher than at all survey sites. Whilst GBH (t=4.826, df=1730, P>0.05) and canopy openness (t=8.959, df=104, P>0.05), along with number of stumps (t=4.799, df=20, P>0.05) and cut saplings (t=8.694, df=20, P>0.05) were all found to be significantly lower than overall Cusuco sites. These trends can be accounted for by the higher elevation of Cantiles survey sites, which tends to reduce tree GBH, and by the relatively undisturbed nature of the habitat found at these elevations which leads to lower canopy openness and human disturbance values.

	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m²	Stumps	Cut saplings
CA	1985.0	82.4	44.5	51.2	37.5	2.8	0.78	1.9	2.9
Cusuco	1581.8	65.0	49.9	51.8	38.1	4.4	0.84	4.9	10.0
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Mean values for Cantiles survey sites and all survey sites in 2017

Cantiles has the highest tree fern percentage cover of any camp in Cusuco, and correspondingly has lower broadleaf, pine and palm percentage cover. The percentage of dead trees found in Cantiles is below the Park average, which again is due to the relatively undisturbed nature of this area of the Park.

	Oper Wall	ratio ace	on ea		
	%	%	%	%	%
	Broadleaf	Fern	Palm	Pine	Dead
CA	60.1	37.2	2.1	0.2	10.9
Cusuco	74.9	19.5	3.6	1.8	12.7
7	, , ,	, ,	^ '''		

Tree percentage breakdown for Cantiles and all survey sites in 2017

ii) Comparison with 2016

At Cantiles survey sites leaf litter depth (t=4.218, df=104, P>0.05), soil density depth (t=5.060, df=104, P>0.05), and canopy openness (t=3.585, df=104, P>0.05) were all found to have significantly increased from 2016 to 2017, with all over variables not significantly changed.

	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m²	Stumps	Cut saplings
CA_2016 ⁺	1989.5	71.5	42.2	35.4	26.5	1.9	0.68	1.7	3.4
CA_2017	1985.0	82.4	44.5	51.2	37.5	2.8	0.78	1.9	2.9
	6 0 111	1 001	0 10	0.17					

Mean values for Cantiles in 2016 and 2017

Percentage covers of each tree category were found to be relatively unchanged between 2016 and 2017, with only a minor increase in the percentage of dead trees present.

	% Broadleaf	% Fern	% Palm	% Pine	% Dead
CA_2016 ⁺	59.0	39.2	1.8	0.1	9.1
CA_2017	60.1	37.2	2.1	0.2	10.9

Tree percentage breakdown for Cantiles in 2016 and 2017 ⁺ one additional site was surveyed in 2016

<u>Cortecito</u>

i) Comparison with all Cusuco survey sites

Cortecito survey sites are situated between 1174m and 1681m above sea level and on average are approximately 120m lower than the average elevation for all sites surveyed across the Park. In Cortecito, sites were found to have no significant difference in soil density depth and sapling count per m² than at all Cusuco survey sites. However, sites were found to a have significantly higher GBH (t=4.264, df=723, P>0.05), canopy openness score (t=4.065, df=69, P>0.05), stump count (t=2.158, df=13, P>0.05), and cut sapling count (t=1.967, df=13, P>0.05). Along with a significantly lower tree count (t=2.677, df=13, P>0.05) and leaf litter depth (t=4.334, df=69, P>0.05). All of these trends are undoubtedly linked to the heavy deforestation and forest degradation found along Cortecito transects. Survey sites 1, 2, and 3 on Transect 1 and (new in 2017) survey site 2 on Transect 2 are completed deforested. The resulting high stump count and cut sapling count for Cortecito survey sites heavily affects the Cusuco mean value for these two variables.



	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m²	Stumps	Cut saplings
C0	1463.0	51.7	58.3	37.1	35.1	9.4	0.68	21.4	52.8
Cusuco	1581.8	65.0	49.9	51.8	38.1	4.4	0.84	4.9	10.0

Mean values for Cortecito survey sites and all survey sites in 2017

Cortecito has an above average percentage cover of broadleaf and palm trees, with well below average cover of ferns and pines. Deforestation along Transects 1 and 2 has led to a huge percentage of dead trees in Cortecito, with over 1 in every 4 trees surveyed being dead.

	% Broadleaf	% Fern	% Palm	% Pine	% Dead
C0	85.5	5.1	9.1	0.3	27.2
Cusuco	74.9	19.5	3.6	1.8	12.7

Tree percentage breakdown for Cortecito and all survey sites in 2017

ii) Comparison with 2016

No measured variables were found to have significantly changed at Cortecito sites between 2016 and 2017, however the mean values for canopy score, stumps and cut saplings can be seen to have greatly increased.

	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m²	Stumps	Cut saplings
CO 2016	1463.0	51.4	55.2	41.5	37.4	6.8	1.58	6.7	4.6
C0_2017	1463.0	51.7	58.3	37.1	35.1	9.4	0.68	21.4	52.8
			-						

Mean values for Cortecito in 2016 and 2017

Percentage cover of the various tree categories were roughly unchanged between 2016 and 2017, meanwhile the percentage of dead trees was found to have more than doubled.

	% Broadleaf	% Fern	% Palm	% Pine	% Dead
CO_2016	86.3	4.3	9.4	0.0	12.9
CO_2017	85.5	5.1	9.1	0.3	27.2

Tree percentage breakdown for Cortecito in 2016 and 2017

These trends are due to the complete deforestation of an additional site in Cortecito (Transect 2 Site 2) in 2017, and the lack of data collected on previously deforested sites (Transect 1 Sites 1, 2, 3) in 2016.

<u>Danto</u>

i) Comparison with all Cusuco survey sites

Danto survey sites are situated between 1536m and 1724m above sea level and on average are approximately 35m higher than the average elevation for all sites surveyed across the Park. Survey sites in Danto show no significant difference in leaf litter depth, canopy openness, sapling count per m², or stump



count when compared to all Cusuco survey sites. However, tree count (t=2.755, df=15, P>0.05) and soil density depth (t=3.618, df=79, P>0.05) were found to be significantly higher at Danto sites than for all sites in the Park. Danto is the only camp to show a significantly different soil density value to the overall Cusuco mean. Additionally, GBH (t=2.648, df=1369, P>0.05) and cut sapling count (t=5.163, df=15, P>0.05) were both found to be significantly lower at Danto sites than at all survey sites.

	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m²	Stumps	Cut saplings
DA	1615.5	85.6	47.2	53.9	45.2	3.9	0.83	3.9	3.3
Cusuco	1581.8	65.0	49.9	51.8	38.1	4.4	0.84	4.9	10.0

Mean values for Danto survey sites and all survey sites in 2017

Danto has a high percentage cover of tree ferns and palms when compared to Cusuco averages, but is still dominated by broadleaf trees. There were no pine trees found at Danto survey sites. The percentage of dead trees found in Danto was slightly above average for the Park.

	% Broadleaf	% Fern	% Palm	% Pine	% Dead
DA	61.8	28.5	9.6	0.0	13.6
Cusuco	74.9	19.5	3.6	1.8	12.7

Tree percentage breakdown for Danto and all survey sites in 2017

ii) Comparison with 2016

In Danto survey sites leaf litter depth (t=5.582, df=79, P>0.05) and soil density depth (t=4.613, df=78, P>0.05) were both found to have significantly increased between 2016 and 2017, with all other variables not significantly changed.

		Soil								
	Elevation	Tree	GBH	Leaf Li	tter	Density	Canopy	Saplings		Cut
	(m)	count	(cm)	depth (mn	n)	(mm)	Score	per m ²	Stumps	saplings
DA_2016 ⁺	1607.7	75.6	48.2	34.7		32.6	2.8	0.69	13.4	8.4
DA_2017 ⁺	1615.5	85.6	47.2	53.9		45.2	3.9	0.83	3.9	3.3
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Mean values for Danto in 2016 and 2017

Percentage tree cover in Danto was found to have shifted slightly between 2016 and 2017 with a decrease in tree fern cover and an increased in broadleaf cover. The percentage of dead trees found at survey sites was similar for both years.

	%	%	%	%	%
	Broadleaf	Fern	Palm	Pine	Dead
DA_2016 [‡]	55.7	34.7	9.6	0.0	12.7
DA_2017 ⁺	61.8	28.5	9.6	0.0	13.6

Tree percentage breakdown for Danto in 2016 and 2017 ⁺ *one site was surveyed in each year but not the other*



<u>Guanales</u> i)

Comparison with all Cusuco survey sites

Guanales has the highest range of elevation of any camp, with survey sites situated between 1197m and 1964m above sea level. On average these sites are approximately 80m below the average elevation for all sites surveyed across the Park. Guanales sites showed no significant difference in GBH, soil density depth, or sapling count per m² when compared to all survey sites. However, there was a significantly higher leaf litter depth (t=3.209, df=104, P>0.05) than at all survey sites; along with a significantly lower tree count (t=1.956, df=20, P>0.05), canopy score (t=17.184, df=104, P>0.05), number of stumps (t=15.075, df=20, P>0.05) and number of cut saplings (t=8.812, df=20, P>0.05). All of these trends correspond to the highly undisturbed nature of the habitat surrounding Guanales camp.

	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m²	Stumps	Cut saplings
GU	1499.4	57.3	50.5	61.0	39.9	1.7	0.93	1.0	2.9
Cusuco	1581.8	65.0	49.9	51.8	38.1	4.4	0.84	4.9	10.0

Mean values for Guanales survey sites and all survey sites in 2017

Guanales is almost entirely dominated by broadleaf trees, with below average percentage cover of pines and well below average cover of tree ferns and palm trees. The percentage of dead trees found in Guanales is well below the Park average due to the camp's highly undisturbed habitat.

	% Broadleaf	% Fern	% Palm	% Pine	% Dead
GU	91.3	6.0	1.2	1.2	8.2
Cusuco	74.9	19.5	3.6	1.8	12.7

Tree percentage breakdown for Guanales and all survey sites in 2017

ii) Comparison with 2016

In Guanales leaf litter depths (t=4.948, df=99, P>0.05) and soil density depths (t=2.735, df=99, P>0.05) were found to have significantly increased from 2016 to 2017, whilst canopy openness (t=4.984, df=99, P>0.05) significantly decreased. Other variables weren't changed significantly.

	Elevation (m)	Tree count	GBH (cm)	Leaf Litter depth (mm)	Soil Density (mm)	Canopy Score	Saplings per m ²	Stumps	Cut saplings
GU_2016	1500.7	56.6	50.0	41.0	33.6	3.8	0.73	0.6	1.9
GU_2017 ⁺	1499.4	57.3	50.5	61.0	39.9	1.7	0.93	1.0	2.9

Mean values for Guanales in 2016 and 2017

Tree percentage cover and percentage of dead trees in Guanales was unchanged between 2016 and 2017.

	%	%	%	%	%
	Broadleaf	Fern	Palm	Pine	Dead
GU_2016	90.7	6.0	1.3	1.9	7.8
GU_2017 ⁺	91.3	6.0	1.2	1.2	8.2

Tree percentage breakdown for Guanales in 2016 and 2017

* one additional site was surveyed in 2017



Summary

i) Comparisons with all Cusuco survey sites

- Camps affected most heavily by deforestation (Buenos Aires & Cortecito) showed below average numbers of trees per site along with above average GBH values, however the most undisturbed site (Guanales) also showed below average numbers of trees per site

- Camps at higher elevations (Cantiles & Danto) showed the opposite trend with above average numbers of trees per site along with lower average GBH values

- Leaf litter depth was below average for the most disturbed sites in the Park (Buenos Aires & Cortecito), and above average for the most undisturbed site in the Park (Guanales)

- Soil density depth differed from the Cusuco average at only one site (Danto)

- Canopy openness score was lower than average at more undisturbed sites (Base Camp, Cantiles, Guanales) whilst being higher than average at the most disturbed sites (Buenos Aires & Cortecito)

- The average number of stumps and cut saplings across the Park was heavily influenced by the large values of each seen in Cortecito survey sites. When removing Cortecito sites from the analysis only Buenos Aires has a noticeable difference in these two values, with higher than average values for both.

- Sapling count per m² was no different to the Cusuco average at any camp

ii) Comparisons with 2016

- Leaf litter depth was found to have increased at the 4 more undisturbed sites (Base Camp, Cantiles, Danto, Guanales) between 2016 and 2017

- Soil density depth was found to have increased at 3 of the more undisturbed sites (Cantiles, Danto, Guanales) between 2016 and 2017

- Canopy openness was found to only have changed at the 2 most undisturbed sites (Cantiles & Guanales) between 2016 and 2017, with canopy openness reducing in Guanales whilst increasing in Cantiles.

- In Base Camp and Buenos Aires the number of cut saplings was found to have increased between 2016 and 2017, and the percentage of dead trees had risen by approximately a third, suggesting an increased level of human disturbance at these camps.

- Tree count per site, mean GBH, sapling count per m², and number of stumps per site were found to have not changed at any camp between 2016 and 2017.

9.7 Bats- End of 2016 Season Report by Dr. Pamela Thompson and Dr. Kevina Vulinec

Mist-Net Report

The bat team conducted mist-netting and acoustic surveys for bats on 78 mist-net nights, across six sites in Cusuco National Park during the Summer 2016 season (16 June 2016 – 7 August 2016). Members of the bat team included Pamela Thompson, Kevina Vulinec, Aniko Kurali, Juan Carlos Hernandez Garcia, Amanda Bush, and Landito Ayala (a highly trained Honduran guide). Results of the acoustic surveys will be addressed below. In terms of mist-netting, 463 individuals of 39 species were captured. Most of the species were captured infrequently; 31 of the 39 species were captured 10 times or less, and frequently only once (see Table 12). The most common species captured was Centurio senex (132 individuals), a highly unexpected result given the capture data for previous years and other reports of the frequency with which this species is usually observed. This unusually high number of C. senex may be potentially driven by site characteristics, as the majority of these individuals (96.2%) were captured at one site, El Cortecito. It is plausible that the mist-nets were unintentionally placed near roosts of this species at this site, or that there was a resource in the area that this species was exploiting. Juan Carlos Hernandez Garcia noted there was



a fruiting palm tree that the C. senex seemed to be visiting, and Aniko Kurali noted C. senex bats frequently dropping fruits into and around the nets. The second and third most common species were Sturnira Iudovici (61 individuals) and Artibeus jamaicensis (44 individuals), which corresponds to trends that were recorded in 2015.

We captured the greatest number of bats at El Cortecito (198 individuals), but this result was driven by the unusually large number of C. senex bats, as mentioned previously. The site with the second greatest number of bats was Buenos Aires (115 individuals). This site also had the largest number of species captured (Table 13). Buenos Aires is the lowest elevation site, which likely has a strong effect on species composition. The highest elevation site, Cantiles, had the lowest number of species captured (Table 13).

The number of total bats captured in 2016 is greater than in 2015, but if we exclude the number of C. senex bats captured in El Cortecito as an outlier, we are left with 336 bats in 2016, as compared to 326 in 2015. These two years represent a decline in total number of bats caught, which may be due to weather conditions (excessive rain) and/or the sites that were included in the survey years. Santo Tomas was not used in 2015 or 2016, and this disturbed and lower elevation site usually yields higher captures.

It is important to note, however, that 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. Combining mist-netting data with acoustic data is the best way to get an accurate picture of species composition at sites.



Table 12 The numbers of individuals of each species mist-netted at each camp

Species	Base camp	Buenos Aires	Cantiles	El Cortecito	El Danto	Guanales	Total
Artibeus aztecus	0	3	0	0	0	0	3
Artibeus jamaicensis	0	27	0	4	0	13	44
Artibeus literatus	0	4	0	1	1	0	6
Artibeus phaeotis	0	0	0	3	0	1	4
Artibeus toltecus	14	7	1	7	0	2	31
Artibeus watsoni	0	1	0	0	0	0	1
Artibeus sp.	0	2	2	0	0	0	4
Bauerus dubiaquercus	2	2	1	1	0	3	9
Carollia perspicillata	0	1	0	0	0	0	1
Carollia sowelli	7	13	1	3	1	0	25
Carollia sp.	0	0	0	0	0	1	1
Centurio senex	0	1	1	127	3	0	132
Chiroderma salvini	1	1	0	0	0	0	2
Choeroniscus godmani	0	0	0	6	0	0	6
Chrotopterus auritus	0	0	0	1	0	1	2
Desmodus rotundus	0	5	0	0	0	0	5
Diphylla ecaudata	2	0	0	0	0	0	2
Enchisthenes hartii	1	0	1	6	0	0	8
Eptesicus brasillensis	1	1	0	0	1	0	3
Glossophaga commissarisi	1	0	0	1	0	0	2
Glossophaga soricina	1	8	0	13	2	1	25
Glossophaga sp.	1	0	0	1	0	1	3
Hylonycteris underwoodi	0	0	0	0	1	0	1
Lonchophylla mordax	0	0	0	0	1	0	1
Micronycteris microtis	0	0	0	0	1	0	1
Myotis albescens	0	0	0	1	1	0	2
Myotis keaysi	7	3	7	5	6	0	28
Myotis nigracans	0	1	0	0	0	0	1
Myotis sp.	0	0	1	0	0	0	1
Phyllostomus hastatus	1	1	0	0	0	0	2
Platyrrhinus helleri	0	2	0	0	0	0	2
Pteronotus davyi	0	2	0	1	0	0	3
Pteronotus parnelli	2	0	0	1	1	0	4
Sturnira lilium	1	21	0	0	0	0	22
Sturnira ludovici	30	0	2	15	13	1	61
Trachops cirrhosus	1	0	0	1	2	0	4
Vampyressa thyone	0	6	0	0	0	0	6
Vampyrodes caraccioli	0	2	0	0	0	0	2
Unknown sp.	2	1	0	0	0	0	3
Total	75	115	17	198	34	24	463

Table 13 The number of species mist-netted at each camp

	Species richness
Base camp	17
Buenos Aires	23
Cantiles	9
El Cortecito	19
El Danto	13
Guanales	9



We calculated the Shannon diversity index for each site (the Shannon index is defined as

$$H' = -\sum_{i=1}^R p_i \ln p_i$$

where pi is the proportional abundance of species i). We also calculated Shannon's Equitability (EH), which is defined as the Shannon index (H) divided by the natural log of the number of species at the site. Equitability assumes a value between 0 and 1 with 1 being complete evenness. We did these analyses for all camps, and uncorrected counts at El Cortecito (including all *C. senex* captures) as well as a "corrected" El Cortecito, which included a count of 3 for *Centurio senex*, as this was the largest number caught at another site (El Danto). Results are presented in Figure 23. The "corrected" El Cortecito site has the most even Shannon's equitability score (0.853).



Figure 23

Recommendations

It is possible that there have been name changes to certain bat species in the recent literature. This should be investigated and keys (and past data) updated if applicable. In particular, the names of *Sturnira ludovici* and *Sturnira lilium* may have changed to *S. hondurensis* and *S. parvidens*, respectively (Velazco and Patterson 2014). Also the genus of the small *Artibeus* species (*A. phaeotis, A. watsoni, A. toltecus,* and *A. aztecus*) may have changed to *Dermanura* (Solari et al. 2009). It is actually quite difficult to distinguish between the small *Artibeus* sp., and it is possible there is a level of hybridization that may be occurring. It would be very interesting to take genetic samples to help resolve these species (or possibly population level) differences. During a discussion with Fiona Reid, she stated that she is waiting to use the new names until they have been confirmed.

There is debate among the keys we have available, whether *Lonchophylla mordax* occurs in this region of Honduras or not. It's in one key for Costa Rica, but not in the Fiona Reid book or the new guide for bats of Nicaragua. *Lonchophylla concava* may be a synonym, and is in both the Reid and Nicaragua key. In addition,



our key does not include the Mormoopidae family, and it is unclear whether there are two or more species that occur in this region of Honduras.

Given the large number of *Centurio senex* we captured this year, it seems clear we need to focus on recording the nature of the habitat surrounding the mist-netting areas, including any conspicuous resources (like fruiting trees) which are observed. A micro-habitat assessment around the mist-nets might also prove easier to analyze than merging the habitat data with capture data, and may improve explanatory power for the captures.

Acoustic Monitoring

We monitored 6 sites around Cusuco National Park using acoustic recorders. These include Base Camp and the satellite camps. We tried to get a sample of the different elevations in the park (range from 60m to 2242m). At this time, we have examined concurrent data for mist net captures and acoustic recordings (analyzed so far) for 17 nights out of 50 nights of concurrent recording and mist_netting in 2016. We set up Pettersson's 500x units during the same time frame as the mist netting, close to the net sites, but far enough away (>50m) that the calls of bats caught in the nets would not be recorded. We set the recorders to record at 1830-000 hrs, and with the following parameters: sampling frequency = 500 kHz, pretrigger = OFF, Length of recording = 5s, Trigger sensitivity = VERY HIGH, High-pass filter = ON. Input gain was set to, the trigger level = 36, and interval = 5s. Bat calls were identified by visual inspection of sonographs using Sonobat v3.1.6. We compared the calls recorded by the Petterssons to voucher calls that were recorded from bats released by hand or from the published literature.

We recorded bats concurrently with mist_netting for 50 nights, and of these 16 had no bat calls. Of the 17 nights where identifications were completed, we recorded 294 calls (average per night 32.67). During those same nights, we recorded 66 captures in nets (average per night 7.33). Six days had no bat recordings, either from equipment malfunctioning or from a lack of bats. Over 3 of these days no bats were captured in mist-nets as well. We recorded calls from 17 species and caught 20 species in the nets. Five species were both recorded and caught; 32 species were only recorded or caught. This result indicates that there is only 16% overlap between bat species that are both recorded and captured in nets.



Figure 24 - The average number of calls recorded vs. the average number of bats captured in nets at each camp for the 17 nights monitored and analyzed. BA = Buenos Aires (n = 4), BC = Base Camp (n = 5), CA = Cantiles (n = 3), CO = Cortecito (n = 2), DA = El Danto

Here we present more detailed data for two camps for which we had more than two nights analyzed through call identifications (5 nights at Base Camp and 4 nights at Buenos Aires).

BASE CAMP

We recorded 72 total calls, and captured 15 total bats in nets. Of these bats, only 7% of species occurred in both our mist net captures and our recordings (Figure 25).

BUENOS AIRES

We recorded 81 total bat calls and captured 37 total bats in nets. There was a 4% overlap between recorded bats and those captured (Figure 26).





Figure 25 - Top graph: Percentage of bat calls by species over 5 days of recording at Base Camp. Bottom graph: Percentage of bats by species captured in mist nets over the same 5 days.





Figure 26 - Top graph: Percentage of bat calls by species over 4 days of recording at Buenos Aires. Bottom graph: Percentage of bats by species captured in mist nets over the same 6 days.

CONCLUSIONS

Monitoring programs using mist-netting alone miss a significant proportion of local species. Insectivorous bats in particular are good at avoiding nets and often fly higher than the nets. Nevertheless, recording alone often misses frugivorous and other "whispering" bats because the amplitude of the call is low. A combination of methods, along with a good call library for reference, is critical for accurate studies of bat biodiversity (MacSwiney et al. 2008).

Note: We (the Vulinec lab at Delaware State University) have finished sorting calls for all 50 nights of concurrent recording with mist net captures. These bat calls are currently being identified and will be sent to Opwall later. We expect to have a publication from these data.



9.8 Odonata by Dr Melijn Jocque

Odonata were collected opportunistically in Cusuco National Park (CNP), Honduras. Honduras. CNP is situated in north-eastern Honduras, within the Merendon Mountain range. The core zone of the park consists of lower montane tropical rain forest (a mix of primary and secondary), with patches of primary cloud forest and upper montane rain forest. Several large rivers drain the water from the mountain, including Rio Cusuco and Rio Cantilles. A large ridge roughly divides the water catchments in two groups with different microclimatic conditions. The west side or the ocean orientated part of CNP receives more rain and overall is more humid, more difficult to access due to the lack of roads and has more undisturbed mid elevation forest habitat. The eastern side is drier and received considerable logging in the 1950's. Only at low elevation in the buffer zone, some small artificial standing water bodies are present. Odonata were collected with a hand net, photographed live for documentation of the colours and fixated in 70% ethanol. In the lab, specimens were prepared in acetone for investigation of the posterior appendages and male genital structures.

At the moment of writing 27 species are identified with certainty. Some of the more common but cryptic forest damselflies are in identification, mostly species in the *genus Palaemnema, Paraphlebia* and *Argia.* An updated checklist of dragonfly species from Cusuco is in preparation.

	Family	genus	species
1	Aeshnidae	Aeshna	williamsoniana
2	Aeshnidae	Oplonaeschna	armata
3	Aeshnidae	Rhionaeshna	cornigera
4	Gomphidae	Epigomphus	subobtusus
5	Libellulidae	Brechmorhoga	pertinax pertinax
6	Libellulidae	Brechmorhoga	rapax
7	Libellulidae	Libellula	herculea
8	Amphipterygidae	Amphipteryx	meridionalis
9	Calopterygidae	Hetaerina	capitalis
10	Calopterygidae	Hetaerina	cruentata
11	Calopterygidae	Hetaerina	majuscula
12	Coenagrionidae	Acanthagrion	trilobatum
13	Coenagrionidae	Argia	chelata
14	Coenagrionidae	Argia	cuprea
15	Coenagrionidae	Argia	eliptica
16	Lestidae	Archilestes	grandis
17	Megapodagrionidae	Heteragrion	alienum



18	Megapodagrionidae	Heteragrion	eboratum
19	Megapodagrionidae	Paraphlebia	n. sp. 1
20	Megapodagrionidae	Philogenia	strigilis
21	Platystictidae	Palaemnema	angelina
22	Platystictidae	Palaemnema	paulina
23	Perilestidae	Perissolestes	magdalenae
24	Polythoridae	Cora	marina
25	Protoneuridae	Protoneura	peramans
26	Pseudostigmatidae	Megaloprepus	coerulatus
27	Pseudostigmatidae	Mecistogaster	modesta



9.9 Longhorns and Click Beetles by Dr Merlijn Jocque

Opportunistically and on light traps longhorns and click beetles were collected in CNP. The collection of these groups of beetles is to gain insight in the diversity of beetles by tackling the larger and more charismatic groups first, and over time make them part of the monitoring protocol. The aim is to have an identification guide for Cusuco NP, that can be used for identifications at the light trap. In this way only smaller and cryptic species that are more difficult to identify in the field, will have to be collected in the future. Animals were preserved in 70% and some in 98% ethanol. Animals are pinned and identification is ongoing. A preliminary list of longhorn identifications is provided here.

Subfamily	Tribus	Genus	Species
Prioninae	Prionini	Derobrachus	sp1
Prioninae	Prionini	Derobrachus	sp2
Prioninae	Prionini	Derobrachus	sp3
Prioninae	Macrotomini	Mallodon	sp.
Prioninae	Macrotomini	Aplagiognathus	SP.
Prioninae	Mallaspini	Scatopyrodes	tenuicornis
Cerambycinae	Callichromatini	Callichroma	cyanomellas
Cerambycinae	Trachyderini	Crioprosopus	nieti
Cerambycinae	Bothriospilini	Chlorida	cincta
Cerambycinae	Eburiini	Eburia	Sp
Lamiinae	Onciderini	Bacuris	sexvittatus
Lamiinae	Parmenini	Echthistatus	kawksi
Lamiinae	Monichamini	Monochamus	clamator
Lamiinae	Hemilophini	Oedudes	spectabilis
Lamiinae	Monochamini	Plagiohammus	inermis
Parandrinae	Parandrini	Parandra (Parandra)	glabra
Parandrinae	Parandrini	Parandra (Parandra)	SP.
Prioninae	Macrodontiini	Macrodontia	castroi
Disteniinae	Disteniini	Novantinoe	agriloides
Lamiinae	Hemilophini	Cirrhicera	
Disteniinae	Disteniini	Disteniazteca	pilati
Disteniinae	Disteniini	Elytrimitatrix	guatemalana
Lamiinae	Monochamini	Taeniotes	scalatus


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





















