COSTA RICA FOREST RESEARCH PROGRAMME (CRF)
Osa Peninsula, Costa Rica

CRF Phase 141 Science Report
01 January – 23 March 2014

Nathan J. Roberts, Delyth Williams, Anna Morris, Jessica Dangerfield,
John Scott, Hal Starkie, Sophie Burns, Kirsty Hunter, Alex Caldwell
### Staff Members

<table>
<thead>
<tr>
<th>Name</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nathan Roberts (NJR)</td>
<td>Principal Investigator (PI)</td>
</tr>
<tr>
<td>Chris Martin (CM)</td>
<td>Project Coordinator (PC)</td>
</tr>
<tr>
<td>Anna Douglas-Morris (AM)</td>
<td>Assistant Research Officer (ARO)</td>
</tr>
<tr>
<td>Delyth Williams (DW)</td>
<td>Assistant Research Officer (ARO)</td>
</tr>
<tr>
<td>Jessica Dangerfield (JD)</td>
<td>Assistant Research Officer (ARO)</td>
</tr>
<tr>
<td>John Scott (JS)</td>
<td>Assistant Research Officer (ARO)</td>
</tr>
<tr>
<td>Hal Starkie (HS)</td>
<td>Conservation Apprentice (CA)</td>
</tr>
<tr>
<td>Sophie Burns (SB)</td>
<td>Conservation Apprentice (CA)</td>
</tr>
<tr>
<td>Jenny Collins (JC)</td>
<td>Field Communications Officer (FCO)</td>
</tr>
</tbody>
</table>

Table of Contents

1. Introduction .................................................................................................................. 5
   1.1 Natural History of Costa Rica and Wildlife Conservation ........................................ 5
   1.2 Osa Peninsula ........................................................................................................... 5
   1.3 Aims and Objectives of Frontier CRF ..................................................................... 6

2. Training .......................................................................................................................... 7
   2.1 Briefing Sessions ...................................................................................................... 7
   2.2 Science Lectures ...................................................................................................... 7
   2.3 Field Training ......................................................................................................... 8
   2.4 BTECs .................................................................................................................... 9

3. Research Work Programme ....................................................................................... 10
   3.1 Survey Areas .......................................................................................................... 10
   3.2 Local Population Density of The Four Primate Species Coexisting in Costa Rica .... 11
      3.2.1 Introduction .................................................................................................... 11
      3.2.2 Methodology ................................................................................................. 11
      3.2.3 Results .......................................................................................................... 13
      3.2.4 Discussion ..................................................................................................... 14
   3.3 Latrine Site Selection in the Neotropical Otter and Characterising the Occurrence of
      Mucous in Spraints ................................................................................................. 14
      3.3.1 Introduction .................................................................................................... 14
      3.3.2 Methodology ................................................................................................. 15
      3.3.3 Results .......................................................................................................... 16
      3.3.4 Discussion ..................................................................................................... 17
   3.4 Using Camera Traps to Estimate Species Abundance of Otters ......................... 19
      3.4.1 Introduction .................................................................................................... 19
      3.4.2 Methodology ................................................................................................. 21
      3.4.3 Results .......................................................................................................... 21
      3.4.4 Discussion ..................................................................................................... 21
   3.5 Avian Diversity within Primary, Secondary and Riparian Forest ....................... 22
      3.5.1 Introduction .................................................................................................... 22
      3.5.2 Methodology ................................................................................................. 22
      3.5.3 Results .......................................................................................................... 23
      3.5.4 Discussion ..................................................................................................... 23
   3.6 CRF and the Osa Conservation Sea Turtle Conservation Programme ................ 23
3.6.1 Introduction ................................................................................. 23
3.6.2 Methodology ........................................................................... 24
3.6.3 Results ...................................................................................... 25
3.6.4 Discussion ................................................................................ 25
3.7 Diversity and Vertical Stratification of Butterflies within Nymphalidae .......... 26
3.7.1 Introduction .............................................................................. 26
3.7.2 Methodology ............................................................................ 27
3.7.3 Results ...................................................................................... 28
3.7.4 Discussion ................................................................................ 29
3.8 The Three-Dimensional Space Use of Mantled Howler Monkeys (Alouatta palliata) 31
3.8.1 Introduction .............................................................................. 31
3.8.2 Methodology ............................................................................ 32
3.8.3 Results ...................................................................................... 33
3.8.4 Discussion ................................................................................ 34
4. Additional Projects ......................................................................... 35
4.1 Testing the Influence of Hiking Trails on Bird Diversity and Abundance within Neotropical Primary and Secondary Lowland Forest ........................................... 35
4.2 Measuring Disturbance Through Novel Techniques: Soundscape Ecology within a Heterogeneous Landscape on the Osa Peninsula, Costa Rica. .............................. 35
4.3 Systematically Comparing Methods of Studying Butterfly Diversity: Canopy Trapping Versus Netting .................................................................................. 36
5. Proposed Work Programme for Next Phase .............................................. 38
6. References ....................................................................................... 39
7. Appendices ....................................................................................... 49

Appendix 1. Capture Frequency of Butterfly Species Within the Two Sample Areas ....... 49
1. Introduction

1.1 Natural History of Costa Rica and Wildlife Conservation

Despite occupying only around 50,000 km$^2$ of the Earth’s surface, the Central American country of Costa Rica is home to around 5 % of all species on Earth (Sanchez-Azofeifa et al., 2002). The extraordinary biodiversity observed here has in part been attributed to the climatic conditions present in this area. Temperature fluctuates little throughout the year, with an average annual temperature of 27 °C. Rainfall however, is much more variable with distinct wet and dry seasons that are particularly prominent in the southern Pacific lowlands which receive an average annual rainfall of 7,300 mm (Baker, 2012). The dry season begins around November/December and continues through to April/May after which the rainy season commences.

Costa Rica has become a world leader in conservation and ecotourism (Fagan et al., 2013). With over 190 reserves and national parks, 11 % of the country’s total area is occupied with these protected natural areas, all created since the 1960s. Prior to this, human activities such as logging and hunting seriously threatened biodiversity in this region with over half of the country’s forests having been cut pre-1960s (Henderson, 2002). Habitat fragmentation and loss due to expanding human populations which encroach into natural areas also threaten wildlife populations by reducing the size of available habitat and bringing them into closer contact with humans. Though some of these issues have been controlled through the implementation of several reforestation programmes and legislation (Sánchez-Azofeifa et al., 2001; Henderson, 2002) others continue to threaten Costa Rican ecosystems today.

Costa Rican law currently protects 166 species from hunting, capture and sale; but illegal hunting still occurs, including in protected areas (Baker, 2012). Poaching of turtles for their fatty calipee and the collection of turtle eggs has severely depleted populations of endangered Black turtles (Chelonia mydas) and vulnerable Olive ridley turtles (Lepidochelys olivacea) that use Costa Rica’s coastlines as nesting sites. Similarly, the hunting of Costa Rica’s wild cat species, peccaries and tapirs for their meat, skins and other animal parts has significantly reduced wild populations and ongoing human-cat conflict may present an additional threat to Costa Rican felid species. Furthermore the projected impacts of climate change in combination with these factors are likely to have significant adverse effects on Costa Rican biodiversity.

1.2 Osa Peninsula

The Osa Peninsula is located within the Southern Pacific lowlands, one of three endemic zones in the country (Henderson, 2002). On the peninsula lies the world-renowned wildlife refuge, Corcovado National Park (CNP) where it is estimated 2.5 % of all species living on Earth can be found. Species that are not only endemic to the country of Costa Rica but also species and subspecies endemic to the region such as the Cherrie’s Tanager (Ramphocelus costaricensis) and the Red-backed squirrel monkey (Saimiri oerstedii) are found on the Osa Peninsula. These endemic species are of conservation significance because local extirpation within their limited range would result also in their global extinction.
The Osa Peninsula has a population of around 12,000 people, mainly in small scattered settlements. The major sources of income in the region are agriculture (rice, bananas, beans and corn), livestock (cattle), gold mining, logging and more recently the expanding eco-tourism industry (Carrillo et al., 2000).

Frontier’s Costa Rica Forest Research Programme (CRF) began in July 2009 in collaboration with the local non-governmental organisation Osa Conservation at the Piro site (N 08°23.826, W 083°20.564) in the south-east of the Osa Peninsula. There are six core study areas within CRF in terms of focal species and groups: primates, birds, butterflies, Neotropical otters, sea turtles and wild cats. The Neotropical otter is one of the Frontier Costa Rica Forest Research Programme’s key study species owing to its status by the IUCN as a Data Deficient mammal. The shorelines adjacent to the property are used by Black turtles and Olive ridley turtles as nesting beaches throughout the year. Beach patrols are conducted in collaboration with Osa Conservation as a part of a long-term monitoring and protection programme. Additionally all four Costa Rican primate species coexist on the Osa property study area, as do numerous bird, butterfly and amphibian species.

1.3 Aims and Objectives of Frontier CRF

Under the umbrella of the research programme, the specific aims and objectives are:

i) To calculate the density and abundance of the four primate species within the Osa Conservation property at Piro and its boundaries.

ii) To record the Neotropical otter’s local distribution, assess the environmental characteristics around latrine sites and investigate the role of scat deposition in intra-specific communication.

iii) To systematically test the reliability of using camera trap images to estimate the abundance of Neotropical otters by individual identification and capture-recapture analysis.

iv) To investigate the occurrence and scale of human-felid conflict within the Osa Conservation Area (ACOSA) and local attitudes towards conservation.

v) To measure the avian diversity within primary and secondary forest and riparian habitats within the Osa Conservation property.

vi) To monitor the activity and health of nesting marine turtles on Piro and Pejeperro beaches and to support the management of the hatchery under the Osa Conservation Sea Turtle Conservation Programme.

vii) To assess the diversity of butterflies of the Nymphalidae family within an area of the Osa Conservation property and investigate inter-species vertical stratification.

viii) To investigate the behaviour of mantled howler monkeys and their three-dimensional space use within the Osa Conservation property at Piro and its boundaries.
2. Training

Volunteer Research Assistants (RAs) and newly appointed staff receive a number of briefing sessions on arrival (Table 1), followed by regular science lectures (Table 2) and field training (Table 3) throughout their deployments and contracts. The Costa Rica Forest Research Programme also supported candidates completing the BTEC Advanced Certificates and Advanced Diplomas in Tropical Habitat Conservation (Table 4).

2.1 Briefing Sessions

All arrivals to CRF are introduced to the aims of the research programme, the methodologies followed and the conservation significance of the individual studies and are also provided with an update as to the achievements of CRF. This information is delivered via the Introduction to the Frontier Costa Rica Forest Research Programme presentation. Additionally all volunteers and staff are given a full health and safety and medical briefing and are tested before participating in field activities, those volunteers undertaking a BTEC and/or CoPE qualification are given an introductory brief before they begin the assessments.

<table>
<thead>
<tr>
<th>Briefing Session</th>
<th>Presenter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Introduction to the Frontier Costa Rica Forest Research Programme</td>
<td>NJR</td>
</tr>
<tr>
<td>Health and Safety Brief and Test</td>
<td>CM</td>
</tr>
<tr>
<td>Medical Brief and Test</td>
<td>CM</td>
</tr>
<tr>
<td>Introduction to the BTEC and CoPE Qualifications</td>
<td>NJR</td>
</tr>
<tr>
<td>Introduction to Media and Communications</td>
<td>JC</td>
</tr>
</tbody>
</table>

2.2 Science Lectures

A broad programme of science lectures is offered at CRF providing information and training in various aspects of research. Lectures are typically presented utilizing PowerPoint giving an opportunity for greater understanding of the ecology and identification of focal species, methods of data analysis used by CRF and the considerations when planning research projects.

Lectures are scheduled with the following objectives:

- Allow every volunteer and staff person to attend each presentation at least once during deployment, regardless of length stay.
- Meet the time requirements for BTEC assessments,
- Avoid conflict with other activities, maximizing attendance.
- Workshops provide detailed training on specific software and applications utilized in conservation purposes.

Attendance of lectures is non-compulsory.
Table 2. Science lectures delivered during Phase CRF141

<table>
<thead>
<tr>
<th>Science Lecture</th>
<th>Presenter</th>
<th>Date(s) Delivered</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mariposas de Costa Rica</td>
<td>JD</td>
<td>10 January 2014; 08 February 2014; 12 February 2014; 21 February 2014; 13 March 2014</td>
</tr>
<tr>
<td>Data Analysis: Studying Biodiversity with EstimateS</td>
<td>NJR</td>
<td>20 January 2014; 29 January 2014</td>
</tr>
<tr>
<td>Common Bird Species of the Osa Peninsula</td>
<td>AM</td>
<td>22 January 2014</td>
</tr>
<tr>
<td>Data Analysis: Studying Biodiversity with DIVERSITY</td>
<td>NJR</td>
<td>29 January 2014</td>
</tr>
<tr>
<td>Data Analysis: Primate Density and DISTANCE</td>
<td>DW</td>
<td>05 February 2014; 28 February 2014</td>
</tr>
<tr>
<td>Survey Planning: BTEC E07 Preparation</td>
<td>NJR</td>
<td>07 February 2014; 22 March 2014</td>
</tr>
<tr>
<td>Introduction to Primate Behaviour</td>
<td>DW</td>
<td>17 February 2014</td>
</tr>
<tr>
<td>Surveying and Monitoring: BTEC E09 Preparation</td>
<td>NJR</td>
<td>20 February 2014</td>
</tr>
<tr>
<td>Studying Abundance of the Neotropical Otter: Ex situ Research with CRF</td>
<td>NJR</td>
<td>21 February 2014; 06 March 2014; 21 March 2014</td>
</tr>
<tr>
<td>Spatial Analyses: The Use of GIS at CRF</td>
<td>NJR</td>
<td>24 February 2014; 04 March 2014</td>
</tr>
<tr>
<td>Anurans of the Osa Peninsula</td>
<td>JS</td>
<td>25 February 2014</td>
</tr>
<tr>
<td>Soundscape Ecology</td>
<td>HS</td>
<td>08 March 2014</td>
</tr>
<tr>
<td>Bird Identification</td>
<td>JS</td>
<td>10 March 2014; 19 March 2014</td>
</tr>
</tbody>
</table>

2.3 Field Training

Volunteers and newly appointed staff receive field training supporting research objectives. Training is hands-on and provides an opportunity for participants to become familiar with and use the field equipment.

- Specific training sessions to prepare volunteers and staff for accurate data collection
- Identification of flora and fauna
- Survey method training
- Skills and tools used for research
  - Turtle Tagging Training
  - GPS functionality
  - Camera trap
  - Trapping (butterfly nets and traps)
  - Proper maintenance of equipment
Table 3. Field training provided during Phase CRF141

<table>
<thead>
<tr>
<th>Field Training</th>
<th>Provider</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reptile and Amphibian Identification</td>
<td>JD, HS, JC, AM</td>
</tr>
<tr>
<td>Camera Trap Workshop</td>
<td>NJR, DW</td>
</tr>
<tr>
<td>Tree Identification</td>
<td>CM</td>
</tr>
<tr>
<td>Turtle Tagging Training</td>
<td>NJR</td>
</tr>
<tr>
<td>Turtle Patrol Data Collection</td>
<td>NJR, AM, DW, JD, SB</td>
</tr>
<tr>
<td>Primate Surveys by Distance-sampling</td>
<td>NJR, AM, DW, JD, JS, SB, HS</td>
</tr>
<tr>
<td>Bird Surveys by Point Surveys</td>
<td>AM, JS</td>
</tr>
<tr>
<td>Butterfly Canopy Trapping</td>
<td>NJR, JD, HS, SB</td>
</tr>
<tr>
<td>Butterfly Netting</td>
<td>JD, HS, SB</td>
</tr>
<tr>
<td>Field Equipment: GPS, Laser Rangefinder and Clinometer</td>
<td>NJR, AM, DW, JD, JS, HS, SB</td>
</tr>
<tr>
<td>Identification of Neotropical Otters and Their Signs, and Recording River Attributes</td>
<td>NJR, DW</td>
</tr>
<tr>
<td>Studying Primate Behaviour</td>
<td>DW</td>
</tr>
</tbody>
</table>

2.4 BTECs

Frontier offers volunteer Research Assistants an opportunity to gain internationally recognised qualifications based around teamwork, survey techniques, environmental conservation and effective communication of results. The BTEC in Tropical Habitat Conservation may be studied as an Advanced Certificate (four week program) or Advanced Diploma (ten week program).

Table 4. BTECs Undertaken in Tropical Habitat Conservation During Phase CRF141

<table>
<thead>
<tr>
<th>Student Name</th>
<th>Project Title (and qualification)</th>
<th>Mentor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jordan Farrant</td>
<td>Effect of Canopy Height and Canopy Cover on Scat Locations of the Neotropical Otter (Advanced Certificate)</td>
<td>DW</td>
</tr>
<tr>
<td>Alex Gordon</td>
<td>The Relationship Between Turtle Track Width and Nesting Success (Advanced Certificate)</td>
<td>NJR</td>
</tr>
<tr>
<td>Jamie Thomas</td>
<td>Inca Project: Understanding Public Attitudes Regarding Wild Cats of the Osa Peninsula and Finding Solutions to Human-Felid Conflict (Advanced Diploma)</td>
<td>NJR</td>
</tr>
<tr>
<td>Laurence Beaumont</td>
<td>Understanding the Important Characteristics of Scat Locations of Neotropical Otter That Have Mucous (Advanced Certificate)</td>
<td>DW</td>
</tr>
<tr>
<td>Danielle Cerrone</td>
<td>Measuring the Effects of Disturbance in Primary and Secondary Forest on the Abundance of Selected Bird Species (Advanced Diploma)</td>
<td>JS</td>
</tr>
<tr>
<td>Jennifer Sumners</td>
<td>Do Sea Turtles (Family: Cheloniidae) Come Ashore To Nest At A Particular Moon Phase? (Advanced Diploma)</td>
<td>NJR</td>
</tr>
<tr>
<td>Nathan Norris</td>
<td>[Title TBC] (Advanced Diploma)</td>
<td>DW</td>
</tr>
<tr>
<td>Isabella Eddington</td>
<td>Movement Paths and Space Use of Mantled Howler Monkeys (Advanced Certificate)</td>
<td>DW</td>
</tr>
<tr>
<td>Laura Sheffield</td>
<td>[Title TBC] (Advanced Diploma)</td>
<td>[TBC]</td>
</tr>
</tbody>
</table>
3. Research Work Programme

3.1 Survey Areas

Field research is conducted within an 11.83 km² privately-owned property in Piro, Osa Peninsula, Costa Rica and its boundaries (8°23'-8°26'N, 83°18'-83°25'W) (Figure 1). Largely within the Golfo Dulce Forest Reserve and Osa Conservation Area (ACOSA) Wildlife Refuge, this property is owned by NGO Osa Conservation (OC) and is a heterogeneous landscape composed of lowland moist primary, secondary and coastal forest. Dominant tree species include; *Ficus insipida*, *Ceiba pentandra*, *Attalea butracea*, *Carapa guianensis*, *Castilla tunu*, *Spondias mombin*, *Hyeronima alchorneoides*, *Chimarrhis latifolia*, *Fruita dorada*, *Caryocar costaricense*, *Ocotea insularis*, *Pouteria torta*, and *Inga allenii*. The property also borders dense forest and pastoral properties to the southeast, encompassing pochote (*Bombacopsis quinata*) and teak (*Tectona grandis*) plantations in the north and north east, each occupying 0.4 km². There are two biological research stations within the study area: Cerro Osa station in the north-west and Piro station in the southern region of the property. Mean annual rainfall and temperature for the area is 5,000-6,000 mm and 26-28 °C; the dry season extends from the end of December until March.

![Figure 1](image_url)

**Figure 1.** The Frontier Costa Rica Forest Research Programme (CRF) study area with surveyed beaches, rivers and trails marked. LS: Laguna Silvestre; NT: New Trail; Rd: Road; TP: Terciopelo; PT: Piro; BT: Beach Trail; LH: Los Higuerones; AT: Ajo; CA: Chiricano Alegre; OT: Ocelote; CO: Cerro Osa; NB: Northern Border; CT: Chicle.
Within the property, there is a forest trail network covering most of the southern and north-eastern portions. Besides Cerro Osa which is an old logging road and the north-south segment of Northern Border and Laguna Silvestre, which are both 5 m-wide cleared tracks, the trails are narrow and machete-cut. New Trail is the most recently cut trail and is approximately one year old, while all other trails are more than five years old.

The property is bordered to the south by the Pacific Ocean and the immediate coastline is separated into two beaches – Playa Piro (2 km) and Playa Pejeperro (4.5 km). The primary rivers intersecting the property are the Rio Piro and the adjoining Quebrada Coyunda; the mouth of the former is at approximately the centre of Playa Piro.

3.2 Local Population Density of The Four Primate Species Coexisting in Costa Rica

3.2.1 Introduction

There are four species of primate found in Costa Rica: the Central American squirrel monkey (*Saimiri oerstedii*), Mantled howler monkey (*Alouatta palliata*), Geoffroy’s spider monkey (*Ateles geoffroyi*) and White-faced capuchin (*Cebus capuchinus*). All four species are present within the study site in Piro. *A. geoffroyi* is listed as endangered and *S. oerstedii* as vulnerable (IUCN, 2013). Population declines exceeding 50% over the past 45 years have been reported for *A. geoffroyi*, principally driven by stresses induced from habitat loss (Cuarón et al., 2008a). *S. oerstedii* has a limited distribution, restricted to Panama and Costa Rica meaning populations exploited for the pet trade and experiencing habitat loss and degradation are already vulnerable to stochastic events. Land conversion for agriculture and development, clear cutting, selective logging and the pet trade have implications for all four Costa Rican primate species (Cropp and Boinski, 2000).

The primate guild in Costa Rica is vital for forest structure integrity. All four species perform important roles as seed dispersers (Julliot, 1997; Garber et al., 2006) and the Osa Peninsula is the only part of Costa Rica where all four species occur in concert (Carrillo, 2000).

Frontier CRF has been surveying the presence and absence of all four primate species in collaboration with Osa Conservation since March 2010. The overall aim of this research is to provide an insight into the habitat use by primates in the area to better aid management and policy decisions at a local level. The principal and foremost objective of this project is to produce estimates of the population density of each of the four species.

3.2.2 Methodology

Data is collected following a standardised line-transect sampling protocol (Buckland et al., 2010; Thomas et al., 2010). The trails are sampled equally across times of day. Surveys are taken at 0600, 0800, and 1400 hrs to cover peak primate activity, increasing the detection probability. The transects for the most part, combinations of forest trails. Transects sampled (Table 5) are walked by two observers at a constant speed of 1.5 – 2 kmhr⁻¹ as recommended by Karanth and Nichols (2002). No transect is surveyed more than once on the same day and
sampling is only conducted in fair weather due to the reduction in detection probability in adverse weather conditions.

Table 5. Transects Sampled and Descriptions

<table>
<thead>
<tr>
<th>Transect Name (code)</th>
<th>Transect Length (km)</th>
<th>Habitat Description and Trail Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ajo – Ocelote (AT – OT)</td>
<td>3.1</td>
<td>Trail through secondary and mature, primary forest</td>
</tr>
<tr>
<td>Terciopelo – Piro (TP – PT)</td>
<td>2.3</td>
<td>Trail through riparian, primary and secondary forest</td>
</tr>
<tr>
<td>Cerro Osa (CO)</td>
<td>1.9</td>
<td>Old logging road and forest trail through secondary and mature, primary forest, abandoned pochote (Bombacopsis quinata) plantation and across a branch of the Quebrada Coyunda River</td>
</tr>
<tr>
<td>Laguna Silvestre (LS)</td>
<td>2.6</td>
<td>Access road through secondary and primary forest</td>
</tr>
<tr>
<td>Beach Trail – Road (BT – RD)</td>
<td>1.6</td>
<td>Trail through secondary forest and main access road dissecting secondary forest</td>
</tr>
<tr>
<td>Chiricano Alegre – Cerro Osa – Los Higuerones (CA – CO – LH)</td>
<td>1.9</td>
<td>Trail through secondary and mature, primary forest</td>
</tr>
<tr>
<td>Chicle (CT)</td>
<td>1.8</td>
<td>Forest ridge trail through primary forest and across Quebrada Coyunda River</td>
</tr>
<tr>
<td>Northern Border (NB)</td>
<td>2.5</td>
<td>Old logging road through a pochote (Bombacopsis quinata) plantation and trail through mature, primary forest</td>
</tr>
<tr>
<td>New Trail-Road (NT – RD)</td>
<td>3.8</td>
<td>Trail through secondary forest and main access road dissecting secondary forest</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>2.4 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21.6</td>
<td></td>
</tr>
</tbody>
</table>

All visual encounters of primates are recorded, logging the spatial location with GPS, noting the number of individuals within the observed group and perpendicular distance from the transect line to the geometric centre of the group at first sighting. All individuals visible at the same time and exhibiting the same general behaviour (e.g., resting, moving or foraging) were considered to be of the same group (Chapman et al., 1995). Agreement on group size was made between the observers where the group could be counted together; otherwise the observers split their search effort into non-overlapping areas (e.g., left and right of the trail) and made independent counts which were then summed. Where possible group composition was also recorded as secondary data. Vocal detections are not included.

The perpendicular distance to the centre of the group was calculated using the radial distance $r$ and the sighting angle $\theta$, which are related to the perpendicular distance $x$ by the formula $x = rsin\theta$. The sighting angle was measured with a compass and the radial distance calculated using the formula $r = dcos\theta$, where $r$ is radial distance, $d$ is the direct distance measured with a laser rangefinder between the observer and the geometric centre of the group in the tree, and $\theta$ is the slope angle between the observer and the geometric centre of the group in the tree measured
with a clinometer. Alternatively, where field conditions permitted, a tape measure was used to measure the perpendicular distance. After every recorded observation, the observers immediately continued along the transect noting any direction of animal movement to eliminate the chance of recording the same individuals more than once per survey.

Data were analysed using DISTANCE ver. 6.0 (Thomas et al., 2010) under the following assumptions: i) all groups at zero perpendicular distance to the line were detected with certainty; ii) groups were detected at their initial locations and were not counted twice, and; iii) accurate measurements of distances were made from the line to the geometric centre of the group (Thomas et al., 2010). As trails were concentrated around the southern edge of the property, our survey effort was not evenly distributed within the study area. The study site was therefore divided into two substrata to avoid bias: an area of 1.67 km² in the southern region of the property (encompassing trails Terciopelo-Piro, Beach Trail-Road, Ajo-Ocelote, Chiricano Alegre-Cerro Osa-Los Higueros and the lower 414 m of Cerro Osa) and an area of 10.16 km² in the northern and western sections of the property (encompassing trails Chicle, Northern Border, Laguna Silvestre, New Trail-Road and the remainder 1052 meters of Cerro Osa). These two sections were considered as two substrata layers in DISTANCE (Buckland et al., 2010).

The conventional distance sampling (CDS) function was employed to permit stratification of the two sections of the property. The data was truncated by 10% (Buckland et al., 2001). Cluster size regression analysis was selected for each model except in circumstances where warnings arose. In these cases, the mean of observed cluster sizes was chosen to estimate cluster size (Link et al. 2010). As there were several replicates of each trail, the number of samples was added as the multiplier to the CDS model to account for the survey effort (Leca et al., 2013).

Several candidate models were tested and the most parsimonious model was selected based on AIC values. The suitability of each model was also determined using chi-square ($\chi^2$) goodness of fit (GOF) tests (cf. Leca et al., 2013): significant results of $\chi^2$ analysis suggest that the model does not fit the data (Buckland et al., 1993).

The data were stratified at the sample level and density estimates were pooled using the mean density estimates of the two substrata, weighted by the strata area to compensate for the large difference in substrata area. Detection function and cluster size were estimated at the global level.

The research was non-invasive and adhered to the legal requirements of Costa Rica. Any and all aggressive behaviour towards the observers by individual primates was responded to by moving on as quickly as possible.

3.2.3 Results

The density of Black-handed spider monkeys on the property was calculated from 15 replicates of each trail and 107 spider monkey encounters over a period of four months. After truncating the data by 10%, 96 recordings were used in the analysis. The most parsimonious model was the uniform key with a cosine polynomial expansion which estimated there to be 27 individuals on the 11.83 km² property and its boundaries; 2.31 individuals/km². At the strata level, a density of 2.34 and 2.12 individuals/km² were estimated for the northern and southern region, respectively.
New Trail-Road had the highest number of encounters with 29 spider monkey records, whereas Northern Border had the lowest with only one spider monkey encounter being recorded.

3.2.4 Discussion
The recommended number of observations per species (60-80) has now been exceeded for the howler monkey; density will be calculated and reported in Phase CRF142. However, insufficient encounters have been made with White-faced capuchins and Central American squirrel monkey to produce robust density estimates of these species. Baseline data of these primate species is vital throughout global biodiversity hotspots (Mittermeier, 1987; Sechrest et al., 2002) owing to their ecological role as seed dispersal agents (Mittermeier, 1987; Stevenson, 2001; Estrada et al., 2005) and CRF will continue with the objective of providing this for all species within the primate guild.

The estimated density of spider monkeys is drastically lower than anticipated and is notably smaller than the number of spider monkeys estimated in CNP; 68.45 individuals/km² (Weghorst, 2007). Even when considering the range of population estimates across Mesoamerica (5.4 to 89.5 individuals/km²; (Piñeros, 1994; Ramos-Fernández and Ayala-Orozco, 2002), our estimation falls below this range. There is an abundance of fruiting tree species on the property that are known to provide resources to spider monkeys and promote their occupancy; Spondias mombin, Ceiba pendandra, Inga spp., Ocotea spp. and Pouteria campechiana (Coelho et al., 1976; Estrada et al., 2004; Méndez-Carvajal, 2013; Weghorst, 2007; White, 1986). Spider monkeys are noted to be very sensitive to disturbance, usually being one of the first Neotropical primates to become extinct following fragmentation (Link et al., 2010; Ramos-Fernández and Wallace 2008). The property has been subjected to extensive anthropogenic modification and despite the surrounding landscape being part of a wildlife corridor, it has been altered by agricultural practices potentially reducing the efficacy of the corridor by limiting species’ natural dispersal.

Spatial data collected from each primate encounter may be used in future studies within CRF to relate the distribution of primates with the level of disturbance, as quantified by novel techniques of soundscaping. Primates are differentially sensitive to disturbance and the density estimates of the remaining three species will potentially provide more of an indication of the impact anthropogenic activity within the property is having on the primate community.

3.3 Latrine Site Selection in the Neotropical Otter and Characterising the Occurrence of Mucous in Spraints

3.3.1 Introduction
The neotropical river otter (Lontra longicaudis) has a widespread distribution inhabiting rivers, lagoons and streams from Mexico through to Uruguay (Emmons and Feer, 1997). Despite this wide distribution, little is known about the species ecology, local distribution and population status; thus hindering the development of appropriate conservation strategies. The IUCN defines the priorities for this species as field surveying of populations and identifying key habitats in order to establish proper conservation status of what is presently listed on the Red List as Data Deficient (Waldemarin and Alvarez, 2008). Urgently updating the status of this species is
critical as its low reproductive potential and dependence on healthy aquatic environments mean that the species cannot respond quickly to population declines, demonstrated by the local extinction experienced between 1950 and 1970 in response to extensive hunting in parts of the species’ former range (Cezare *et al.*, 2002; Waldemarin and Alvarez, 2008).

Spatial use by otters can be cost-effectively determined by recording the location of their spraints and other signs within and around aquatic environments (Davison *et al.*, 2002). Where spraints, urine and anal secretions are repeatedly deposited by an otter, a latrine is established (Bowyer *et al.*, 1995). The exact purpose of latrines is not conclusive, but is believed to play a role in intraspecific communication (Melquist and Hornocker, 1983; Rostain *et al.*, 2004), signalling reproductive state to the opposite sex (Rostain *et al.*, 2004), marking areas of key resource use (Kruuk, 1992) and conveying the social status of males (Rostain *et al.*, 2004). It is further suggested that despite otters being carnivorous and generally solitary, they are not territorial in the classical sense as it would cost too much time and energy to patrol to the polar extents of their home range to mark territorial boundaries (Melquist and Hornocker, 1983; Remonti *et al.*, 2011). Latrines are, therefore located within the home range of otters (Melquist and Hornocker, 1983) and as these sites are indicative of habitat quality and suitability, determining which environmental characteristics otters will preferentially mark reveals insights into their ecology.

While some aspects of the environment are relatively consistently reported to influence latrine site selection, such as conspicuous substrata (Quadros and Montiero-Filho, 2002; Depue and Ben-David, 2010), prey availability (Melquist and Hornocker, 1983) and vegetation structure (Prenda and Granada-Lorencio, 1996); numerous other interacting factors are likely also promote latrine site selection. There are however discrepancies as to the importance of each factor. These other factors include water depth (Anoop and Hussain, 2004; Depue and Ben-David, 2010; Remonti *et al.*, 2011; Carrillo-Rubio and Lafón, 2004) and water edge topography (Swimley *et al.*, 1998; Bowyer *et al.*, 1995; Anoop and Hussain, 2004).

Furthermore, the study of seasonal variation in detection rates of spraints and other signs has been advocated as well as monitoring frequency of use of latrine site (Rheingantz *et al.*, 2004; Rheingantz *et al.*, 2008; Santos and Reiss, 2012). Seasonal changes in sprainting behaviours are also expected to be synchronous with the breeding season (Stevens and Serfass, 2008) and temporal peaks may be explained by prey availability (Remonti *et al.*, 2011; Almeida *et al.*, 2012) and changes in distribution (Gori *et al.*, 2003).

3.3.2 Methodology

Two linear transects were established along the course of the Rio Piro and Quebrada Coyunda, measuring 5.2 km and 2.8 km respectively. Each river was sampled once each week, typically as two distinct transects. One began at CRF camp and surveyed 3.5 km north on the Rio Piro and the other began at CRF camp, surveyed south on the Rio Piro to the river mouth (1.7 km) and then surveyed 2.8 km of the Quebrada Coyunda east from the intersection with Rio Piro. Surveyors walked within the river course and typically began sampling early morning.

During the course of the survey all rocks, logs, fallen trees, roots and river margins were actively searched for otter spraint. Fresh spraint was distinguished from old spraint based on
moisture content and how contained the spraint was. Old spraint which was dry and broken apart would be recorded as such for consideration in analyses as environmental characteristics may have changed significantly since the time of spraint deposition. The presence/absence of mucous in spraint was also recorded in addition to the substrate on which the spraint was deposited.

Each site was determined to be a new site or a reutilised site where new spraints were found in previously used sites; this was verified ex-situ from GPS data recorded at the time of each spraint encounter mapped onto GIS. At each site, river width was measured as well as the river depth at 1 m intervals of the river cross-section, escape cover distance; defined as the distance from the water’s edge to the point where the undergrowth starts was measured for both river margins.

3.3.3 Results
Between 1st October 2013 and 21st March 2014, latrine utilisation by Lontra longicaudis was recorded on the Rio Piro and Quebrada Coyunda (Figure 2). Spraint presence along with its relative occurrence of mucous was surveyed over a length of 170.8 km. During the study period, 497 spraints were recorded. The majority of spraints encountered were deposited on conspicuous rocks, rock platforms, (n = 152; 39.1%) fallen tree trunks and roots (n = 215; 55.2%); very few were on finer loose substrate on river margins (n = 22; 5.7%). Further, within months over 80% of spraints were found on previously visited latrines and excluding the southernmost extent of the Rio Piro, the full study area was utilised for scat deposition during the sampling period at least once.

On average, 7.6% of spraints encountered per month contained mucous. There was an observed peak in the frequency of spraint encountered per kilometre and the relative occurrence of mucous in January and February, respectively.

Figure 2. Latrine utilisation by Lontra longicaudis in the Rio Piro and Quebrada Coyunda and relative occurrence of mucous in spraint

The distribution of spraint containing mucous varied within the study period. In October these spraints occurred exclusively on the Rio Piro (RP), in December the extent expanded into the
Quebrada Coyunda (QC), and within February and into March these spraint were found primarily at the RP-QC intersection and along the course of the QC (Figure 3). Ninety six per cent of spraint containing mucous was also found on a reutilised site. Environmental conditions at latrine locations with spraint containing mucous were comparable to those without mucous in spraint (Table 6).

![Figure 3. Spatial and temporal distribution of scat containing mucous in relation to all other spraint where mucous was absent](image)

<table>
<thead>
<tr>
<th>Spraint Type</th>
<th>Average River Width (m ±SD)</th>
<th>Average River Depth (cm ±SD)</th>
<th>Average Escape Distance (m ±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spraint with mucous</td>
<td>7.23 ± 3.95</td>
<td>18.95 ± 8.87</td>
<td>3.01 ± 1.9</td>
</tr>
<tr>
<td>Spraint without mucous</td>
<td>7.39 ± 3.9</td>
<td>20.76 ± 14.95</td>
<td>3.09 ± 4.2</td>
</tr>
</tbody>
</table>

3.3.4 Discussion
The preference for spraint deposition on conspicuous substrata observed here concurs with previous studies (Kasper et al., 2008), although here, use of these features is more exclusive. It
would suggest an abundance of these features as it has been reported that if these raised platforms are not available, *L. longicaudis* will scratch at the ground surface to create a small mound which they will then defecate on (Quadros and Montiero-Filho, 2002). These behaviours enhance visual and olfactory cues for communication (Quadros and Montiero-Filho, 2002; Depue and Ben-David, 2010). The even distribution of logs and rocks within the study area would confirm a preference for these features; further investigation could be performed with respect to the positioning of these substrata within the river course; either at the margin or within the river course.

In the first month reported here, many of the sites were recorded as reutilised. This is due to the study being an expansion of previous work which recorded latrine locations but did not encompass the full extent of the present study. Reutilised sites are therefore, considered since the former study began in July 2013. The present study is therefore more representative of the study area than in previous phases and eliminates an inevitable spike in frequency of new sites reported at the beginning of sampling, evident in other studies (e.g., Kasper et al., 2008).

Here all records were considered independently rather than explicitly creating spraint encounter histories for unique latrines, reporting the frequency of spraints deposited at reutilised sites rather than specifically how many sites were reutilised in any given period. Although it may be possible to perform analysis of the spatial data to identify latrines for which the encounter histories can be produced, defining latrines by spatial isolation is only one method of many which have been used (e.g., Kruuk et al., 1986; Swimley et al., 1998; Quadros and Monteiro-Filho, 2002; Depue and Ben-David, 2010; Almeida et al., 2012; Crowley et al., 2012). It remains contested as to what defines a latrine, hindering the comparison of data between location and otter species.

A methodological consideration when collecting data on otter sprainting behaviour is seasonal variation in intensity of use. The seasonal changes observed for *L. longicaudis* in Argentina that were linked with changes in distribution (Gori et al., 2003). A peak in latrine use in January followed by a decline in February and March observed here concurs with findings in Brazil (Kasper et al., 2008). In *L. canadenisis*; peaks in October, November and February and March are believed to correspond to the breeding season (Stevens and Serfass, 2008) and when the pups begin to move around in family groups (Prigioni et al., 2005). Further, temporal variation in *L. lutra* sprainting activity has been linked to prey availability (Remonti et al., 2011; Almeida et al., 2012).

Owing to the dietary preference of otters (Gori et al., 2003) and expected sprainting behaviours around resource areas (Melquist and Hornocker, 1983), it is likely that sites with environmental characteristics that increase prey availability will promote latrine site selection. Deep pools create a suitable lentic environment for the preferred slow-swimming fish, thus providing otters with plentiful prey (Carrillo-Rubio and Lafón, 2004; LeBlanc et al., 2007). It has also been reported that deeper water may provide an effective escape route from predators (Depue and Ben-David, 2010). Conversely, for smooth-coated otters (*L. perspicillata*) a preference for shallower water has been documented, though it may be explained by close proximity to larger water bodies where prey abundance was greater (Anoop and Hussain, 2004). Here it is advocated that utilised sites are directly compared against non-utilised sites to better determine
sprainting preferences and integration of prey availability into the present study would provide further understanding of the contributing factors in otter space use.

Groundtruthing may be required to verify records of 0 m escape cover distance, where it was recorded irrespective of bank slope. It is assumed that a vertical high bank will not provide otters with an escape route and therefore these records collected here should be excluded from future analyses, using only values of 0 m where the vegetation is at the water edge and the bank slope and height are considered practical for escape. Whilst other studies have included water-edge topography in their analyses of latrine site selection (Remonti et al., 2011; Crowley et al., 2012), it has not consistently appeared to be a key determining factor in latrine site selection. Despite local sites with spraint containing mucous being comparable with those with mucous absent, the process of groundtruthing and reanalysing data may clarify the significance of river margin topography in sprainting behaviour.

Considering otters are found on almost every continent, in a range of habitats; there is a distinct paucity of data available across and within species. There may be severe limitations in the ability to compare results between studies, but care needs to be taken when comparing sites of latrine activity and extrapolating results, as scent marking behaviours can vary depending on population density, habitat features, human disturbance, season and there may be interspecific behavioural differences in sprainting behaviours (Quadros & Monteiro-Filho, 2002; Guter et al., 2008). Therefore much more intense study on the habitat preference of specifically L. longicaudis is required.

This study provides valuable insight and progression in understanding the habits of wild otters, expanding on the small foundation of knowledge which exists on the ecology and specifically latrine site selection by L. longicaudis. Provided there is a large sampling effort, it has been advocated that relating latrine site selection and environmental characteristics is a favoured method of accurately investigating otter ecology (Guter et al., 2008) and has to date not been studied in this part of the species’ range. The study here has potentially expanded our knowledge of reproductive behaviours by quantifying the occurrence and distribution of spraint containing mucous (R. MacKenzie, personal communication). Finer scale analyses are recommended to consider whether the apparent shift in distribution of latrines with mucous present can be explained by changes in environmental conditions. The role of mucous in otter ecology and the value it can present in research and conservation should continue to be studied.

### 3.4 Using Camera Traps to Estimate Species Abundance of Otters

#### 3.4.1 Introduction

Basic information on population abundance is vital in conservation research in order to assess a species’ current status, monitor temporal trends and define conservation priorities (Mackenzie, 2005; Oliveira-Santos et al., 2010). Without robust data on distribution and abundance, conservation strategies and management practices may be misguided, ineffective and inefficient (Langbein et al., 1999; O’Brien, 2008; Hájková et al., 2009; Oubbard et al., 2010).
The Neotropical river otter (*Lontra longicaudis*) is a Data Deficient species, (Waldemarin and Alvarez, 2008) and at present no standardised methods to estimate the abundance of the species exist. However, it is thought that the species is rare and declining throughout its range from north-western Mexico to Peru and Uruguay, therefore field surveys of populations are one of the IUCN conservation priorities for the species (Waldemarin and Alvarez, 2008). Furthermore, developing standardised methods is essential for reliably comparing data at multiple spatial and temporal scales.

The difficulties associated with assessing the abundance of otters (Family: Mustelidae) by direct observation are associated with low encounter frequencies, thus obligating the implementation of alternative approaches (Hájková et al., 2009), such as recording distinct otter spraint (Davison et al., 2002). However, estimating abundance and therefore assessing population status of elusive and rare species is often difficult (Balme et al., 2009; Hájková et al., 2009), and using sprainting intensity as an index of otter abundance is unreliable as it may reflect the abundance of suitable, conspicuous sprainting sites and activity rather than true population size (Guter et al., 2008; Calzada et al., 2010).

Camera-trapping is a relatively new methodological advancement (Pettorelli et al., 2009) that uses specialised equipment to detect and take photographs of passing animals (Rowcliffe et al., 2008). Since its early applications in the 1920s, camera traps have become an increasingly popular tool in non-invasive wildlife research (Rowcliffe and Carbone, 2008). The most common application of camera-trapping is for estimation of abundance for wild cat species (Rowcliffe and Carbone, 2008), which is most often calculated by simultaneously photographing the left and right flank of animals with paired camera traps. This results in capture histories of individuals identified by their unique stripe or spot patterns and an estimation of population parameters by capture-recapture analysis (Karanth, 1995). However, camera trap studies of non-striped and non-spotted species are less represented and are generally limited to species inventories, rather than estimating abundance or density (Rowcliffe et al., 2008). Applying camera-trapping methodology to these species will indefinitely increase the value of this tool, no longer limited to only those species with unique identifying markers (Rowcliffe and Carbone, 2008).

Trolle et al. (2008) argue that species lacking conspicuous stripes and spots may be identified to individual level by subtle marks, coat colouration, scars, body structure and sex, this approach has been applied in a number of studies concerning various taxa (e.g., coyotes (*Canis latrans*) (Larrucea et al., 2007); foxes (*Vulpes vulpes*) (Sarmento et al., 2009); Maned wolf (*Chrysocyon brachyurus*) (Trolle et al., 2007); tapirs (*Tapirus spp.*) (Holden et al., 2003; Noss et al., 2003; Trolle et al., 2008); and pumas (*Puma concolor*) (Kelly et al., 2008). However, individuals may be misidentified if shallow scratches fade (Trolle et al., 2008), photographs may be captured in different light and humidity conditions, as well as varying approach path angles and distances relative to the camera (Oliveira-Santos et al., 2010). Investigation into the reliability of individual identification of non-spotted and non-striped species has begun using tapirs as a case study, reporting very poor levels of accuracy (Oliveira-Santos et al., 2010).

It is believed that otters may be individually identified using markings on the chin (Chanin, 2003); however the reliability of this method has not been systematically investigated. It has
been strongly advocated that future studies validate these theories using data from captive individuals to test whether inter-individual variation at the population level is sufficient to reliably distinguish between individuals (Foster and Harmsen, 2012). Furthermore, for camera trap data to be analysed by capture-recapture methods, unique identifying markers must be visible in every photograph (Foster and Harmsen, 2012). Alternatively, where individuals of a species cannot be identified, novel methods reveal potential to estimate abundance from measurements of animal and camera parameters using the images themselves without the need to identify individuals (Rowcliffe et al., 2008).

The specific objectives of this study are to: i) investigate the ability of researchers to identify individual Neotropical river otters (Lontra longicaudis) in a set of photographs with known a population size; ii) investigate which characteristics are being used by researchers to identify individuals within the population; iii) quantify the proportion of camera trap photographs which are discarded on the basis of the inability to identify individuals; iv) assess the effect of any inter-researcher variability in individual identification on reliability of species abundance estimates by capture-recapture analysis, and; v) provide informed recommendations towards developing standardised methodologies for estimating the abundance of Neotropical river otters in the wild.

3.4.2 Methodology
Camera-trapping will be conducted within the captive areas of animal collections holding at least one individual Neotropical river otter. Cameras will be active 24 hr/day. The resulting images will be grouped into three subsets: (1) right flank of animal; (2) left flank of animal; (3) animal facing camera. Cameras will be active until more than 50 images within each subset have been captured and it is agreed with animal collection staff that all individuals were photographed at least once. Animal collection staff will also be asked to identify the individual(s) in each photograph.

The photographs will be sent to members of the IUCN Otter Specialist Group and other otter researchers who will be invited to respond to the following questions: (1) How many individuals were identified?; (2) What parts or characteristics of the animals were considered relevant in the identification of individuals?; and; (3) How many photos were discarded as inappropriate for identification? Respondents will also be asked to identify the individual(s) in each photograph, giving a unique numerical identifier to each individual.

The number of individuals identified by each participant will be expressed as a simple calculation of error, proportion of over-estimation or under-estimation in relation to the known population size. Characteristics used by respondents to identify individuals will be assessed to investigate any trends in accuracy of estimate and characteristic(s) used. The proportion of photographs discarded will be expressed as a percentage of the total number of photographs collected. Capture-recapture methods will be applied to the capture histories created from participants’ responses containing unique numerical identifiers for individuals in each photograph and abundance estimates calculated in CAPTURE. These values will be compared with the estimate derived from the capture history based on responses from animal collection staff.
3.4.3 Results
Camera trap images are still being collected at the time of writing.

3.4.4 Discussion
No results to discuss this Phase.

3.5 Avian Diversity within Primary, Secondary and Riparian Forest

3.5.1 Introduction
Costa Rica has a very rich avifaunal diversity, hosting approximately 850 species, more than the United States and Canada combined (Henderson, 2010; Sánchez-Azofeifa et al., 2001), 160 of these bird species are endemic to Costa Rica (Henderson, 2010). The most frequently historically encountered bird species for the Frontier Costa Rica Forest Research Programme include: Blue-crowned Manakin (Pipra coronata), Black-hooded Antshrike (Thamnophilus bridgesi), Chestnut-backed Antbird (Myrmeciza exsul), Black-mandibled Toucan (Ramphastos ambiguus), Great Tinamou (Tinamus major), Green Kingfisher (Chloroceryle americana), Long-billed Hermit (Phaethornis longirostris), Scarlet Macaw (Ara macao), Short-billed Pigeon (Patagioenas nigrirostris) and White Ibis (Eudocimus albus).

The distribution of birds and the species richness of a given area can often be explained by the habitat type and characteristics of the environment (Wilme and Goodman, 2003). From this information, it has been possible to classify species as habitat generalists or specialists (based on the extent of their occurrence in different environments), the former being more resilient to landscape change through deforestation, fragmentation and anthropogenic activities (Pejchar et al., 2008). Birds are good indicators of the health of the habitats they occupy due to their sensitivity to change, therefore recognising habitat specialists and monitoring trends in avifaunal diversity may help to identify species at greater risk of population decline as a result of anthropogenically-induced environmental change. Furthermore, declining bird populations will affect rates of pollination and seed dispersal, two fundamental functions of a healthy ecosystem, facilitated by birds (Pejchar et al., 2008). Considering this and the high levels of endemism in Costa Rica, it is important that bird communities in this region are regularly and closely monitored.

The aim of this study is to identify and compare the bird communities found within primary and secondary forest as well as within the course of a river and to further understand which species are habitat generalists and specialists based on presence/absence data at each sampling point.

3.5.2 Methodology
A total of 20 survey sites were established within three different habitat types at the Piro site, comprised of primary and secondary forest as well as a river course. Stations were set along Rio Piro, New trail, Ocelot trail, Piro trail, Tercipelo trail, Ajo trail and Cerro Osa trail at 200 m intervals. Each station was visited a minimum of three times, with a total of 75 points completed between the 3rd and 22nd of February 2014. In order to reduce the ‘time of day’ effect, the order in which stations were visited was reversed for each survey. Surveys were performed in the morning between 0600 and 0800 hrs. Each point count lasted 10 minutes, with a one minute settling period occurring before the commencement of each survey. Any species seen or heard
within a 50 m radius of the station point was recorded during each point count. Each survey was led by ARO Anna Morris with assistance from ARO John Scott. Birds were identified to species level by sight using binoculars or by song recognition. When birds could not be identified to species level, the bird group was still recorded (e.g. as Woodcreeper spp.) but was not included in the data analysis. An audio recording was taken during each survey and when possible, photographs of unidentifiable birds were taken to aid with species identification. The main references used were Garrigues and Dean (2007) and Jongsma et al., (2005).

3.5.3 Results
Now that the data collection phase has been completed; ARO Anna Morris has begun analysing audio recordings in order to make alterations and add any species which were previously unidentified by the survey team. The data will then be analysed to identify patterns in the occurrence of each species and family with relation to habitat in order to achieve the aim of the study, which is to gain an understanding of which species are more likely to be habitat specialists and which are likely to be habitat generalists. The full results and discussion of the study are expected to be published in Phase CRF142 or CRF143.

3.5.4 Discussion
No results to discuss this Phase.

3.6 CRF and the Osa Conservation Sea Turtle Conservation Programme

3.6.1 Introduction
Sea turtles are both a flagship species for conservation due to their iconic nature and an excellent indicator species for climate change due to their temperature-dependant sex determination; where increased temperatures create a sex bias skewed toward females which could cause entire populations to collapse. Additionally, temperature-induced changes in plant community composition together with rising sea levels may result in increased incidences of beach erosion and inundation of nests (Janzen, 1994). Turtle late maturation in conjunction with anthropogenic threats such as beach development, long line fishing and pollution mean that turtle populations are highly vulnerable and often unstable (Govan, 1998). Poaching and the illegal trade of turtle eggs cause further reductions to turtle populations, which may result in entire clutches being destroyed. Turtle hunting was most severe from the 1950s until the 1970s, with half of the world’s turtle catches being made in Mexico where as many as 350,000 turtles were harvested annually (Marquez et al., 1996).

A number of conservation strategies have been established throughout Costa Rica, including legalised limited commercial egg harvesting on a nesting beach in Ostional during the first 36 hours of wet season arribadas (Campbell, 1998) and an annual catch of 1,800 Black turtles being granted to fishermen in Limón (Troëng and Rankin, 2004). Though the latter may have increased extractive use along with illegal hunting in the mid-1990s, the ban on black turtle fishing and increased law enforcement since 1999 may have largely increased female turtle survivorship (Troëng and Rankin, 2004). In other regions such as Tortuguero, the Costa Rican government has made egg poaching illegal, in addition to prohibiting the trade of calipee (the edible part of the shell) (Government of Costa Rica 1963 and 1969; Troëng and Rankin, 2004). Meanwhile, the growing ecotourism industry in Costa Rica has provided locals with an
alternative source of income and has promoted conservation throughout the country. To evaluate the effectiveness of such strategies, it is imperative that monitoring programmes are long-term as it can take decades for species with late maturity to show a population response (Troëng and Rankin 2004; Bjorndal et al., 1999).

On the Osa Peninsula where turtles are threatened primarily from predation by dogs, coastal development, illegal trade of turtle eggs and to a lesser extent turtle meat (Drake, 1996), NGO Osa Conservation has been patrolling Piro and Pejeperro beaches since 2003. Their aim is to monitor the frequency and health of the local nesting turtle population and manage nest relocations to the associated hatchery. The Frontier Costa Rica Forest Research Programme works in partnership with the Osa Conservation Sea Turtle Conservation Programme concerning the four species which nest here: Olive ridley (*Lepidochelys olivacea*), Pacific black turtle (*Chelonia mydas agassizii*), leatherback (*Derochelys coriacea*) and hawksbill (*Eretmochelys imbricata*). Of these, the latter two nest only rarely on these beaches. The Olive ridley is most commonly found nesting here and is listed by the IUCN as Vulnerable, the black turtle as globally Endangered (Troëng and Rankin 2004; Honarvar et al., 2008; IUCN, 2013).

### 3.6.2 Methodology

Phase 141 is in the peak black turtle season; night and morning patrols are conducted on both Playa Piro and Playa Pejeperro managed between Frontier and Osa Conservation. Morning patrols commenced at 0400 hrs on Playa Piro and 0300 hrs on Pejeperro (high tide permitting) to minimise surveyor exposure to unavoidable direct sunlight and high temperatures. Night patrols typically started at 1930 hrs on both beaches. To minimise disturbance to nesting females, surveyors used red lights during night patrols and survey teams were a maximum of six persons. In this phase CRF led 37 turtle patrols across Piro (n = 19) and Pejeperro beaches (n = 18).

The transect area for both beaches was divided into 100 m sectors; this constituted a two kilometre stretch of Playa Piro and a 4.4 km stretch of Playa Pejeperro. For every turtle track encountered details of the previous day’s date were recorded on morning patrols (i.e., the date of turtle activity) or the date of the night patrol along with time, name of data recorder, sector number; the nest type associated with the tracks either as *in situ* (IS), false crawl (FC; i.e., turtle returned to sea without nesting), or NA when the nest type could not be determined; track symmetry defined as either symmetrical (S) or asymmetrical (A), and distance to the vegetation. The track was then crossed through with a deep heel drag in the sand to avoid the track being recorded again in subsequent patrols. Track characteristics were used as indicators of species where the turtle was absent (i.e., asymmetrical tracks suggest olive ridley, symmetrical tracks suggest black). If a turtle was encountered, the species and distance to the tide was recorded, a health assessment conducted and tagging of both the individual’s flippers completed.

*In-situ* nests were confirmed by inserting a stick into the sand to locate the egg chamber (indicated by a marked change in resistance with applied pressure) followed by careful digging to confirm the presence of eggs. A false crawl was defined by the absence of a nest or where it was clear that the turtle returned to sea without digging a nest. In the case of predated nests (typically evident by the presence of predator tracks, egg shells and signs that the nest had been dug up), the predator was identified by the tracks and egg shells counted and recorded.
All turtles encountered pre- or mid-nesting were tagged whilst they were laying, during which time the female enters a haze and therefore the process reduced the associated stress. To reduce the risk of infection during tagging, the tag sites were washed firstly with water, then alcohol and iodine before applying the alcohol-cleaned tag. National’s ear tags (National Band and Tag Co., KY, USA) each with a unique identifying number engraved were used. Two tags were used, one on each front flipper, both through the third nail from the body (Figure 4).

Figure 4. Turtle flipper tag placement

While the turtle was laying (or at latest when returning to the ocean, stopping the turtle en route by covering the eyes and kneeling in front of her), a health assessment was completed. In addition to the date, flipper tag numbers and sector; the following health parameters were observed: head (including the eyes, ears and nostrils), the front and rear flippers, the carapace and skin, fibropapilomas (e.g., barnacles) and body fat were all recorded and anything unusual noted.

3.6.3 Results
Between the 1st of January and the 23rd of March 2014 a total of nine night and 16 morning turtle patrols were conducted by Frontier and nine night and 28 morning patrols were led by Osa Conservation on Piro beach. On Pejeperro beach 10 morning and 10 night patrols were carried out by Frontier and Osa Conservation conducted 19 and 7 morning and night patrols respectively. During these patrols two Olive ridley turtles and one black turtle were tagged on Piro beach and four Olive ridley and five black turtles were tagged on Pejeperro. On Piro beach a total of 29 Olive ridley tracks and 114 black turtle tracks were recorded and 13 predated nests were excavated. On Pejeperro beach 39 Olive ridley tracks and 206 black turtle tracks were observed and 14 predated nests were excavated.

3.6.4 Discussion
The greater frequency of encounters of black turtles than Olive ridley turtles is in line with the peak seasons of these two species. The intensity of tagging has been very insignificant this phase owing to management of the programme by Osa Conservation such that efforts are concentrated on morning patrols when turtle encounters are comparatively rare. New sampling strategies to increase night patrol effort by means of two simultaneous patrol groups per night
patrol have been trialled successfully this phase. It is still under trial and any decision as to developments or changes to patrol survey effort and protocol will be made by the programme managers at Osa Conservation. An increased frequency of tagged turtles will progress towards the aim of estimating population size by capture-recapture analysis and also to monitor the health of individual turtles through recaptures.

3.7 Diversity and Vertical Stratification of Butterflies within Nymphalidae

3.7.1 Introduction

It is widely thought that we may be currently undergoing a sixth mass extinction event (Wake and Vredenburg, 2008; Barnosky et al., 2001; Thomas et al., 2004) largely driven by anthropogenic influences. Some estimates suggest extinction rates have increased to levels between 50 and several thousand times higher than the background level (Ceballos et al., 2010). Understanding and documenting biodiversity is therefore of ever increasing importance.

Despite being one of the most biodiverse taxonomic groups; insects are not well studied compared to other groups such as mammals and birds. Lack of knowledge concerning particular groups has prevented an accurate estimation of how much biodiversity is being lost and the rate it is being lost at (Pimm et al., 1995). The study of insects has been impeded by the difficulties concerned with sampling and issues involved with their identification. This has driven the study and search for indicator species which provide researchers with a useful short term solution for rapid biodiversity assessments of insects and potentially other taxonomic groups.

Butterflies have long been considered indicators of biodiversity (Kremen, 1992; Beccaloni, 1995; Blair, 1999; Thomas et al., 2004; Thomas, 2005), habitat disturbance (DeVries et al. 1997; Lien and Yuan, 2002) and environmental change (Thomas et al., 2004). Through studying butterflies, much information can be learnt about the environment and potentially act as a proxy for levels of biodiversity for other insect groups which may be more difficult to study (Elmes et al., 1999).

Compared to other insect groups, butterflies are easily surveyed by methods such as pollard transect walks and point counts, commonly used in temperate regions, with netting and bait trapping used in the tropics. Species are comparatively easy to identify and have gained popularity as a charismatic invertebrate species; combined, these characteristics have resulted in much volunteer-based research and monitoring programmes in Europe (The UK Butterfly Monitoring Scheme; Botham et al., 2008). However, despite being one of the more well studied insect groups, very little is known about many species, particularly those in the tropics where many species have yet to be evaluated by the IUCN. Currently, very little work has been conducted on butterfly diversity on the Osa Peninsula, despite values of species richness often being used in making conservation decisions, delineating protected areas and to monitor temporal trends.

Many factors impact butterfly diversity, including habitat heterogeneity (Bonebrake and Sorto, 2009; Horner-Devine et al., 2003), vertical stratification and resource partitioning (Schoener, 1974; DeVries, 1997), understanding these is essential to uncovering the mechanisms which
support high levels of biodiversity in tropical environments. Much research on butterfly diversity has determined a positive correlation between species diversity and habitat disturbance (Johnson, 2011; DeVries et al., 1997; Lien and Yuan, 2002). However, it is important to note that habitat specialists of conservation importance may not benefit, but in fact be vulnerable to the effects of habitat disturbance (Lien and Yuan, 2002; Horner-Devine et al., 2003). For example the Nymphalid species *Zaretis ellop*, a species present on the Osa Peninsula, requires undisturbed habitat such as primary forest to thrive (Thomas, 1991).

Species assemblages also vary within vertical space and research has found a level of height stratification between species occupying the canopy and understory. Despite species richness being similar in both canopy and understory, species trapped in these two height classes differ, supporting the theory of vertical stratification amongst butterflies (DeVries, 1997). Morphological differences have also been observed between canopy and understory species, suggesting different selective processes acting in the two microhabitats in Costa Rican rainforests (DeVries, 1997). Vertical stratification has been explained by varying light levels acting on the ectothermic nature of insects; however it is unlikely that this is the sole factor maintaining vertical stratification (Walla et al., 2004). Vertical stratification may be in part related to the height of butterflies’ food plants and therefore resource partitioning determines height as opposed to light levels. There is also a positive correlation between height of larval host plants and adult flight height (Beccaloni, 1997). The underlying causes responsible for vertical stratification currently remain unresolved and require further investigation to clarify fully.

Although extensive research has been conducted establishing the state of vertical stratification between canopy and understory butterfly assemblages (DeVries, 1988; DeVries and Walla 2001; DeVries et al. 2010), only a small amount of research has been focused exclusively on vertical stratification within the understory or canopy layers individually.

Here alpha diversity and vertical stratification of frugivorous nymphalid butterfly species in the understory were assessed on the Osa Peninsula using a bait trapping methodology. The investigation aimed to inventory butterfly species in this area to gather baseline data on species presence for future monitoring. Members of the family Nymphalidae were targeted owing to their ability to act as an indicator species of other lepidopteran families (Horner-Devine et al., 2003) and their ease of sampling. It was hypothesized that butterfly species would be randomly distributed vertically in the canopy.

3.7.2 Methodology

Data were collected by baited live capture canopy traps from 10th December 2013 to 31st March 2014. Twelve Van Someren-Rydon style (Rydon, 1964) traps were constructed, each with a cylinder net height of 1m sealed to a plastic tray above and a base tray secured 5cm beneath the cylinder. This trap design maximized the capture success by exploiting butterflies’ flight take-off pattern. To increase the completeness of the inventory and representativeness of the study, traps were non-identical to increase probability of capturing both shade-loving and photophillic butterfly species (Shuey, 1997); traps were either constructed with opaque or semi-transparent trays.
Twelve trapping locations were systematically chosen where it was practical to erect canopy traps. Some occupied light spots whilst others were in more shaded areas, all traps were set within the southern portion of the property. Traps were placed either directly on, or within 5 m of the river or trail. Six traps were set on the Rio Piro and six on a forest trail (Chiricano Alegre). The precise trap locations were subject to minor changes during the study period owing to fallen trees which interfered with the original trap placements.

Traps were suspended from branches at least 11 m above the ground to allow for sampling at heights of one, two, four, six, eight, and 10 metres at each trap throughout the sampling period to survey low- and high-flying species. In any given week within each of the two sampled areas, no two traps were set at the same height; between weeks individual traps rotated between these heights to ensure sampling effort was equal across traps, heights and sample areas. Therefore, in a six week period all traps had sampled each height.

Traps were baited with approximately 20g of fermenting banana to attract fruit-feeding nymphalid butterflies (Family: Nymphalidae); bait was placed in the centre of the lower tray. Bait was prepared 48 hrs in advance and administered on alternate days unless all bait was consumed within the first 24 hrs (Hughes et al., 1998). Traps were checked five consecutive days each week for the duration of the sampling period; traps were opened and baited one day prior to checks and closed on the fifth day each week. Baiting and checking was conducted once daily in the afternoon.

Captured individuals were identified in the field to species level using field guides (Chacon Gamboa and Montero, 2007). Photographs were taken whilst individuals were held in the hand (where possible) to create an image database of both the dorsal and ventral sides to allow for identification and three-way ex-situ independent verification following identifications made in the field. All individuals were released alive. Ex-situ identification verification was undertaken using additional reference guides (DeVries, 1997; Warren et al., 2013).

Data were analysed using EstimateS (Colwell, 2009) and DIVERSITY (Henderson and Seaby, 1998). Total species richness was estimated in DIVERSITY by Chao Presence/Absence. Species accumulation curves were formed using $S_{obs}$ (Mao Tau) values produced in EstimateS. In EstimateS data were pooled by sampling date, in order to eliminate bias 100 randomisations were run without replacement. On occasion, individuals escaped traps during the trap check process; these individuals were excluded from final analyses as were butterfly individuals whose identity could not be agreed upon by the independent identifiers.

### 3.7.3 Results
A total sampling effort of 867 trapping days was achieved using twelve traps operating five consecutive days a week over the course of 15 weeks. Over that sampling period, a total of 266 butterfly individuals were captured across 45 species. Of those individuals; nine (3.38%) escaped when manoeuvring traps, when handling butterflies or immediately prior to trap checks. These individuals were excluded from data analyses, as were individuals whose wing condition was too poor for confident identification to be made ($n = 2; 0.75$%).
The diversity of butterfly species and subfamilies within the family Nymphalidae are sampled on the property at the riparian, Rio Piro and secondary forest, Chiricano Alegre, locations. A total of 129 and 137 butterflies were caught on the river and forest trails respectively (Appendix 1). Thirty six species were caught on the river and 37 in the forest. Seven species were caught exclusively on the river that were not sampled on the forest trail and eight species exclusively on the forest trail, but not on the river. Thirty species (66.7 %) were captured on both the river and forest trails suggesting high similarity in species assemblages between the two sites.

As expected, all individuals captured belonged to the family Nymphalidae. The butterflies sampled comprised five subfamilies. Subfamily diversity was equal across the two sites. The greatest diversity was sampled in the Charaxinae subfamily, for which a total of 16 species were observed across all traps at both sites. At the species level, Archaeoprepona amphimachus amphiktion was the most frequently trapped across the entire survey composing 10.5 % of all individuals trapped. On the river the most frequently trapped species was Taygetis laches laches (n = 10) and on the forest trail A. a. amphiktion (n = 19).

Species richness was estimated by Chao Presence/Absence (Figure 5); therefore, our sampling effort resulted in a survey completeness of 89 %.

Figure 5: Species accumulation curve and expected number of species computed by Chao Presence/Absence.

3.7.4 Discussion
Obtaining a survey completeness value of 89 % suggests that the survey effort was sufficient for encountering the majority of the species in this area within the sampling period. The data collection is still underway, as is the verification process so these figures and species reported here may be subject to change.

A large proportion of the species sampled on the river were also sampled on the forest trail (66.67 %). High species assemblage similarity between the two sampling sites is expected given the close proximity of the river and the trail as well as the biological similarity of the two sites (both located on or close to a river and bordered with or within secondary forest habitat). However, there are some differences between the species sampled on the river and within the forest. Comparing the species caught in each of the two sites provides an interesting view of some site-specific differences in species assemblages.
The high frequency of *A. a. amphiktion* may be explained given that this species is known to spend a lot of time on the forest floor (DeVries, 1988) so we would expect to capture this butterfly when trapping in the understory. *Consul fabius cerops* was captured 15 times within the sampling period (five on the river and 10 on the forest trail) and is known to fly within both the canopy and understory layers of the forest. It has also been observed flying along forest and river edges which may explain its presence in this study (DeVries, 1988). Another species that was commonly encountered was the *Colobura direce*, which is associated with secondary forest habitat between 3-8 m (DeVries, 1988). This species was caught at heights ranging from 1-8 m suggesting a greater vertical range in flight height than originally reported. The presence of the closely related *Tigridia acesta acesta* suggests that the disturbance level of this area to be moderate owing to the fact that *Tigridia* spp. is intolerant of high disturbance levels (DeVries, 1988).

*Hamadryas amphinome mexicana* is also associated with secondary forest growth. *Catonephele mexicana* is also reported to be tolerant of secondary forest habitat, (DeVries, 1988) for which further support is found in the results here. Many of the species encountered here, including *Hamadryas* spp. occur in all rainforest environments (DeVries, 1988).

Moderate levels of disturbance have been found to have positive impacts on butterfly diversity (Horner-Devine et al., 2003; DeVries et al., 1997; Johnson, 2011). So it would be expected that high species diversity would be sampled in a secondary growth area. CNP has 220 butterfly species (belonging to all families, not just Nymphalids). Considering that 460 of the 1250 species of butterfly in Costa Rica are Nymphalid butterflies (36.8%), if we assume that roughly this percentage of butterfly species present in CNP belong to the Nymphalidae family, approximately 81 species of Nymphalid butterfly occur here. This may seem unusual given that higher species diversity should be expected on the property surveyed at Piro given the level of disturbance compared to CNP which should be relatively undisturbed. This disparity in results may be in part due to the small area surveyed here and the surveying of the understory species only and therefore direct comparisons with CNP should be interpreted with caution.

Some species encountered are thought to be rare and of conservation concern such as *Historis acheronta acheronta*, which was only encountered once within the sampling period. Some more unusual findings include the presence of *Memphis aracana*, known to occur mostly within primary forest habitats and occupies the canopy layer. However this species was documented four times during the study and the majority of these were along the river. This may indicate that this butterfly is more of a generalist with respect to the heights it occupies and the habitat it can inhabit. However males are known to perch at riparian edges which may account for this species’ presence, particularly given that three out of the four were sampled along the Rio Piro. Similarly *Zaretis ellops* is reported to occur in primary growth and undisturbed habitats; yet was sampled in our study within secondary forest, although not often. This may suggest that either the property is not significantly disturbed or that these butterflies are not as restricted with respect to their ecology and habitats as previously alluded to.

*Hamadryas arinome ariensis* is also documented to occur on the Atlantic slope within the canopy, so this is also an unexpected find on the Osa Peninsula. *Nessae aglaura aglaura* was
sampled twice over the course of this study and is usually noted for inhabiting swamp environments within primary forests. Interestingly, this species was only sampled along the river traps and never along the forest trail. *N. a. aglaura* is noted for its intolerance of habitat disturbance (DeVries, 1988), which could account for the low encounter frequency of this species here. The greatest species diversity was within the Charaxinae subfamily and the genus *Memphis* which would be expected given the high diversity of this genus, particularly when compared to other genera of the area (for example *Tigridia acesta* is the only species of its genus).

Seasonality impacts butterflies’ abundance and although the wet season is a better time for butterfly diversity, it is impractical to sample via bait trapping in the wet season. Over the course of the study some trends have arisen; for example *Nessoa aglaura aglaura* was only captured at the beginning of the sampling period. These trends may be related to seasonality of specific butterfly species. A year round study would make an interesting addition to the data collected here.

In conclusion, the butterflies present on the Osa property suggest that the area has some degree of moderate disturbance. A number of species known to inhabit primary forest have now been documented on the site, suggesting that the habitat requirements of some species are not as restricted as previously documented. However, the low frequencies at which these species were sampled may suggest that they are present but rare in this environment; perhaps because they are not well suited to the more disturbed secondary growth habitats. The study area contains areas of primary forest and plantation. Sampling in these areas would make an interesting comparison and perhaps supplement the inventory. The presence of some unexpected species suggests that this area should be monitored to assess change in butterflies in this area. Mark-release-recapture studies could also complement the information found in this study and provide an idea of the abundance of different species.

### 3.8 The Three-Dimensional Space Use of Mantled Howler Monkeys (*Alouatta palliata*)

#### 3.8.1 Introduction

Mantled howler monkeys (*Alouatta palliata*) have an extensive range in Central and South America from Mexico through to Peru. The IUCN classifies *A. palliata* as Least Concern however their current population trend is not known (Cuarón et al., 2008b). They are primarily folivorous but will supplement their diet with other vegetative substrate. Mature fruits of *Ficus* species are an important dietary component and there is a correlation between scarcity of fruits, dietary stress and mortality (Milton, 1982). The large range and different forest types that *A. palliata* can occupy has been attributed to their relatively flexible diet (Cuarón et al., 2008b). Unlike other species of New World primates, *A. palliata* is fairly resilient to anthropogenic disturbance and can tolerate moderate levels of habitat fragmentation as observed in the *A. palliata mexicana* population in Los Tuxtlas, Mexico. However, the level of fragmentation and patch size does impact their population density, group size and behaviour, meaning they are still under threat from habitat loss (Arroyo-Rodriguez and Dias, 2010).
The diversity and density of vegetative species has a positive impact on Mantled howler monkey populations (Cristóbal-Azkarate et al., 2005; Estrada and Coates-Estrada, 1996). An abundance of large and fruting trees (diameter at basal height >60cm) are important for howler monkey populations and scarcity in either of these resources leads to altered foraging behaviour; groups will feed from smaller, more widespread food resources when big, fruiting trees are scarce (Dunn et al., 2009). An increase in distribution of food resources inevitably increases a group’s foraging effort, thus allowing less time for other behaviours and an elevated energetic output (Dunn et al., 2009) which may be detrimental to the health of individuals as their diet is primarily composed of leaves - a low energy source. There are numerous large fruiting tree species on the property, including Ficus species, so investigating their behaviour in these trees and the time spent foraging may provide an indication about the suitability of the area for mantled howler monkeys.

The aim of the project is to investigate the behaviour of Mantled howler monkeys and their use of three-dimensional space within the Osa Conservation property at Piro and its boundaries. Specifically, the objectives are to: i) record the behaviour of all visible individuals every 10 minutes using a scan sampling method; ii) map the distributions of the different groups within the study region, and; iii) estimate the average canopy height of the group.

3.8.2 Methodology

Data were collected from 0600 until 1730 hrs, effectively dawn until dusk, six days per week during March 2014. Attempts were made to find different groups on the property by varying the search area to ensure that data were representative of the population. However, due to safety concerns, northern areas of the property were not utilised due to the topography of the area. When a group was encountered, they were followed for the full day. On the hour and every 10 minutes thereafter, scans were taken and the following data collected: canopy height; GPS point; and behaviour of visible individuals. As the members of the group could not all be seen at once, three minutes was used to gain as much behaviour data as possible.

The canopy height was calculated using a laser range finder, clinometer and trigonometry: sin(angle) * radial distance. Notes were also made regarding the substrate the group was feeding on and any identifiable characteristics of individuals in the group. In addition, any intergroup species interactions were recorded, which ranged from the presence of another species in the area to any physical contact between individuals (for example, we observed a fight between a female howler monkey and a spider monkey where the howler monkey fell 20 m to the ground).

The GPS waypoints will be mapped using GIS and the behavioural data will be statistically analysed to see whether the canopy height has a significant impact on mantled howler monkey behaviour.

An ethogram (Table 7) was developed and used to assist in the definition of the behaviours. Any additional behaviours were recorded and added to the ethogram.
### Table 7. Ethogram describing the different behaviours observed

<table>
<thead>
<tr>
<th>Behaviour (acronym)</th>
<th>Definition</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting (R)</td>
<td>Individual lying/sitting and did not move for the duration of the scan</td>
<td>Resting</td>
</tr>
<tr>
<td>Hanging Tail (HT)</td>
<td>Individual was hanging from tail</td>
<td>Resting</td>
</tr>
<tr>
<td>Feeding Leaves (FL)</td>
<td>Individual was feeding on leaves</td>
<td>Feeding</td>
</tr>
<tr>
<td>Feeding Fruits (FF)</td>
<td>Individual was feeding on fruits</td>
<td>Feeding</td>
</tr>
<tr>
<td>Feeding Stems (Fstems)</td>
<td>Individual was feeding on the stems of leaves</td>
<td>Feeding</td>
</tr>
<tr>
<td>Locomotion (L)</td>
<td>Individual was moving in the same tree for more than 10 seconds (i.e. not engaged in another behaviour)</td>
<td>Locomotion</td>
</tr>
<tr>
<td>Travelling (T)</td>
<td>Individual was travelling from tree to tree with obvious purpose</td>
<td>Locomotion</td>
</tr>
<tr>
<td>Playing (P)</td>
<td>Individual was playing either alone or with a conspecific</td>
<td>Social Interaction</td>
</tr>
<tr>
<td>Aggressive (AGG)</td>
<td>Individual screeches or fights with another individual (in these cases the recipient and the aggressor was noted)</td>
<td>Social Interaction</td>
</tr>
<tr>
<td>Howling (H)</td>
<td>Individual was howling</td>
<td>Social Interaction</td>
</tr>
<tr>
<td>Grunting (G)</td>
<td>Individual was grunting</td>
<td>Social Interaction</td>
</tr>
</tbody>
</table>

#### 3.8.3 Results

Data collection is still underway for this project so as of yet, there are no presentable results. The spatial data from 1st March until 17th March has been mapped onto GIS as part of a Research Assistant’s BTEC project (Figure 6). Each coloured line denotes the daily movement of the group followed that day. The green circles represent where each waypoint was taken; the larger the diameter of the circle, the more time the howler monkeys spent at that location. Resting and feeding were the main behaviours observed in these large circles.
3.8.4 Discussion
It can be seen that going off trail and following the groups for the whole day provides a great deal of data on the movement of the groups (Figure 6). The clumping of some locations suggests that the same group is being followed on different days, so it is anticipated that the continuation of this project will provide information about group home ranges. Gaining an insight into the movement and activity patterns of the Mantled howler monkey population on the property expands the primate project within CRF. Hopefully, as we learn more about the population, new research projects that will promote the conservation of these important seed dispersal agents can be carried out.
4. Additional Projects

4.1 Testing the Influence of Hiking Trails on Bird Diversity and Abundance within Neotropical Primary and Secondary Lowland Forest.

Within the project’s study area there are 16 km of hiking trails, primarily focused around the southern region within primary and secondary forest. There is evidence that hiking trails create an internal edge effect within natural habitat, with the implication that this edge may lead to changes in the species composition of bird communities (Miller et al., 1998). Human presence on such trails is also linked to negative impacts on some species, affecting feeding strategies (Fernández-Juricic, 2000) and nesting (Fernández-Juricic, 2002).

The methodology was designed and trialled between 23rd February and 13th March 2014 by ARO John Scott and PI Nathan Roberts. Geographical Information System (GIS) mapping was used to generate 20 locations to carry out bird point counts. Fifty percent of the points occur on hiking trails, while 50% of the points were placed 100 m away from any hiking trails. The selected points are also split between primary and secondary forest. There is expected to be a difference in diversity and abundance of different species between on-trail and off-trail points, and the aim of the study is to discover which species are likely to be affected most (either negatively or positively) by the presence of hiking trails.

This study will focus only on resident species, with data being collected over a four month period finishing at the end of July 2014. During this period, a target has been set to visit each point 12 times, amounting to 240 points surveyed. It is expected that this work will be reported in Phase CRF142.


Surveying a habitat such as a tropical forests, assessing biodiversity can be long, arduous and impractical (Lawton et al., 1998). Traditionally these assessments have depended on species inventories, however soundscaping offers a viable way to conduct rapid biodiversity assessments which is cheap, less labour intensive, less invasive and can be carried out by amateurs (Penman et al., 2005, Frommolt et al., 2008, Snaddon et al., 2013). Currently soundscape ecology offers a great platform to conduct general biodiversity assessments.

Soundscape ecology differs from other fields of acoustic biology by focusing not on species or individual levels, but by looking at the broader picture and studying macro or community acoustics (Pijanowski et al, 2011). Like landscape ecology, soundscape ecology looks at the interaction of pattern and ecological processes across broad spatial regions.

A soundscape is everything that can be heard in an environment. Soundscapes are comprised of three components, biophony (sounds created by organisms), geophony (non-biological sounds; rain, wind, thunder etc) and anthropophony (sounds that emanate from humans and their inventions) (Krause, 1987, Pijanowski et al., 2011).
Due to sound being a core property of nature that can be radically affected by anthropogenic disturbance, it is easy to find a relationship between biophony and environmental quality; a rich and diverse biophony will likely reflect a healthy ecosystem (Pijanowski et al., 2011). This assumption, along with the acoustic complexity index, is vital in the analyses of soundscapes in determining the disturbance levels of a ecosystem.

The aim of this study is to make a health assessment of the Osa Conservation property at Piro. This will be done by soundscaping nine locations to gain an understanding of past and present disturbance levels.

Nine random locations have been chosen across the Osa conservation property at Piro. Recording will take place twice a day, with one hour being recorded at the dawn chorus (5:30a.m.-6:30a.m) and one hour during the dusk chorus (5:00pm-6:00pm). From these recordings, the first minute of every five minutes will be analysed, using the package soundecology in the statistical program R for a rapid biodiversity assessment and acoustic complexity index.

While waiting for the soundscape proposal to be cleared by Osa conservation, the project is set up and ready to start as soon as permission is granted.

4.3 Systematically Comparing Methods of Studying Butterfly Diversity: Canopy Trapping Versus Netting.

Accurately assessing species diversity is crucial to assessing levels of biodiversity in a given area. Often conservation depends on protecting certain areas based on levels of biodiversity and species richness (Kery and Plattner, 2007). Butterflies (specifically Nymphalidae) are often studied to gain an understanding of biodiversity, disturbance and environmental conditions because they act as indicator taxa for other butterflies, insects as well as vertebrate species (Horner-Devine et al., 2003, Thomas, 2005).

Despite high species diversity of butterflies in the tropics, very little work has been conducted on butterfly diversity and abundance. The work that has been conducted used bait traps which exploit the feeding behaviors of certain butterfly species depending on the bait used (Austin and Riley, 1995). A second method for assessing butterfly diversity is netting specimens in the field. Which method is best is still debated, as there have been no direct comparisons between these two methods to determine which is more effective and efficient for compiling species inventories of tropical butterflies.

This study will compare the two methods, aiding future research into butterfly diversity in tropical environments. The site for this study is the road that runs along the coast of the Osa Peninsula connecting Puerto Jimenez with Carate. The two kilometre section of road to be surveyed cuts through the Osa conservation Piro property and has dense tropical forest on either side. Ten Van Someren-Rydon style bait traps will be used, each spaced 200 m apart from their neighboring traps. All traps will be set at 1.5 m above ground and will be monitored every 24 hrs with re-baiting occurring every 48 hours. Along the same stretch of road, netting will occur every day that the bait traps are open. The hours of netting will equal the same number of human effort hours that occur when monitoring the traps.
After the data collection, comparisons between the two methods in terms of species/number of individuals caught will take place in order to determine which, if any method is most efficient in tropical habitats. The work is subject to permit approval.
5. Proposed Work Programme for Next Phase

Primate surveys will continue sampling evenly across trails and times until sufficient data is collected for all four primate species. It is expected that in Phase CRF142 it will be possible to report on howler monkey density and that this result will be prepared for peer-reviewed publication as the research article of spider monkey density.

The in-situ otter research will continue to follow the same protocol in Phase CRF142. Towards the end of Phase CRF141 there were no encounters of spraint on the Rio Piro (N) transect for two consecutive weeks. This will continue to be explored as will any temporal changes in distribution and environmental variables. In the next phase it is also anticipated that the ex-situ project will make considerable progress.

The next phase will be an intermission between the two peak turtle nesting seasons experienced on Piro and Pejeporro beaches. It is expected that turtle patrols will continue at the same frequency and we will continue to work with Osa Conservation on the programme. Leading on from a successful BTEC project, CRF also anticipates further investigation on the effects of lunar phase and tide on marine turtle nesting behaviour. This will involve using historical data and new data from night patrols in Phase CRF142.

In the next phase butterfly monitoring will continue. Further analysis is to be completed following completion of species verification after which, the hypothesis regarding vertical stratification will be tested. Future work will incorporate additional study sites for canopy trapping in the primary forest and plantation areas of the property, which will provide a more comprehensive species inventory of the property as a whole. Trapping in these locations would also make for an interesting comparison study of the butterfly diversity present in these different areas. This expansion of the butterfly programme will probably be best suited to the next year owing to the approaching wet season and the associated impracticalities for canopy trapping.

Following the success of the new primate behaviour project, it is anticipated that this work can be expanded upon, continuing with the recording and analysis of spatial data, supported by individual group identification. This study would aim to give us a clearer understanding of the species use of the property.

Between June and December 2013, 40 interviews were conducted with workers at livestock and agricultural produce properties, with the objective of: i) understanding the occurrence and scale of human-wildlife conflict; ii) understanding local attitudes towards conservation, and; iii) providing a rapid assessment of the status of local wildlife populations. The sampled area was between Puerto Jimenez and Carate on the Osa Peninsula. To maximise the representativeness of this study prior to analyses, it is anticipated that more farm surveys will be conducted within the Osa Conservation Area (ACOSA) in the northern part within Phase CRF142. This is expected to form the first phase of a larger interdisciplinary study which will shed light on the human dimensions and ecological constructs of conflict in this previously unreported locality and will build a novel framework for resolving human-wildlife conflict. Ultimately, this project will allow for the conservation of wildlife, including one of the most human-impacted wildlife species, the jaguar (Quigley and Crawshaw, 1992).
6. References


Calzada, J., Delibes-Mateos, M., Clavero, M., & Delibes, M. (2010). If drink coffee at the coffee-shop is the answer, what is the question? Some comments on the use of sprainting index to monitor otters. Ecological Indicators, 10, 560-561.


## Appendices

### Appendix 1. Capture Frequency of Butterfly Species Within the Two Sample Areas

<table>
<thead>
<tr>
<th>Sub Family</th>
<th>Species</th>
<th>Chiricano Alegre</th>
<th>Rio Piro</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biblidae</td>
<td><em>Catonephele mexicana</em></td>
<td>3</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td><em>Catonephele numilla esite</em></td>
<td>4</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>Hamadryas amphinome mexicana</em></td>
<td>2</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Hamadryas arinome ariensis</em></td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Hamadryas februa ferentina</em></td>
<td>9</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td><em>Hamadryas feronia farinulenta</em></td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Hamadryas iptheim iptheim</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Hamadryas laodamia saurites</em></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>Nessaea aglaura aglaura</em></td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Nica flavilla</em></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Charaxinae</td>
<td><em>Archaoprepona amphimachus amphikton</em></td>
<td>19</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td><em>Archaoprepona camilla Camilla</em></td>
<td>4</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Archaoprepona demophon centralis</em></td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Consul fabius cerops</em></td>
<td>10</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td><em>Fountainea eurypyle confuse</em></td>
<td>3</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>Fountainea halice chrysophana</em></td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>Memphis arginussa eubaena</em></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>Memphis artacaena</em></td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>Memphis beatrix</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Memphis lyceus</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Memphis morusus boisduvali</em></td>
<td>6</td>
<td>9</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td><em>Memphis oenomais</em></td>
<td>5</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>Memphis pithyusa pithyusa</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Prepona laertes demodice</em></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Prepona omphale Octavia</em></td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Zaretis ellips</em></td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Morphinae</td>
<td><em>Caligo atreus dionysos</em></td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>Caligo brasiliensis sulanus</em></td>
<td>5</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td><em>Caligo illioneus oberon</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Caligo telamonius menmon</em></td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td><em>Morpho menelaus amathonte</em></td>
<td>7</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td><em>Morpho peleides marinita</em></td>
<td>3</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Opsiphanes bogotanus Alajuela</em></td>
<td>3</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td><em>Opsiphanes cassina chiriwenquis</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Opsiphanes tamarinidi tamarinidi</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Nymphalinae</td>
<td><em>Colobura annulata</em></td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td><em>Colobura dirce dirce</em></td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td><em>Historis acheronta acheronta</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Temenis laothoe hondurensis</em></td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td><em>Tigridia acetica acet</em></td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Satyrinae</td>
<td><em>Chloreuptychia arnaca</em></td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td><em>Cissia confuse</em></td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Magneuptychia alcinoe</em></td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td><em>Pareuptychia metaleuca</em></td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Pareuptychia ocorrhoe ocorrhoe</em></td>
<td>1</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td><em>Taygetis laches laches</em></td>
<td>2</td>
<td>10</td>
<td>12</td>
</tr>
</tbody>
</table>