The conservation of Baird's tapir (*Tapirus bairdii*) in Honduras

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The conservation of Baird’s tapir (*Tapirus bairdii*) in Honduras

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Summary

This thesis tested hypotheses about the response of Baird's tapir to rising anthropogenic pressure in Honduras. Baird's tapir is the largest land mammal native to the Neotropics, and was previously abundant in all countries between Mexico and Ecuador. Patterns in Baird's tapir occupancy in Honduras were modelled to test the effect of a range of environmental variables on the distribution and abundance of the species.

Analyses of the genetic structure of Baird's tapir in Honduras revealed very little genetic diversity at the mitochondrial genome. Only two haplotypes were identified from 69 samples successfully genotyped, representing a remarkably low level of diversity, which is likely to affect the viability of the species in the long-term.

Occupancy and population estimates were generated for four protected areas, and for the remaining regions in Honduras where Baird's tapir have been confirmed to remain. Occupancy was found to be associated with environmental variables relating to accessibility. Although the population estimates generated here exceed previous estimates, evidence of ongoing poaching and deforestation of tapir habitat suggests that Baird's tapir populations in Honduras may not be viable outside of the protected areas of La Moskitia. Population Viability Analyses for the remaining areas of tapir occupancy in Honduras demonstrate the vulnerability of the species to habitat fragmentation and hunting pressure.

Annual surveys in Cusuco National Park (PNC) from 2009 to 2012 investigated the conservation status of the species in this tapir stronghold over the duration of this PhD project. Patch occupancy analysis demonstrated that Baird's tapir display a distribution bias in PNC associated with accessibility, and identified that Baird's tapir are suffering a population decline that has the potential to cause the extirpation of the species from this Park within the next few years.

For Dad.

The single greatest inspiration in the lives of your three sons, your legacy lives on in us and in all who knew you.

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Abbreviations

Ā	Allele Frequency
ASL	Above Sea Level
BP	Base pairs
BRP	Rio Platano Biosphere Reserve
BSA	Bovine Serum Albumin
CITES	Convention on International Trade of Endangered Species of Wild Fauna and Flora
DEFRA	Department for the Environment, Food and Rural Affairs
DNA	Deoxyribose Nucleic Acid
DNTPs	Di-Nucleotide Tri-Phosphates
Eps	Extinction Probability in PRESENCE
Gamma	Colonization in PRESENCE
GTR	General Time Reversible
Ha	Hectares
Ho	Observed Heterozygosity
ICF	Instituto Nacional de Conservacion y Desarrollo Forestal, Areas Protegidas y Vida Silvestre (Institute of Conservation and Forestry)
IAPPPO	Importation of Animal Products and Poultry Products Order (UK)
IUCN	International Union for Conservation of Nature
K	Carrying Capacity
LSU	Landscape Units
ML	Maximum Likelihood
mtDNA	Mitochondrial DNA
MVP	Minimum Viable Population

Nc	Census population
nDNA	nuclear DNA
Ne	Effective population
NGO	Non-Governmental Organisation
NJ	Neighbour Joining
Npb	Non-Parametric Bootstrapping
P	Detectability in PRESENCE
PCR	Polymerase Chain Reaction
PE	Probability of Extinction
Pi	Initial Population
PNC	Cusuco National Park
PNPB	Pico Bonito National Park
PNSRT	Sierra Rio Tinto National Park (Proposed)
Psi	Occupancy in PRESENCE
PHVA	Population and Habitat Viability Analysis
PVA	Population Viability Analysis
SSRs	Single Sequence Repeats
STRs	Short Tandem Repeats
TE	Time to Extinction
TMP	Tapir Monitoring Protocol
TSG	Tapir Specialist Group
UN-GEF	United Nations-Global Environment Facility
UNODC	United Nations Office on Drugs and Crime
UV	Ultra Violet

Chapter 1. General Introduction

1.1. Summary

In the following introduction, I review the biology of Baird's tapir (*Tapirus bairdii*), present an assessment of the conservation status of the species, and outline the contribution this study aims to make to the field. I provide an overview of the methods I propose to use for studying Baird's tapir populations in Honduras, and demonstrate the importance of Honduras for Baird's tapir conservation.

Tropical forest-dwelling mammals are among the most threatened taxa in the world, and population declines are frequently correlated with the intensity of human activity (Ceballos & Ehrlich 2002; Sodhi & Ehrlich 2010). The study of such species can be difficult as they are often secretive, solitary and survive at low population densities (Robinson & Redford 1986; Kindberg *et al*. 2009). These difficulties may be exacerbated by the challenging nature of the habitat, which may constrain visibility and disguise spoor. The monitoring and management of wildlife populations depends upon the accurate reporting of population estimates and trends (Carbone *et al*. 2001). This study aims to test and develop techniques for studying rare and elusive species in a challenging landscape. Baird's tapir is thought to be declining rapidly throughout its range in Central and South America due to the human impacts of habitat loss and hunting (Castellanos *et al*. 2008). Remaining populations are little-studied and live at low densities in the most inaccessible parts of their habitat (Garcia *et al*. 2012).

This study attempts to apply a combination of non-invasive genetics and patch occupancy analytical techniques to elucidate aspects of Baird's tapir biology and population ecology, particularly the response of the species to anthropogenic stress. The principle aim of this study is to improve our knowledge of the conservation status of Baird's tapir in Honduras, and to develop suitable techniques for use in the research of similarly difficult-to-study species.

1.2. The genus *Tapirus*

The family Tapiridae are classified in the order Perissodactyla, the odd-toed ungulates, along with the Rhinocerotidae (rhinoceroses) and Equidae (horses). The Tapiridae separated from the Rhinocerotidae and the Equidae in the early Eocene, around 54-56 MYA (Trifonov *et al.* 2008; Steiner & Ryder 2011). The last surviving genus in the Tapiridae, *Tapirus*, originated in Northern Europe during the Oligocene (30-37 MYA), from where they dispersed across existing land bridges to occupy much of Asia and the Americas (Ruiz-Garcia *et al.* 2012).

Like most extant megaherbivores that survived the mass extinction that extirpated many species during the Pleistocene, tapirs today have a much reduced global distribution. Their current distribution was determined by the location of forest fragment refuges during times of climatic and environmental upheaval (Ruiz-Garcia *et al*. 2012). Tapirs once ranged throughout the Northern Hemisphere, but their distribution is now restricted to parts of Central and South America, and South East Asia (Matola *et al*. 1997). The three distinct mitochondrial lineages of modern day tapirs of the genus *Tapirus* diverged approximately 10-20 MYA, resulting in the emergence of two Neotropical and one Asian lineage (Ashley *et al*. 1996; de Thoisy *et al.* 2010; Ruiz-Garcia *et al*. 2012). Four species of tapir remain extant: the Malaysian tapir (*Tapirus indicus*), the lowland tapir (*Tapirus terrestris*), the mountain tapir (*Tapirus pinchaque*) and Baird's tapir (*Tapirus bairdii*), all of which are represented on the IUCN Red List of threatened species (Castellanos *et al.* 2008).

Only a very small number of genetic studies have been carried out on Neotropical tapirs, and either contradictory (Ashley *et al*. 1996; Norman & Ashley 2000) or apparently non-replicable (Norton and Ashley 2004a, b) results have confounded attempts to elucidate the molecular history of Neotropical tapir species. Two recent studies (de Thoisy *et al.* 2010; Ruiz Garcia *et al*. 2012) have more definitively characterized the phylogenetic history of Neotropical tapirs, and have shown that *T. bairdii* is considerably divergent from the two extant South American species *T. pinchaque* and *T. terrestris*, with an estimated divergence time of 10.9 MYA (Ruiz-Garcia *et al*. 2012).

1.3. Baird's Tapir

1.3.1. The Ecology of Baird's Tapir

Baird's tapir, the largest of the three species of Neotropical tapir, is the largest terrestrial mammal native to the Neotropics. Now locally extinct in El Salvador, the species' range extends from Oaxaca province in south eastern Mexico to the Andes of Colombia, with remnant populations in Ecuador (Medici *et al.* 2005; Figure 1.1).

Fully grown adults weigh over 250 kg, are 200 cm from nose to tail and stand 120 cm at the shoulder. Baird's tapir are relatively long-lived, with a life span of up to 29 years. Female Baird's tapir typically give birth to one young after a 13 month gestation period. The young stay with their mothers for up to 18 months before weaning, and reach sexual maturity at

18-24 months (Medici *et al.* 2005). The International Union for the Conservation of Nature (IUCN) suggest a generation time of 11 years in Baird's tapir (Castellanos *et al.* 2008), which reflects the low recruitment rate of this species. Generation time is calculated as the average age of the parents of the current cohort of offspring in the population, though many individuals may have been reproductively active for several years (IUCN 2001).

Tapir may be active both day and night, but are generally more active during nocturnal hours (Foerster & Vaughan 2002) and may become preferentially nocturnal in areas with increased human disturbance (Eisenberg 1989). Baird's tapir are forest dwelling ungulates inhabiting a range of forest types including rainforest, broadleaf and cloud forest. They associate strongly with water (Matola *et al.* 1997) and, as such, are also found in marsh and swamp areas, in mangroves and other riparian habitats. Tapirs rely upon water for many activities including feeding, temperature regulation, defecation and protection from predators (Garcia *et al.* 2012).

Previous studies have suggested that the main factor determining occupancy in Baird's tapir is likely to be the level of human disturbance (Medici *et al*. 2005; Naranjo & Bodmer 2007; Tejeda Cruz *et al.* 2009). Several studies have shown that tapir avoid disturbed habitats (Lira-Torres *et al*. 2004; Tejeda-Cruz *et al.* 2009; Tobler 2002), and Flesher and Ley (1996) have suggested that an effective exclusion zone of nearly 300m exists around all habitats affected by human activity, into which tapir will rarely venture, potentially limiting the colonisation and migration of tapir in disturbed areas. Contrasting this, in a mosaic landscape of mature and logged habitat, tapir may preferentially seek out food in the disturbed habitat due to the greater availability of plants, trees and shrubs at browsing height (Fragoso 1991; Naranjo & Bodmer 2007); and tapir have been observed to forage in plantations even in areas where they are hunted (Dunn *et al*. 2012).

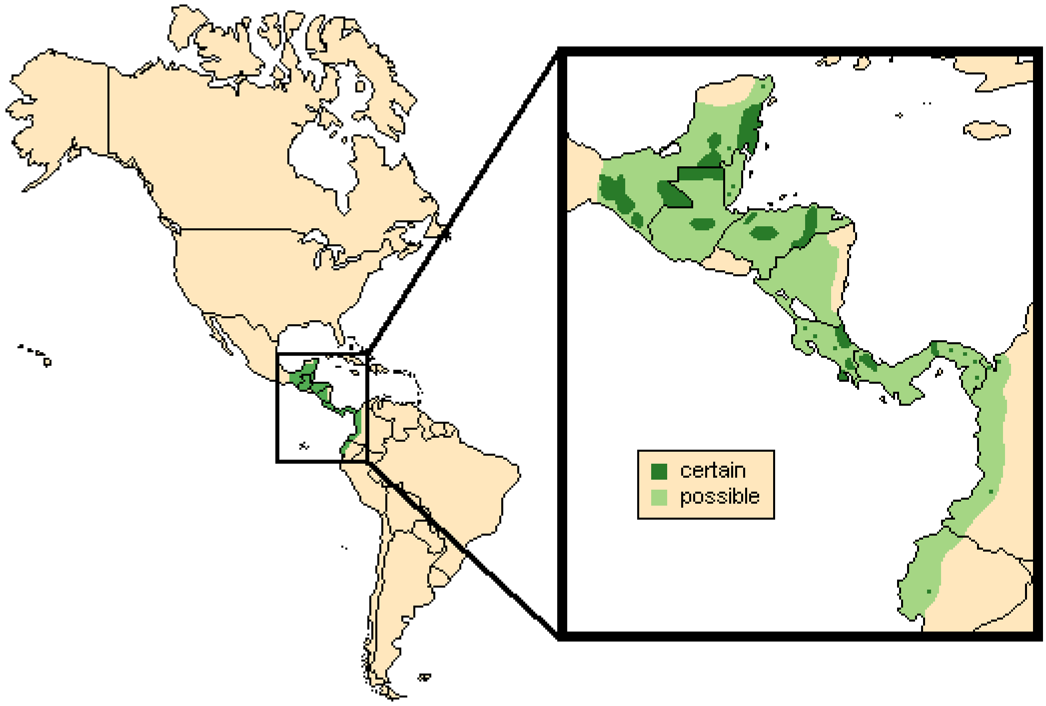


Figure 1.1: Range map for Baird's tapir (*Tapirus bairdii*).
Map redrawn from Matola *et al.* (2007). 'Certain' refers to localities within the overall 'possible' range of Baird's tapir, where Baird's tapir presence has been confirmed by member researchers of the Tapir Specialist Group (TSG).

Tapirs are generalist herbivores with a varied diet that may include well over 100 species of plant (Naranjo 2009), consisting of leaves, shoots, fruits and fibres, depending on the availability of food stuffs in the environment (Garcia *et al*. 2012). Examples are given by three studies, performed in different areas, which document the percentage of food that is comprised of leaves: the Cordillera de Talamanca, Costa Rica: 12-26 % (Tobler 2002); Triunfo Biosphere Reserve, Mexico: 45 % (Lira-Torres *et al.* 2004); Corcovado National Park, Cost Rica: 67 % (Tobler 2002). This generalist herbivory enables tapir to colonise a range of habitats, provided certain landscape criteria such as suitable fluvial features are present (Matola *et al.* 1997). Their adaptability in the face of environmental change has been a feature of their ability to survive where many other species of similar body size have become extinct (Garcia *et al*. 2012).

Baird's tapir occupancy is seen as an indicator of habitat quality (Tobler 2002; Lira-Torres *et al*. 2004; Tejeda-Cruz *et al.* 2009), and the species has been reported to be an important seed disperser for many species of plant including economically important trees such as *Manilkara zapota* (Fragoso *et al.* 2003; O'Farrill *et al*. 2007; O'Farrill *et al.* 2012; O'Farrill *et al.* 2013). O'Farrill *et al*. (2012) showed that the probability of germination increased after passage through the tapir gut, which they concluded was due to the favourable growing environment provided by tapir latrines. Campos-Arceiz *et al.* (2012) demonstrated that Malayan tapirs act as seed predators for large-seeded plants, but effective dispersers for small-seeded plants. It is clear that, irrespective of the commercial value of the plant species distributed by Baird's tapir, they play an important role in seed dispersal and forest regeneration (Figure 1.2). Tapir demonstrate an apparent preference for defecating in water (Naranjo 1995, 2009), which could create a bias for or against the dispersal and germination of certain plant species depending upon their ability to survive when immersed.

Some studies have shown that tapirs use latrines consisting of multiple dung piles in the same location, either deposited by the same or by different individuals (Naranjo & Cruz-Aldan 1998; Fragoso *et al*. 2003). The precise function of these latrines remains unknown, but chemical communication of reproductive state, or territorial demarcation, as reported in the Asian one horned rhino (Dinerstein 2003), would be a strong candidate.

Figure 1.2: Baird's tapir as seed dispersers.
Baird's tapir may play an important role in seed distribution and in assisting in the germination of seeds passed through their gut, as demonstrated in the pictures above. 1: seeds in fresh Baird's tapir faeces. 2: seedlings growing from a pile of Baird's tapir faeces. Images taken in Cusuco National Park (PNC), Honduras, in 2012.

Ungulate home ranges are determined by a range of species- and context-specific variables (Grant *et al.* 1992). Home range defence costs increase with range size due to the necessity of spending more time patrolling and the probability of encountering a larger number of rivals, therefore undefended ranges tend to be much larger than defended ranges (Sodhi & Ehrlich 2010). Range sizes also vary between habitat types depending upon the availability of resources, with defended home ranges being more common in areas of high resource abundance (Grant *et al*. 1992). Home range sizes have been estimated in Baird's tapir using a variety of methods including telemetry (Foerster & Vaughan 2002), camera trapping

(Christopher Jordan, unpublished data) and line transects (Naranjo 2009), in a variety of landscapes across much of their range. These studies have recorded a large variation in the home range sizes of tapir. For consistency in conservation planning, the IUCN Tapir Specialist Group (TSG) have applied a 'standard' home range size whereby male Baird's tapir maintain a home range of approximately 100 ha (1 km^2), and females range more widely (Medici *et al.* 2005). Studies of home range sizes in *T. terrestris* and *T. pinchaque* have also been published (e.g. Downer 1996; Noss *et al.* 2003), and although caution must be applied when making cross-species assumptions, given the similar life history patterns of the three species, these studies may provide a useful reference when investigating *T. bairdii* territorial dynamics.

Tapir home ranges may overlap considerably, with an average overlap of over 30% (Foerster 1998). This is of particular importance to conservation as it suggests that several individuals can coexist in an area much smaller than if home ranges were conserved and defended, provided the area is sufficiently rich in resources. This overlapping of home ranges is a classical pattern for tropical forest species such as collared peccary (Judas & Henry 1999), white tailed deer (Hirth & McCullough 1977) and jaguar (Grant *et al.* 1992), where movements are dictated by the availability of food and territories are not generally defended. Population density is easier to estimate than home range size, and has been estimated for Baird's tapir in several studies (range 0.05-1.6 tapirs/km^2, mean 0.43 tapirs/km^2, from a total of nine studies; reviewed in Naranjo 2009). Higher population densities, up to 3.03 tapirs/km^2 have been recorded in isolated locations in Costa Rica, where hunting is controlled and emigration is made impossible by human activity, resulting in elevated population densities where resources are sufficiently abundant (Gonzalez-Maya *et al*. 2012).

1.3.2. Species Status and Threats

1.3.2.1. Status

Baird's tapir is classified as endangered throughout its range due to the ongoing decline in numbers assumed to result from the loss and fragmentation of habitat, and from hunting pressure. It is estimated that the population has declined by 50% in the past three generations (33 years) and is predicted to decline by more than 50% over the next three generations (Castellanos *et al*. 2008). Baird's tapir have become locally extinct in El Salvador, and the latest estimates suggest that fewer than 5,500 adult Baird's tapir remain across their range, with approximately 500 individuals in Honduras (Castellanos *et al*. 2008).

1.3.2.2. Overhunting

Overhunting is perhaps the most serious threat to large vertebrates in tropical forests (Sodhi & Ehrlich 2010). Persistent hunting of Baird's tapir has been identified as a major threat to ongoing species survival (Brooks *et al.* 1997), and tapir are still hunted for food across much of their range (Naranjo 2009; Dunn *et al*. 2012). Hunting pressure may become unsustainable when hunted populations are not replenished by immigration. Metapopulation theory describes the balance between local extinction and recolonisation (Hanski & Ovaskainen 2002). There are three general models in metapopulation theory. The 'mainland-island model', where the 'mainland' or 'source' population is a source of emigrants to smaller 'island' or 'sink' populations that may regularly go extinct but are recolonised by immigrants from the mainland. The 'many island model' describes a number of smaller populations with an equal chance of local extinction, where the pattern of local extinction and colonisation ensures the overall population persists through time (Sodhi & Ehrlich 2010). The third metapopulation model is known as the 'source sink model', whereby positive population growth in a central 'source' population replenishes peripheral sink populations that would display negative population growth in the absence of immigration (Hanski & Simberloff 1997). Metapopulation dynamics have been previously described in Baird's tapir. A study by Naranjo & Bodmer (2007) explored source-sink populations of hunted forest ungulates, including Baird's tapir, in the Lacandon Forest in Mexico. They concluded that as long as harvesting rates did not exceed 20% of the estimated capacity for annual growth of the population, Baird's tapir can continue to survive, if sufficient levels of immigration exist from neighbouring areas.

1.3.2.3. Loss of genetic diversity

The maintenance of genetic diversity has become a primary objective of conservation geneticists in developing conservation strategies. Population bottlenecks, fragmentation and founder effects are key drivers in the loss of genetic diversity (heterozygosity or allelic diversity), which may cause a loss of evolutionary potential in a species (Allendorf & Luikart 2007), and have been shown to be contributing to the decline and vulnerability of several mammalian taxa (Lacy 1997). A classic study on loss of heterozygosity leading to extinction was conducted by Saccheri *et al.* (1998), who demonstrated that extinction risk is greatly increased by the loss of heterozygosity in the Glanville fritillary (*Melitaea cinxia*), leading to reduced rates of larval survival, adult longevity and egg hatching rate. The authors of this study conclude that their findings are of particular relevance to species with small and isolated populations, such as those affected by the loss and fragmentation of their habitat. The long-term genetic effects of isolation and population decline in Baird's tapir are likely to be severe, and suggest that genetic factors be included at the forefront of conservation planning for the species (Frankham *et al*. 2014). Baird's tapir display very low mitochondrial DNA (mtDNA) diversity when compared to the other Neotropical tapirs and to other Neotropical mammals examined at the same loci (Ruiz Garcia *et al*. 2012; Cozzuol *et al*. 2013). This suggests a history of isolation, genetic bottlenecks and genetic drift, which has accelerated in the last few decades with an increase in the level of habitat removal in Central America due to deforestation for timber and agricultural conversion (Garcia *et al*. 2012).

1.3.2.4. Habitat loss and fragmentation

Habitat fragmentation is of primary concern in conservation biology, and has been described as a leading cause in the global decline in biodiversity (Fahrig 2003; Segelbacher *et al.* 2010). Ninety percent of threatened species are primarily affected by habitat loss (Sodhi & Ehrlich 2010). Fragmentation can create conditions that lead directly to extinction by exposing the population to a higher risk of edge effects, habitat loss and pressure from hunting, disease etc. Fragmentation also inhibits the regulatory effect of metapopulation dynamics that would replenish depauperated populations under normal conditions (Sodhi & Ehrlich 2010). When considering a species such as Baird's tapir, which is extremely sensitive to human activity and lives in a highly fragmented landscape with rapid ongoing loss of habitat, these factors become increasingly important.

Across their entire range, as little as 30% of original tapir habitat remains (Revington 1992), much of which is highly fragmented. The publication of a fine-scale (30 m x 30 m) dataset on global forest loss (Hansen *et al*. 2013) allows the analysis of the effects of even small levels of local deforestation on tapir distribution. This thesis will look at the conservation status of Baird's tapir in Honduras, where very few studies have been undertaken to date. By assessing the level of habitat fragmentation in Honduras, and the connectivity -or otherwise- of the areas where Baird's tapir are expected to remain, it will be possible to discuss whether metapopulation dynamics are likely or able to maintain a stable national population.

1.3.3. Conservation of Baird's Tapir

Baird's tapir is an important flagship species for conservation in Central America and the conservation of the species has been recognised to be a priority by national governments in the region. Conservation directives relating to *Tapirus* species are drawn up and enacted by members of the TSG. In 2005 the Baird's Tapir Action Plan was created to define the most important objectives for Baird's tapir conservation and research for the coming years (Table 1.1).

Baird's tapir is included under CITES Appendix I, which bans the trade of all tapir products. It is the National Animal of Belize, and has been granted protected status in all countries across its range (Castellanos *et al*. 2008). The practicalities of this protection on the ground are, however, limited, as many of the protected areas in which it survives are not patrolled by rangers, and despite government interest, in reality all conservation directives are generated by the TSG, with no basis in law, and enacted by member-researchers in the field. As such Baird's tapir are still hunted for food and for sport across a large part of its range, and individual populations continue to decline (Castellanos *et al.* 2008).

Large amounts of baseline data, such as accurate distribution maps and population predictions, are still missing for this poorly-studied species. At the present time, Baird's tapir distribution is imprecisely defined, with many areas that were formerly considered strongholds for the population now affected by deforestation, hunting and other pressures (Castellanos *et al*. 2008). Accurate estimates of abundance or census population size are important factors in the conservation and management of endangered species (Carbone *et al*. 2001). Where estimates of actual abundance are difficult to obtain, other indices of abundance such as occupancy are important surrogates for designing conservation strategies (Mackenzie *et al*. 2002). Even estimating occupancy in Baird's tapir may be problematic, because the remaining populations of Baird's tapir are assumed to be highly fragmented and found in the most inaccessible parts of their range, where individuals are elusive and difficult to observe (Garcia *et al*. 2012).

One of the goals of the TSG is to define the amount of genetic variation required to maintain a stable population in perpetuity. Perhaps a more realistic goal is to define the minimum population size required for the species to be viable. Traill *et al.* (2007) suggest that minimum viable populations for mammals are typically over 4000 individuals and while there are numerous examples of species that have recovered from considerably lower numbers, these have tended to require significant human intervention.

As part of ongoing conservation efforts, two Population Viability Analyses (PVAs) have been conducted to assess the viability of Baird’s tapir across its entire range and to update the Baird’s Tapir Action Plan (Medici *et al*. 2005; Barongi *et al.* 2006). The existing Baird’s tapir PVAs provide an overall picture of the status of the species, but PVAs can also be used to provide estimates of population trajectories for individual populations under different environmental parameters (e.g. Bach *et al*. 2010). This is of particular interest in Baird’s tapir conservation, where isolated populations are likely to be experiencing highly differing environmental conditions and threats to survival, and will form an important part of this study.

Table 1.1: Primary objectives of the Baird's tapir action plan (Medici *et al.* 2005).
Objectives were agreed upon by delegates at the International Tapir Symposium in 2005, and represent the research priorities and conservation objectives deemed most important for the conservation of the species.

1. To quantify and document habitat loss and transformation, hunting, and disease introduction that reduce the population sizes, reducing their viability in the long run and generate information on abundance, population dynamics, genetics, health assessment, habitat availability and use.
2. To produce a map of the priority areas for conservation in the distribution range of the species.
3. To initiate inter-institutional international cooperation in shared areas between neighbouring range countries for the development of conservation strategies, and generating information on abundance, population dynamics, genetics, health assessment, habitat availability and use.
4. To produce a diagnostic (to identify necessities) about the isolated populations of Baird's tapir and propose strategies for their conservation in the long run.
5. To produce a current map of the distribution range of Baird's tapir in each country, using comparable methods.
6. To define a viable population.
7. To focus the generation of information about abundance, population dynamics, genetics, health assessment, habitat availability and use on the five bioregions (Zoque Forest, Maya Forest, Mosquitia Forest, Lower San Juan River Basin, Darien-Talamanca), where the largest populations of Baird's tapir are found.
8. To define the necessary habitat to maintain a viable population in the long run.
9. To define the minimum amount of genetic variation in order to guarantee a viable population.
10. To document the main issues associated with incoherence in public policy, deficiencies in education and communication, and political instability that make it hard to design and implement conservation and management actions.
11. To produce a synthesis of all the available information about the natural history of the species.
12. To estimate the frequency and intensity of natural phenomena of varying intensity in each one of the range countries.

1.4. Molecular studies of *Tapirus* species

Molecular studies have been carried out on all four extant members of the *Tapirus* genus using a range of molecular markers. A recent study by Ruiz-Garcia *et al*. (2012) aimed to define the phylogenetic relationships between the Neotropical tapirs using the mitochondrial *cytochrome-b* gene, and concluded that Baird's tapir has considerably lower levels of genetic diversity than the other Neotropical species, as described above.

Three studies have investigated the molecular phylogeny of Baird's tapir. Ashley *et al.* (1996) and Norman and Ashley (2000) attempted to analyse the genetic relationship between *Tapirus* species and calibrate molecular clocks in *Tapirus* against well-defined palaeontological dates. Both studies supported the existing theory that Neotropical tapirs belong to two separate clades, with the predominately Central American Baird's tapir in one clade, and the exclusively South American lowland and mountain tapirs in the other. The results they obtained from the two mitochondrial genes analysed, *cytochrome oxidase subunit II* (*COII*) and *12S* ribosomal RNA, suggested greatly different divergence times and evolutionary rates, so considerable caution must be applied before drawing any precise conclusions from these studies.

The study by Ruiz-Garcia *et al*. (2012) contributed considerably more detailed information. They sequenced 30 wild Baird's tapir samples from sites across a wide expanse of the species' range, in a range-wide study on all three Neotropical tapir species. They identified seven polymorphic sites on the *cytochrome-b* gene, which is considerably less than has been identified in the mountain tapir (*Tapirus pinchaque*; 47 sites) and the lowland tapir (*Tapirus terrestris*; 107 sites), despite the population size of Baird's tapir being estimated as approximately five times that of *T. pinchaque* (Castellanos *et al*. 2008). These findings strongly suggest that Baird's tapir has experienced a genetic bottleneck, or that genetic drift has greatly reduced the genetic diversity of this species.

Two further studies (Norton & Ashley 2004a, b) have investigated the current genetic status of the species. These studies analysed genetic variability among wild Baird's tapir in southern Costa Rica and southern Panama, and captive tapir in North and Central America. Using six polymorphic microsatellite markers, they identified that the populations they studied displayed low levels of allelic diversity and heterozygosity, but that they showed no sign of having experienced a recent bottleneck. The statistical power of this study could have been improved through the use of more molecular markers, which would have provided a more accurate assessment of genetic diversity in the populations sampled; and by sampling a greater number of individuals (Berthier *et al*. 2002).

Signatures of bottlenecks may be particularly difficult to identify when the sample size is small. Studies have shown that increasing the number of markers may be more important than increasing the sample size. Hoban *et al*. (2013) demonstrated that doubling the number of markers gives equal or greater gains in power than tripling the sample size, and increasing the number of microsatellite markers up to 100 shows continued additive gains in power. In cases where obtaining large numbers of samples is difficult, it may be advisable to increase the number of markers rather than attempting to increase the sample size, which is increasingly feasible for modern conservation studies, given advances in the techniques used to identify suitable markers (Landguth *et al*. 2012; Hoban *et al*. 2013).

Given the paucity of genetic information available for Baird's tapir there is a pressing need for a detailed population-level genetic study to be carried out on this species, particularly as the data that do exist suggest that populations are both highly fragmented and low in genetic diversity (Norton & Ashley 2004a; Ruiz Garcia *et al.* 2012; Cozzuol *et al.* 2013). As yet, no genetics studies have been carried out on Baird's tapir from Honduras, which is an important gap in our understanding of the species.

Tapirs and rhinoceroses (collectively the ceratomorph Perissodactyls) have been shown to display a very slow rate of karyotypic evolution in comparison to other species such as horses (the hippomorph Perissodactyls), with Baird's tapir and the lowland tapir displaying particularly slow rates of karyotypic change, up to 80 times slower than in the genus *Equus* (Trifonov *et al.* 2008). The highly conserved nature of the Tapiridae genome and the relatively slow recruitment rate means that tapirs may be unusually susceptible to loss of genetic diversity, and also suggests that heterozygosity could only be reliably estimated using a large number of molecular markers. The relatively large number of conserved autosomes between the karyotypes of the extant tapir species (Houck & Kingswood 2000) does, however, increase the probability of successful cross-amplification of molecular markers.

A study by Gonclaves da Silva *et al.* (2009) isolated eight loci in lowland tapir (*Tapirus terrestris*), and characterised five loci in lowland tapir that had been previously isolated in Baird's tapir. Sanches *et al*. (2009) isolated a further seven loci in *T. terrestris*. Work carried out by these two studies strongly suggests the potential for high levels of cross-amplification of polymorphic microsatellite loci within the Tapiridae.

1.5. Conservation Genetics

Conservation genetics has emerged as an effective method for improving the precision of conservation programmes. Conservation genetics addresses 11 key issues: inbreeding depression, loss of genetic diversity, reduced gene flow, genetic drift, the accumulation or purging of deleterious mutations, genetic issues relating to ex-situ breeding and reintroduction, genetic management units, resolving taxonomic relationships, forensic studies, understanding biological processes so as to better manage populations, and outbreeding depression (Sodhi & Ehrlich 2010).

As many genetic traits directly impact reproductive or evolutionary fitness (Sodhi & Ehrlich 2010), genetic analysis is able to reveal survival-critical variables that would be impossible to identify using non-genetic studies (Sarre & Georges 2009). Despite this, genetics research rarely translates into management strategies for endangered species, largely due to genetics having an ill-defined role in conservation and wildlife management, though this is improving (Moyle *et al*. 2003; Howes *et al*. 2009; Pereira *et al*. 2013).

The primary function of conservation genetics, in the management of threatened species, remains identifying genetic trends, such as isolation and inbreeding, in populations under threat from extinction. This can enable the implementation of targeted conservation strategies that use genetic data as a basis for the allocation of resources for conservation; and in 'genetic rescue' projects, i.e. the translocation of genetically distinct individuals into a genetically depauperated population, as per Hogg *et al*. (2006).

Conservation genetics is particularly relevant to Baird's tapir conservation. Evidence suggests that the species contains considerably less genetic diversity than the other Neotropical mammals and other tapir species (Norton & Ashley 2004a; Ruiz-Garcia *et al.* 2012), and that many of the remaining populations are likely to be isolated and not sustained by metapopulation dynamics (Saccheri *et al*. 1998). Current research priorities must include gathering data on the genetic diversity of the remaining populations, and identifying whether corridors for gene-transfer exist between spatially separate populations, which would facilitate population stability through metapopulation dynamic processes (Naranjo & Bodmer 2007). These data will be important in enabling the TSG to better understand how to manage wild populations of Baird's tapir to ensure the viability of the species in perpetuity (Frankham *et al*. 2014).

1.6. Non-Invasive Genetics

Tissue and blood biopsies have the advantage over faecal or hair samples in that they allow a higher quality and volume of DNA for analysis, and thus increase the feasibility and accuracy of DNA profiling (Wehausen *et al*. 2004). To perform a statistically robust study it is necessary to sample large numbers of individuals, therefore the use of invasive techniques for endangered large mammals is ethically questionable due to the potential threat of accidental euthanasia during capture or under anaesthetic while obtaining tissue or blood samples. In addition, invasive sampling may often be impractical given the elusive or dangerous nature of many species that would make capture highly challenging and potentially hazardous. Non-invasive genetics is therefore the safest method of genetic sampling for routine studies on wild populations of endangered large mammals.

The problems encountered in non-invasive genetic sampling are well documented in the literature (e.g. Taberlet *et al.* 1999; Broquet *et al.* 2007) and typically fall into three categories: low DNA quantity; low DNA quality (due to degradation of DNA); and poor extract quality (i.e. the presence of PCR inhibitors; Taberlet *et al.* 1999). The development of reliable molecular markers and methods of statistical analysis (Luikart *et al*. 2010), and of multiplex PCR kits specifically designed to reduce error rates and improve amplification of low-quality faecal DNA (e.g. Piggott *et al.* 2004) have greatly improved the accuracy of results obtained from faecal sampling. The advent of whole genome amplification and the invention of specific PCR and DNA extraction kits are reducing these problems yet further (Bertorelle *et al.* 2009). A fourth category should also be added to this list: the difficulty in encountering a sufficient number of faecal or hair samples in the field to support a statistically robust study.

As with any form of genetics study there are considerable associated costs in terms of reagents and the equipment required for extraction, amplification and analysis of the DNA. The costs of this form of sampling are hugely outweighed by the ability to generate unparalleled data on population and family structure, inbreeding rates, gene flow etc., which would otherwise be impossible to collect. Highly informative studies on elusive species such as the okapi (*Okapia johnstoni*; Stanton *et al*. 2014) and the giant panda (*Ailuropoda melanoleuca*; Zhan *et al*. 2006) have demonstrated the utility of non-invasive sampling in correcting population distribution and census size estimates previously generated using non-genetic methodologies. Through this technique, it is possible to collect highly robust and useful data from animals that are extremely difficult to monitor through other methods. The use of non-invasive genetic techniques has great potential to expand the range of studies available to tapir researchers, and remains one of the most powerful tools in ecological sampling.

1.7. Molecular Markers

1.7.1. MtDNA

Mitochondrial DNA (mtDNA) has been in common use in population genetics and phylogenetic studies since the late 1970s (Ballard & Whitlock 2004). Initial enthusiasm around the use of mtDNA as an excellent population and evolutionary biology tool was largely due to the relative ease of isolating and comparing sequences, its simple sequence organization, maternal inheritance, absence of recombination and both rapid and approximately standardized rate of sequence divergence (Harrison 1989). MtDNA has been used extensively in studies on both between-species genetic differences, to determine phylogenetic relationships between taxa, such as the work by Ruiz-Garcia *et al*. (2012) on the *Tapirus* genus, and within-species population structure, such as the range-wide study of *T. terrestris* by de Thoisy *et al*. (2010). Due to its utility in a wide range of studies, and the relative ease of extracting DNA and analysing sequences, mtDNA remains a major tool for population genetic and phylogenetic studies (Hurst & Jiggins 2005).

The mammalian mitochondrial genome consists of 35 genes and a control region. Different regions within the mitochondrial genome may be sequenced depending on the level of resolution required in the study. For fine-scale studies below the species level, sequence analysis of the highly variable control region or displacement loop is advised (Brown *et al*. 1986). For lower-resolution studies such as regional population structure or higher taxonomic relationships, the less-rapidly evolving *cytochrome-b* gene is recommended (Irwin *et al.* 1991). There are, of course, exceptions: Ruiz-Garcia *et al*. (2012) found sufficient variability in the *cytochrome-b* gene during their study on the evolution of the Neotropical tapirs to assign structure to within-country populations of these three tapir species.

The assumption that mtDNA is a neutral marker has been challenged (e.g. Ballard & Whitlock 2004; Bazin *et al.* 2006). This has implications for the use of mtDNA in phylogenetic and conservation genetic studies. Despite its almost ubiquitous use in population biology, it has been suggested that mtDNA is of limited use in conservation biology, as mtDNA diversity may not consistently reflect species abundance or ecology. It is recommended that comparative studies be carried out using a range of nuclear DNA markers to confirm any conclusions on phylogenetic data arrived at through the use of mtDNA markers (Ballard & Whitlock 2004).

Information derived from mtDNA can, however, still be highly relevant to species conservation, due to its power in resolving population structure. Metapopulation theory assumes that connected populations are more robust and resistant to extinction than fragmented populations (Sodhi & Ehrlich 2010). Populations that display obvious genetic structure can be assumed to be more fragmented, and thus at greater risk of extinction, and these data can be used in the implementation of conservation policies. As no genetics studies have been carried out on Baird's tapir in Honduras, the use of mtDNA to identify population structure is particularly relevant to on-going conservation efforts.

1.7.2. Microsatellites

Microsatellites, also known as Simple Sequence Repeats (SSRs) or Short Tandem Repeats (STRs) are short (usually 70-200 bp) repeats of 2-6 base pairs that occur in very large numbers throughout the nuclear genome. Microsatellites are highly variable, with each locus typically exhibiting considerable polymorphism within a population, enabling fine-scale genetic relationships to be resolved with a high degree of statistical confidence. Microsatellites are widely distributed throughout the genome and are largely located in non-coding regions. As such they are excellent candidates for studies into individual identity, relatedness of individuals and populations, dispersal patterns, and population structure (Vignal *et al.* 2002).

The discovery of microsatellites as a viable molecular marker led to great advances in the molecular analysis of populations (Bruford & Wayne 1993). Increasingly, microsatellite analysis using faecal DNA has become the preferred method of estimating population size in rare and elusive animals (e.g. Taberlet *et al*. 1997; Zhan *et al.* 2006). This method has been instrumental in monitoring fluctuations in population size, and in refining and updating previous censuses in particularly difficult-to-observe species such as the giant panda (Zhan *et al.* 2006). Errors in assigning a genotype to a sample are, however, relatively common due to the high frequency of PCR artefacts such as allelic dropout and false alleles that affect the scoring of microsatellite genotypes. These problems, as discussed previously, are exacerbated in non-invasive genetics due to the presence of poor-quality DNA. Typically five percent of PCR reactions may produce a PCR artefact, so performing a genotyping error-rate study is necessary in assigning confidence limits to your genotypes (Taberlet 1999).

The advent of next-generation deep sequencing provides researchers with the opportunity to obtain highly accurate whole-genome data. These techniques do, however, require high quality DNA such as is not possible to obtain from faecal samples, thus the use of microsatellite analysis is likely to remain prevalent in non-invasive genetic studies for the foreseeable future. It must be added that data from deep sequencing studies so far largely confirm the results already provided by microsatellite data, which suggests that we can be confident of the accuracy of microsatellite analysis techniques (Hohenlohe *et al.* 2010).

Microsatellite analysis is an obvious choice for any study on tapir populations. This is particularly true in this instance due to the paucity of population data on Baird's tapir in Honduras, and the lack of robust studies on wild populations of tapir using microsatellites.

1.8. Non-molecular survey tools

1.8.1. Transect surveys

Transect surveys, the use of purpose-designed survey lines for biological monitoring, are a simple way to conduct standardised, repeatable surveys of biological populations, which enable the robust analysis of population trends over time (Anderson *et al*. 1979). Transects necessarily only encompass a fraction of the overall area being surveyed. The principal assumption of transect surveys is, therefore, that data collected from the transects are representative of the overall habitat. Transects may be particularly useful for monitoring species that live in difficult-to-access regions such as dense forests, where survey capability is inhibited by landscape features.

Transect surveys can be used to obtain a range of count data, depending upon the suitability of the environment. In ecological surveys of large mammals, transects are often used to estimate population density and abundance (e.g. Plumptre 2000). Typically, data on the encounter rate with the target species, and the perpendicular distance between the transect – or the observer – and the target species, are required in order to calculate density and abundance (Buckland *et al.* 1993).

It may not be possible to obtain a distance metric in all circumstances, so indices of relative abundance may be favoured over indices of absolute abundance (Blake 2002; Mackenzie *et al*. 2002). Due to the complex nature of the survey sites for the present study, and the likelihood of not encountering live tapir in the field, the collection of distance-metric data for tapir spoor was expected to be unreliable (Plumptre 2000), and estimating relative abundance was determined as more practical.

1.8.2. Patch occupancy analysis

Patch occupancy analysis is a method of assessing occupancy and detection probability of a species within a statistical framework, providing an index of abundance that allows for the analysis of population trends. Occupancy, the proportion of a habitat that is occupied by a target species, is a fundamental population parameter, and understanding patterns of occupancy is critical in the practical management of wildlife populations. Estimating the proportion of the survey site that is occupied by the target species is increasingly being used as an inexpensive and rapid alternative to estimating actual population size or density (Mackenzie *et al*. 2006). Occupancy is particularly useful as a surrogate for abundance when working with elusive species at large spatial scales, due to the difficulties in deriving accurate estimates of abundance of wide-ranging species on a landscape scale (Hines *et al*. 2010).

Not only can estimates of absolute abundance be expensive and logistically impractical, particularly when relying upon spoor counts in complex terrain (Mackenzie *et al*. 2002), but they can potentially give a misleading impression of the conservation status of a species. A population of the target species may exist in relatively high numbers in a very limited proportion of its habitat, and therefore be susceptible to the increased extinction risk arising from edge effects and isolation (Lawes *et al*. 2000; Fahrig 2003). It is possible to model relative abundance as a function of occupancy, though caution should be applied to such estimates due to inaccuracies that can arise from any variation in detection probability across sites (Royle & Nichols 2003; Royle 2006).

Occupancy estimates are constrained by the detection probability of the target species (Mackenzie *et al* 2002). In most biological surveys there is no guarantee that a species will be recorded during each sampling effort (i.e. the probability of detection is less than one). As such, the formulae used to estimate occupancy must take into account the probability of encountering a species if it is present at the sampling site (Mackenzie *et al.* 2002). Detection probability is calculated from the pattern of detection and non-detection over repeat sampling occasions. Environmental heterogeneity can affect detection probability and confound assessments of presence and absence (Royle 2006). This problem is dealt with by performing repeat surveys of the survey sites to enable occupancy and detection to be modelled as separate parameters. Several studies discuss the importance of correct experimental design before conducting an occupancy survey (Guillera-Arroita *et al.* 2010; Kery *et al.* 2010; Mackenzie *et al.* 2005; O'Connell *et al.* 2006), and all emphasise the need for high numbers of replicates as being a key element to the experimental design.

Mackenzie *et al.* (2005) provide important guidelines to researchers planning on performing an occupancy study depending on the ease of detecting the study species. One of the most important recommendations relates to the allocation of survey effort: when the probability of encounter (p) is greater than 0.5, it is advised to conduct a minimum of three replicate surveys and to increase this if $p < 0.5$. It is recommended that replicates be completed in rapid succession to maintain the closed system assumptions of the model and to minimise temporal detection heterogeneity (Mackenzie *et al.* 2005). If it is not possible to perform temporal replicates, sampling several localities within a survey 'site' can be used as a surrogate for temporal replication (Mackenzie *et al*. 2006).

Presence/absence data can be gathered using a range of survey methodologies. The development of software (PRESENCE; Mackenzie *et al.* 2006) specifically for the analysis of presence-absence data has greatly assisted the estimation of patch occupancy and relative abundance, and in quantifying the impact of different environmental variables on occupancy. From the data inputted, models can be created that offer potential explanations for the data that are observed, and which describe competing hypotheses about the study system relating to occupancy (*Psi*), colonisation (*gamma*), extinction (e*ps*) and detectability (*p*; Table 1.2). The model that best describes the data is chosen using 'goodness of fit' concepts such as Akaike's Information Criterion (AIC) or other maximum likelihood (ML) tests (Johnson & Omland 2004), which provide an information-theoretic method for model selection (Mackenzie *et al.* 2003).

Table 1.2: Description of the parameters for occupancy analyses using the programme PRESENCE. PRESENCE 5.1 (Hines 2010) allows for a spatio-temporal analysis of relative abundance depending upon patterns of occupancy and detectability.

Parameter	**Description**
Psi	Occupancy - the probability that a site is occupied.
gamma	Colonisation - the probability a site which is unoccupied in one year is occupied the next.
eps	Extinction - the probability a site which is occupied in one year is not occupied the next.
p	Detectability - the probability that tapir will be detected in an occupied site.

Patch occupancy analysis is a rapid and economic method of generating the kind of data that the TSG require on tapir presence and relative abundance. This technique has been used on a range of large mammals e.g. blue duikers (*Philantomba monticola*; Lawes *et al.* 2000) and Bengal tigers (*Panthera tigris tigris*; Hines *et al.* 2010), and is currently being used in another study on Baird's tapir in Nicaragua (Christopher Jordan, pers. comm.). Patch occupancy analysis using the software PRESENCE is now being recommended for all ecological studies on tapir by the Tapir Specialist Group.

1.9. Honduras and its significance for Baird's tapir

Honduras has traditionally been seen as a stronghold for Baird's tapir. Tapir are found across Honduras, largely located in National Parks and protected areas in the belt of mountains that runs parallel to the Caribbean coast, which comprises the Honduran section of the Mesoamerican Biological Corridor (Kaiser 2001). The vast area of forest straddling the border between Honduras and Nicaragua on the Caribbean coast, known as La Moskitia, is the largest area of tropical forest in Central America and is likely to retain the largest single population of Baird's tapir across its entire range. Current population estimates, although likely to be imprecise, suggest that Honduras contains approximately 10 % of all Baird's tapir left in the wild (Castellanos *et al*. 2008).

In the 40 years up to 1990, Central America has seen almost 70% of its forests felled to make way for agricultural and urban expansion (Revington 1992), and the rapid rate of land conversion has continued since then, with Honduras reporting an annual deforestation rate of 2.88% between 2000 and 2005 (Rhett Butler, pers. comm.). Despite these levels of deforestation, Honduras has followed the trend in Central American countries in gazetting large areas of land as National Parks. As of 2012, 25,336 km^2 of protected areas existed in mainland Honduras (Figure 1.3) out of a total landmass of 112,492 km^2, amounting to almost 21% of its terrestrial territorial area (data calculated using ArcGIS v 10.1 from the map of protected areas in Honduras provided by N. Estrada, pers. comm.).

In principle this level of preservation is a coup for conservation in Honduras; but in practice the landscape is of a highly fragmented nature, with many small and isolated protected areas being surrounded by agricultural land, which is predicted to be unsuitable for habitation by species such as Baird's tapir. Many of these protected areas are small, with only five over 1000 km^2 and one over 10,000 km^2; and many are at higher altitudes and are isolated by being surrounded by agricultural land in the adjacent lowlands. This would suggest that many of these protected areas may be unable to sustain viable populations of large mammals, if they are experiencing severe edge effects (Woodroffe & Ginsberg 1998).

Figure 1.3: Map of the protected areas of Honduras, including important cities and regions.
Key: Protected areas surveyed for Baird's tapir spoor during this study. 1 = Cusuco National Park (PNC); 2 = Pico Bonito National Park (PNPB); 3 = proposed Sierra Rio Tinto National Park (PNSRT); 4 = Rio Platano Biosphere Reserve (BRP). La Moskitia, including the BRP, is the largest area of tropical forest north of the Amazon, and is expected to be one of the most important regions for Baird's tapir occupancy throughout its range.

Honduras has considerable societal challenges that hamper the implementation of conservation programmes in the country: it has the highest murder rate in the world (over 90 people per 100,000 per annum), huge problems with drug-trafficking, very poor school attendance figures, poverty, and extremely rapid rates of land conversion (UNODC 2012; McSweeney *et al*. 2014). Another concerning trend in Honduras has been the rise of deforestation linked to the narcotics trade. Forest loss in Eastern Honduras, including the La Moskitia region, increased nearly seven times between 2007 and 2011 with a corresponding five-fold increase in the reported movement of cocaine through the region (McSweeney *et al*. 2014). This increase has been attributed to the cartels moving their operations into Honduras after Mexico strengthened their anti-narcotics policing in 2006, and is an example of the way in which unforeseeable social events can put huge pressure on natural systems.

Despite this, several studies have attempted to characterise Baird's tapir distribution in Honduras, all of which have focussed on the extensive protected area network, as hunting renders tapir occupancy outside the protected areas essentially impossible. Tapir have been confirmed to be present in 11 protected areas (Cusuco National Park, El Carbon National Park, Nombre de Dios National Park, Patuca National Park, Pico Bonito National Park, Rio Platano Biosphere Reserve, Rus-Rus National Park, Sierra de Agalta National Park, Sierra Rio Tinto National Park [proposed], Tawahka Asangni Biosphere, Texiguat Wildlife Refuge; N. Estrada pers. comm.) and it is possible that they also still remain in a few of the Parks yet to be surveyed.

To date, only one published study has attempted to estimate tapir numbers in Honduras, which estimated the population of Baird's tapir in the North East of the country to be between 520 and 2760 mature individuals (Flesher 1999). In practical terms, this estimate is too imprecise to derive an accurate population estimate, and was discounted by the Tapir Specialist Group when they most recently estimated that the Honduran population numbers approximately 500 mature individuals (Castellanos *et al*. 2008). Despite this, the area in question, La Moskitia, is clearly a very important region for Baird's tapir conservation due to its size and relative inaccessibility.

Baird's tapir conservation receives good political support in Honduras. During the International Tapir Symposium of 16–21 October 2011, the conservation of Baird's tapir in Honduras received a boost with the signing of a memorandum of understanding between the Minister Director of the Honduran Institute of Conservation and Forestry (Instituto Nacional de Conservación y Desarrollo Forestal, Áreas Protegidas y Vida Silvestre; ICF) and the TSG. In addition to the MOU between the Honduran Government and the TSG, Baird’s tapir has been designated as a priority indicator species by ICF and considerable funds are available for research and educational outreach programmes.

Due to the amount of remaining habitat suitable for tapirs - in particular in La Moskitia, the political support afforded to Baird’s tapir in Honduras, and the amount of conservation work already ongoing in the country, Honduras remains an important country in the Baird’s tapir range. Due to the complex nature of the landscape, and the scarcity of Baird’s tapir in Honduras, Honduras is a suitable study site to develop survey techniques for studying rare and elusive species in a challenging landscape.

1.10. Objectives

This study focuses on the conservation status and threats to Baird’s tapir in Honduras, by analysing the genetic structure of four isolated populations, and through spatial and temporal analysis of occupancy and distribution patterns in a range of landscapes. By surveying Baird’s tapir in four protected areas under different levels of anthropogenic pressure, this study will further elucidate the response of the species to the effects of hunting and habitat alteration. The long-term viability of Baird’s tapir populations is poorly understood, and the use of multiple techniques to determine the conservation status of the species in Honduras, which is considered a stronghold for Baird’s tapir, is important in determining the viability of the species across its range.

Understanding the response of Baird’s tapir to habitat loss and fragmentation, to hunting, and to other forms of anthropogenic disturbance, are the most pressing issues that need to be answered, and are applicable for the study of threatened large mammals in all environments. In presenting an analysis of the conservation status of Baird’s tapir, a poorly-understood species living in a highly challenging environment, this study aims to improve our understanding of the viability of Baird’s tapir populations in Honduras, and provide a template for other researchers working on threatened and elusive taxa.

1.11. Hypotheses

Very few studies have attempted to monitor changes in tapir populations over time and space, or to define the genetic structure of wild populations of tapir in general, and Baird’s tapir in particular. Given the incredibly elusive nature of Baird’s tapir, very little reliable information exists about their natural history. With the limited information available, I hypothesised the following:

1) Baird's tapir populations in Honduras are declining as a result of anthropogenic pressures.

   Predictions:

   a) Encounter rates with tapir spoor will reduce over time.

2) Baird's tapir distribution is mediated by human activities on both a local and national scale.

   Predictions:

   a) Tapir spoor will be observed at higher densities in areas with low human activity.
   b) Tapir occupancy will be negatively correlated with deforestation on both a local and landscape scale.
   c) No gene-flow will be found between study sites where forested corridors do not exist.

3) Habitat fragmentation and the isolation of populations will play a role in defining the genetic structure of Baird's tapir in Honduras.

   Predictions:

   a) Genetic diversity will be higher in the Rio Platano Biosphere Reserve than in Cusuco National Park, due to its larger size.
   b) Gene-flow will be lower in the most isolated Baird's tapir populations compared to more proximate sites.
   c) Genetic structure will be identifiable between isolated populations of Baird's tapir.

To test the hypotheses above, I conducted a total of eight surveys in four protected areas in Honduras to search for evidence of Baird's tapir occupancy. I non-invasively sampled Baird's tapir in all four protected areas, collecting a total of 120 faecal samples, and over 1,500 records of tapir spoor.

1.12. Thesis Structure

Chapter 2 presents a description of the field methodology and the genetic methodological development used in this study.

Chapter 3 explores the phylogenetic structure of Baird's tapir using a 676bp sequence of the cytochrome-b gene, and also describes the efforts to characterise Baird's tapir genetic diversity using previously-published microsatellite primers. The importance of low genetic diversity in Baird's tapir is discussed in terms of the conservation implications of low genetic diversity for the viability of the species.

Chapter 4 explores the use of patch occupancy analysis in identifying the environmental determinants of Baird's tapir occupancy. Evidence is presented that Baird's tapir occupancy is partially determined by a complex suite of factors relating to accessibility and anthropogenic disturbance.

Chapter 5 expands on the conclusion in Chapter 4 that occupancy is negatively correlated with accessibility, and presents the discovery that the distribution of Baird's tapir across Cusuco National Park has altered over time as a result of transect avoidance. In addition, evidence is presented that the population has suffered a demographic decline in the same period, likely to be attributable to the improvement of access into previously remote parts of the Park. This chapter is in preparation for submission as:

Niall McCann, Ryan Gardiner, Ruth Cox, Scott Schilds, Tim Coles, Mike Bruford, and Phil Wheeler (in prep). Creating a new transect network introduces an observation bias in surveys of Baird's tapir.

Chapter 6 presents a PHVA for Baird's tapir across its range in Honduras, and defines sustainable hunting levels for Honduras and for each of the protected areas where Baird's tapir still occur. This chapter concludes that under current hunting levels the population could be extirpated from PNC within the next 20 years, and that the survival of the species in Honduras is partially dependent upon the maintenance of connectivity between remaining populations. Population estimates are generated for the remaining areas of tapir occupancy, and the viability of these populations is discussed in terms of current theory on minimum viable populations and metapopulation theory. A shortened version of this chapter, included as Appendix 1, has been published as:

McCann, N. P., Wheeler, P. M., Coles, T., and Bruford, M. W., 2012. Rapid ongoing decline of Baird’s tapir in Cusuco National Park, Honduras. Integrative Zoology 7: pp. 420-428.

Chapter 7 presents an overview of the outcomes of the whole project, discusses its successes and limitations, and describes the positive impact that this project has had on the conservation of Baird’s tapir in Honduras.

Chapter 2. Field methodology and Genetics methodological development

2.1 Summary

This section includes a site-specific summary of the sampling methodologies used in the field, and an outline of the techniques used in the collection, storage, extraction and amplification of faecal DNA for genetic analyses.

2.2. Field Sampling methodology

2.2.1. Overview

The conservation status of Baird's tapir in Honduras was assessed during surveys in four protected areas (Figure 1.3), spread across the Caribbean coast of Honduras: Cusuco National Park (PNC), Pico Bonito National Park (PNPB), the proposed Sierra Rio Tinto National Park (PNSRT), and the Rio Sikre – in the Rio Platano Biosphere Reserve (BRP). Between June 2009 and August 2012, PNC was surveyed five times, while PNSRT, the BRP and the northern and southern parts of PNPB were each surveyed once.

Permission to work in Honduran protected areas and export permits for faecal samples were granted by the Honduran governmental department Instituto Nacional de Conservacion y Desarrollo Forestal, Areas Protegidas y Vida Silvestre (Institute of Conservation and Forestry; ICF). Import permit IAPPPO1 for the importation of faecal samples was granted by DEFRA.

2.2.2. Spoor sampling methodology

Surveys were undertaken to identify Baird's tapir spoor (sightings, prints, faeces, evidence of foraging; figure 2.1) for patch occupancy analysis, and to collect faecal samples for genetic analyses. Due to inherent differences in the physical and human geography of the five sampling sites, it was not possible to maintain a rigorously standardised sampling regime between sites. Sampling was standardised as much as possible to reduce the potential for sampling bias (Tyre *et al*. 2003). The site-specific methodology for all five sampling sites is given in Section 2.3.

Baird's tapir spoor is highly conspicuous given the size and mass of tapir, and the soft soil substrate found in tropical Honduras. Footprints and dung are unmistakeable and easy to identify by a diligent observer. Differences in observational effort and observer bias have been shown to confound efforts to identify trends in population abundance and distribution (Kery *et al.* 2010). A standardised sampling regime, using a minimum of two trained personnel, travelling at approximately the same speed during all surveys, was adopted to mitigate these potentially confounding variables.

Evidence of foraging is more inconspicuous than tapir footprints and dung. It may sometimes be difficult to authenticate the origin of foraged plants, as Baird's tapir are sympatric with other browsing herbivores (red brocket deer, white tailed deer; Emmons & Feer 1997). Evidence of foraging was typically encountered in association with footprints. On rare occasions where footprints were not also identified, evidence of dentition or the height-off-the-ground of the defoliated plant was usually sufficient to confirm the identification of the species responsible. In situations where it was not possible to confirm the foraging species, the spoor sample was not recorded.

All encounters were recorded on a Garmin Vista HCX GPS unit. A separate protocol for faecal sampling is described in section 2.2.3.

Figure 2.1: Examples of Baird's tapir spoor encountered during field surveys in Honduras.
1: Typical evidence of foraging in Cusuco National Park (PNC) showing bark chewed by a tapir. 2: Baird's tapir footprint in mud in the Rio Platano Biosphere Reserve (BRP).

2.2.3. Faecal sampling methodology

The collection of faecal samples was standardised for all field sites. The age and condition of a faecal sample can affect the amplification success of faecal DNA and the reliability of genotyping (Piggott 2004). After conducting a degradation experiment (see Chapter 3), samples were collected if they were assumed to be no older than 72 hours. The age of samples encountered in the field was estimated based upon appearance, moisture content, the presence or absence of fungal fruiting bodies and on the estimated age of other tapir spoor surrounding the sample. The ageing of samples was always performed by myself, or either of my two guides (Roger Alvarenga and Mito Alvarenga) from whom I learned to age samples, mitigating observer bias and minimising the inevitable variance in the age attributed to each sample. Sample age was estimated to the nearest 24 hours.

When any novel faecal pile was encountered and estimated to be less than 72 hours old, approximately 5 ml of faeces was removed from the exterior of the bolus and stored in 90 % ethanol in a 15 ml falcon tube. Previous studies have shown that the majority of host (target) DNA in a faecal sample can be located on the exterior of the bolus, whereas the interior of the bolus is predominately composed of dietary material (Broquet *et al*. 2007). The sample was transferred into silica gel (Type III indicating; Sigma S-7625) after 24 hours as per the protocol outlined by Roeder *et al*. (2004). For each faecal sample a GPS reading was taken. On return from the field samples were frozen at -20°C for long term storage at Cardiff University.

2.3. Site specific methodology

2.3.1. Baird's tapir in Cusuco National Park

Cusuco National Park (PNC) is located at 15° 30' – 15° 32' N, 88° 13' – 88° 16' W in north western Honduras. The Park covers a total area of 234.4 km^2, with a 76.9 km^2 core zone and a 157.5 km^2 buffer zone. It has an altitudinal range of just above sea level to 2425 m. The Park was recently ranked as the 123rd most irreplaceable protected area for biodiversity globally, the 48th most irreplaceable for threatened taxa, and the 25th most irreplaceable for threatened amphibians (Le Saout *et al*. 2013).

Dedicated biodiversity monitoring programmes have been ongoing in PNC since 2006, when the Park was identified as a stronghold for Baird's tapir. The population of tapir was estimated by local hunters as being between 50 and 70 individuals, representing a significant fraction of the national population, estimated as 500 individuals (Castellanos *et al*. 2008).

Operation Wallacea maintain seven camps around the Park, which are used as bases for scientific research between June and August every year. In 2006, four or five transects up to 3 km in length were cut at each camp to provide a transect network for standardising the sampling methodology used by Operation Wallacea researchers (Figure 2.2). Due to the extremely complex nature of the landscape it was not possible to make these transects either straight or uniform in length, (range 400 m – 3,000 m, mean 1,597 m), but their creation has made it possible to conduct repeatable surveys in large swathes of the Park.

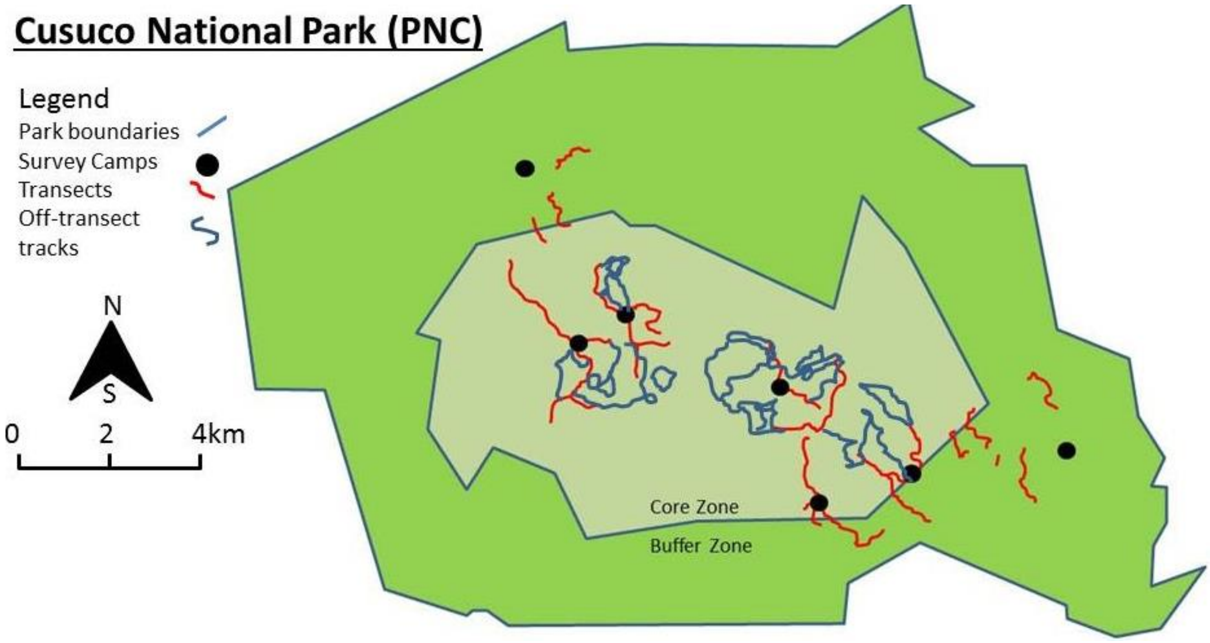


Figure 2.2: Map of Cusuco National Park (PNC), Honduras.
The locations of the seasonal research camps, transects (red), and all off-transect survey tracks (blue), used to survey for Baird's tapir spoor are included. Transects (n=29) were walked twice per year from 2009-2012. Off-transect tracks (n=23) were each walked once between years 2011 and 2012.

PNC was comprehensively surveyed over a total of 24 weeks between June 2009 and August 2012. Annual surveys were conducted in the warmer months of June-August (four weeks in 2009, seven weeks in 2010, eight weeks in 2011, four weeks in 2012); an additional one-week survey was conducted in November 2010, when the Park is much colder and wetter.

2.3.1.1. Cusuco National Park: on-transect surveys

Pre-existing transects, created to monitor spatio-temporal trends in biodiversity, were used to conduct surveys for tapir spoor in PNC. A total of 29 transects were walked twice a year during field seasons in 2009-2012 by a minimum of two trained observers. Observers walked at approximately 1.5 km/hour and recorded all encounters with tapir spoor. Details of the approximate age of the spoor and spoor type were recorded. Surveys were carried out at all times of the day, as the identification of spoor was assumed not to vary depending upon time of day. A total of 194.3 km was walked along the transect network between 2009 and 2012. Data from surveys in 2006, 2008 and 2013 were added to the dataset for the analysis described in Chapter 5.

2.3.1.2. Cusuco National Park: off-transect surveys

A number of surveys were carried out off-transect in 2011 and 2012 in order to assess the distribution of tapirs beyond the transect network. Off-transect trails were designed to radiate out from each of the five core-zone camps, to sample regions of the Park not intersected by the transect network (Figure 2.2). A total of 23 off-transect tracks (seven in 2011 and 16 in 2012) were each walked once by a minimum of two trained observers. Observers walked at an average speed of 1 km/hour and recorded all encounters with tapir spoor. A total of 101.85 km was walked off-transect in 2011 and 2012. Data from an additional eight off-transect trails, surveyed in 2013, and totalling 20.87 km, were included in the analysis presented in Chapter 5.

2.3.2. Baird's tapir in Pico Bonito National Park

Pico Bonito National Park (PNPB) is located in the Cordillera Nombre de Dios, the long mountain range that runs parallel to the central portion of the northern coast of Honduras. It was established on 1 January 1987 and covers an area of 1126 km^2, including a 564 km^2 core zone and a 562 km^2 buffer zone. It has an altitudinal range of between 60m and 2,480 m. PNPB has been shown to be even more important than PNC for biodiversity, being ranked as the 70th most irreplaceable protected area in the world for biodiversity, the 26th for threatened taxa, and the 15th for threatened amphibians (Le Saout *et al*. 2013).

PNPB was chosen as a field site due to its central position in the Honduran part of the Mesoamerican Biological Corridor, and due to information from researchers from the NGO Panthera, who have been conducting an extensive survey of jaguar distribution in Honduras, attesting to the presence of Baird's tapir (F. Castaneda, pers. comm.). Due to the incredibly inaccessible nature of parts of the Park, this location would also enable a comparison of tapir distribution and density patterns in areas with different levels of human disturbance. Access into PNPB is very limited, with a small number of trails used by the Pico Bonito Lodge and other tour groups, and a small number of additional trails created by Panthera for the purpose of surveying jaguar in the Park. A few hunting trails also exist. Due to the highly complex nature of the landscape, expert local guides are essential to successfully carry out any work in the area.

PNPB was surveyed on two occasions for a total of three weeks. 12 tracks, covering 107.17 km, were surveyed for the presence of tapir spoor with the assistance of guides hired locally

(Figure 2.3). The northern edge of PNPB and the hills immediately west of the Park core zone were surveyed in December 2010. Seven locations were surveyed for tapir spoor, covering a range of habitat types including tropical dry forest, tropical broad-leaved upper-montane forest and cloud forest. Survey sites were accessed on foot from local villages; survey tracks totalled 52.2 km in length. Tracks were designed to encompass as much potential tapir habitat as possible in the time available, and followed existing trails, or obvious lines of weakness such as streams and ridges where available. Five trained observers walked at approximately 1 km/hour and recorded all encounters with tapir spoor.

The south side of PNPB was surveyed in June 2012. The expedition started from the village of Los Horcones on the south of PNPB. A base camp was established at an elevation of 1640 m (N 15^{o} 33' 24.8" W 86^{o} 55' 04.8") on the northern slope of a mountain ridge. In addition to the 34.25 km long trail cut to reach base camp, four further survey tracks, covering a total of 20.72 km, were designed to radiate out from the camp and cover as much potential tapir habitat as possible. These tracks were each walked once, at approximately 1 km/hour, by four trained observers, to identify tapir spoor and collect faecal samples. A full report detailing the results of the expedition and a follow-up expedition conducted in 2013 is expected for publication in the near future (Jocque *et al*. unpublished data).

Figure 2.3: Map of Pico Bonito National Park (PNPB), Honduras.
Survey tracks, used to survey for Baird's tapir spoor, in 2010 (black; n=7) and 2012 (red; n=5) are indicated. The location of the village of Los Horcones, the starting point for the survey in 2012, and the site of the base camp in 2012 are also given.

2.3.3. Baird's tapir in the proposed Sierra Rio Tinto National Park

The proposed Sierra Rio Tinto National Park (PNSRT) is an area of national forest located in the northeast of Honduras, covering a total of 882 km^2, with a core zone of 230 km^2 and a buffer zone of 652 km^2. PNSRT was chosen as a suitable field site as Baird's tapir were suspected but not confirmed to be present.

Three survey sites were identified, covering the northern, central and southern portions of the Park (Figure 2.4). Each site was surveyed on foot in June 2011 with the assistance of guides hired locally. In each case, a base camp was established inside the Park from which a total of six surveys, covering 57 km and radiating out from the three base camps, were made into the surrounding forest in search of tapir spoor. Survey tracks followed existing trails or obvious lines of weakness. Observers walked at approximately 1 km/hour and recorded all encounters with tapir spoor.

In the case of Site 2, Campo Santo, problems with the local guides precluded the team from surveying inside the core zone of the Park, where tapir occupancy is suspected to be higher.

This highlights the importance of hiring suitable guides and communicating accurately what is required of them.

Figure 2.4: Map of the proposed Sierra Rio Tinto National Park (PNSRT), Honduras.
Survey tracks (n=6) used to survey for Baird's tapir spoor in June 2011 are marked in red, in addition to the three survey sites. Tracks at the Campo Santo site did not reach the Park core zone.

2.3.4. Baird's tapir in the Rio Platano Biosphere Reserve

The Rio Platano Biosphere Reserve (BRP) is an 8,331 km^2 protected area with a core zone of 5250 km^2 and a buffer zone of 3081 km^2. The BRP is located within La Moskitia, the largest wilderness area in Central America, and the largest area of tropical rainforest north of the Amazon. La Moskitia covers a vast area straddling the border between Honduras and Nicaragua, on the Atlantic coast of Central America (see Figure 2.5). La Moskitia is seen as an important refuge for Baird's tapir due to its size and low human population density (Flesher 1999; Dunn *et al*. 2012). The BRP is home to five groups of indigenous peoples, and in the absence of any roads the area has seen very little development or exploitation; hunting pressure on Baird's tapir is thought to be minimal.

The BRP was chosen for this study due to its remoteness and the expectation that tapir populations here, in the absence of significant human disturbance, would achieve densities representing maxima for the species. The BRP would therefore provide a comparison with isolated and impacted populations elsewhere in Honduras, providing a baseline of genetic diversity and patch occupancy against which other populations could be compared.

The Rio Sikre in the BRP was surveyed as a single line transect in May 2011 by ascending 94.1km of the Rio Sikre by boat from the village of Brus Laguna, where local guides were hired. Tapir spoor was observed on the river banks, and counted by four trained observers while ascending the river at a speed of approximately 5km/hour. The soft mud and latterly sand banks allowed for easy identification of spoor where tapir had approached or crossed the river. There is evidence to suggest that tapir associate strongly with water (Matola *et al.* 1997), but as the forests of the BRP are filled with streams and swamps there is no reason to believe that tapir would cluster along the Rio Sikre in a way that would give an overestimate of general occupancy. Faecal sampling was conducted in the forests on both banks of the river.

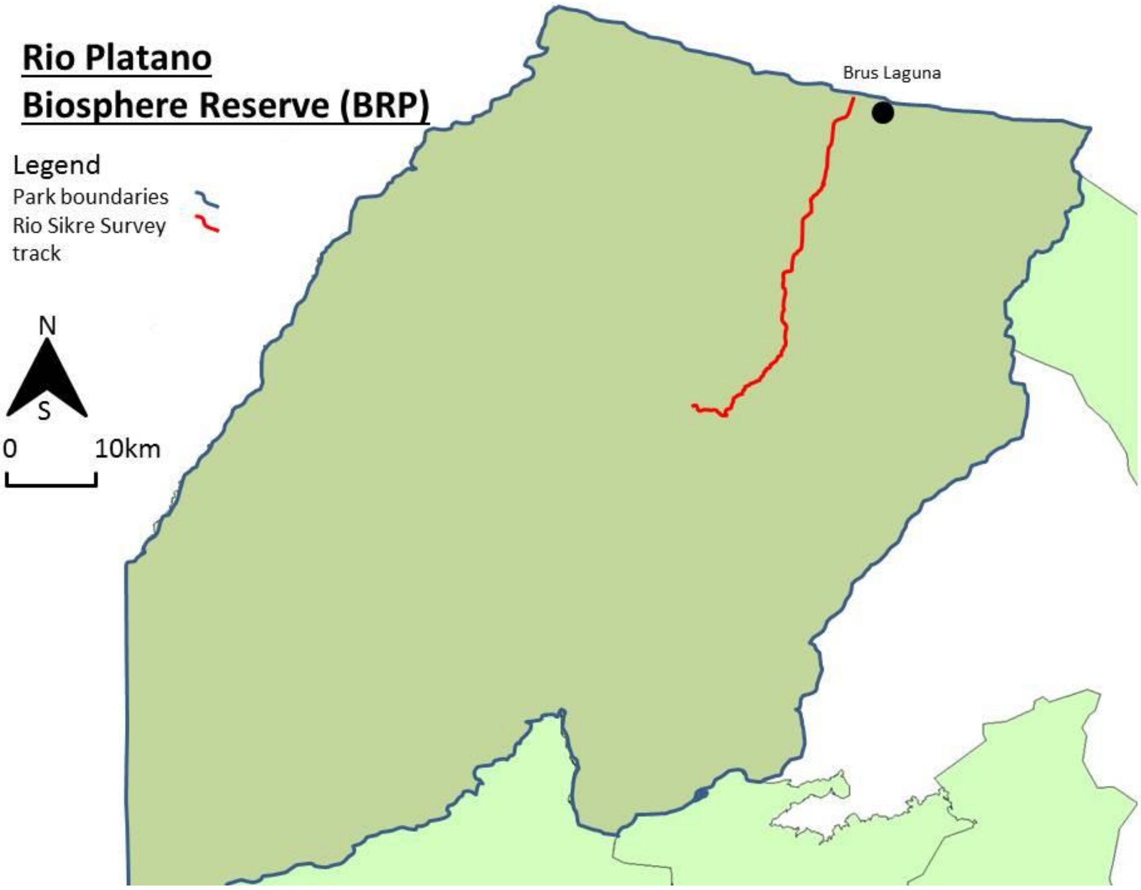


Figure 2.5: Map of the Rio Platano Biosphere Reserve (BRP), Honduras.
The survey track on the Rio Sikre (red), used to survey for Baird's tapir spoor in May 2011, is indicated. Details of the location of the boundary of the BRP core zone were not available.

2.4. Genetics methodology

In addition to Baird's tapir faecal samples (n = 120) collected in the field, five millilitres of blood from a captive *T. terrestris* was provided by Bristol Zoo Gardens for use as a positive sample in testing all stages of the genetics methodology.

2.4.1. DNA Extraction

Extraction of DNA from faecal samples was performed using two commercially available kits: QIAamp DNA Stool Mini Kit (QIAGEN; catalogue number 51504), and HighPure PCR Template Preparation Kit (Roche Diagnostics; catalogue number 11796828001). Small modifications to the manufacturer's protocol were made to the QIAGEN process to maximise the likelihood of DNA being successfully extracted: carrier RNA was added to the extraction process to enhance DNA extraction as per the protocol outlined by Shaw *et al*. (2009), and the centrifugation time was doubled for a number of steps to maximise supernatant volume. Once extraction was complete, samples were returned to storage at -20^{o}C. PCR reactions were performed using 1.5 µl of DNA added to 3µl of QIAGEN multimix, 1 µl of 0.001 mg/µl bovine serum albumin (BSA) and 1 µl of each primer (forward and reverse) at 2 µM, to a total volume of 6.5 µl. Amplification was confirmed by electrophoresis on a 2% agarose gel for 40 minutes. The two kits were discovered to be equally successful in the quantity of the DNA extracted (judged by the intensity of bands in the electrophoresis gel). The Roche Diagnostics kit was chosen as the extraction process took one quarter of the time.

Extraction of DNA from the *T. terrestris* blood sample was performed using a DNeasy Blood & Tissue Kit (QIAGEN; catalogue number 69504). To prepare the sample, 200 µl of blood was mixed with 40 µl proteinase K and 200 µl phosphate buffered saline. 200 µl buffer AL was added to the mix, and then incubated for 10 minutes at 56 ^{o}C. The extraction process was then completed by following the DNeasy Blood & Tissue handbook from step 3 onwards. Extraction success was confirmed by PCR using the protocol outlined in Section 2.4.2.1.

2.4.2. Microsatellites

Microsatellite analysis remains the preferred methodology for genetics studies that aim to determine a range of fine-scale population statistics (Vignal *et al*. 2002), especially with non-invasively collected material (Broquet *et al*. 2007). Microsatellite analysis has previously been used to study wild Baird's tapir populations (Norton & Ashley 2004a).

2.4.2.1. Microsatellite primer selection and PCR optimisation

A total of 23 microsatellite primers were identified from the literature (Norton & Ashley 2004a & 2004b; Goncalves da Silva *et al.* 2009; Sanches *et al.* 2009). Norton and Ashley designed two polymorphic microsatellite primers for Baird's tapir and four for *Tapirus terrestris*; Goncalves da Silva *et al*. designed seven, and Sanches *et al.* designed eight polymorphic microsatellite primers for use in *Tapirus terrestris* (Table 2.1). Goncalves da Silva *et al.* suggested that cross amplification within Tapiridae should be possible due to what they described as the relatively recent divergence of extant tapir species, and due to the high degree of genetic conservation among the Tapiridae, as demonstrated by Trifonov *et al*. (2008). Due to this conclusion, existing primers were utilised for the purposes of this study, in lieu of designing a new microsatellite library for Baird's tapir.

An initial study was performed to optimise the primers and the PCR conditions. Amplifications of all 23 microsatellite loci were performed using DNA extracted from the *T. terrestris* blood sample obtained from Bristol Zoological Gardens and five *T. bairdii* faecal samples from PNC. PCRs were performed using a Techne Flexigene thermal cycler. Reaction conditions for the preliminary optimisation experiments for primers Tap1 – Tap6 were conducted as per Norton and Ashley (2004a & 2004b), using 1.5 µl of DNA added to 3 µl of QIAGEN multimix, 1 µl of 0.001 mg/µl BSA and 0.5 µl of each primer (forward and reverse) at 2 µM, to a total volume of 6.5 µl. A range of annealing temperatures were trialled depending on the recommended annealing temperature of each primer (Table 2.1), using the PCR reaction conditions as follows: 94^{o}C for 5 minutes, 50 cycles of 94^{o}C for 45 seconds, annealing temperature for 30 seconds, 72^{o}C for 90 seconds, then a final extension of 72^{o}C for 10 minutes.

Table 2.1: Characterisation of 23 microsatellite loci developed from genomic libraries of *Tapirus terrestris* and *T. bairdii.*

Loci Tap1-Tap6 were developed by Norton and Ashley (2004a); loci Tap7-Tap13 were developed by Goncalves da Silva *et al*. (2009); loci Tap 14-23 were developed by Sanchez *et al*. (2009). PCR conditions and amplicon details for all loci are also presented.

Locus	**Motif**	**Primer sequences 5'-3'**	**Annealing Temperature (°C)**	**Size (bp)**	**Alleles**
Tap1	(AC)25	Forward ATTAAGCAGATGCCAACCTGAAG Reverse CCCTGTGGTGTTTTTTGGATC	60	144–154	6
Tap2	(TC)10(AC)10	Forward TCAACCCCCTCCTTGTCTCTTC Reverse ACTGATGGAACCGAGAAGCCAC	63	144–154	4
Tap3	(TC)11(AC)10	Forward GCAGGCAAGGCTGAGGTTTT Reverse CTGCATTCTCCTTTGCCGACGGC	60	114–124	3
Tap4	(AC)19	Forward TTAGGGAAATAACAGGTCTGG Reverse GTTGTTTTGCATCCAAATTGG	55	162–170	3
Tap5	(AC)19	Forward TTGACCTTTTCATAAGCAGCC Reverse CCATCTCTTCCATTCCAGTTC	53	214–238	7
Tap6	(AC)14	Forward ACAGTTTGTTCCCTCCAAGGTTG Reverse GCAGGTCAAATATACTGTCAGCCTGG	53	218–238	4
Tap7	(AC)13	Forward TGGCAACATGGAATATAAAGC Reverse GAAGCCCACGATGTTACAGG	59 - 51	307–315	4
Tap8	TT (TG)20	Forward TCACTTGAGTGACCCACACC Reverse TTACAGACTCACCGGGCTTC	59 - 51	172–180	5
Tap9	TT (GT)12	Forward GCAATTCCCACACAAACTG Reverse CCCTCCTTTTGACGATGAAC	59 - 51	161–171	4
Tap10	(CT)2CATA(CA)16	Forward TGCTCCAGCAAAAGAATCTG Reverse GGCTGGTGGCTACCATACTG	59 - 51	234–256	8
Tap11	(GT)17	Forward ACCATATGCCAAGGGTTTTG Reverse GCTGCCTTCATAGTGGCTTC	59 - 51	262–286	9
Tap12	(GT)16	Forward AGGAACAGACACACCGCTTC Reverse AACTGAAGGAGCAGATCTTGG	59 - 51	194–202	5

Tap13	(CA)7CT(CA)4CTCA	Forward AGGCAGACTGTGACTGCTCA Reverse GGGGGATTTCACTGTTAAGG	59 - 51	250–268	4
Tap14	(CATT)6	Forward CGTTAGCATGATCTCTAGACC Reverse GCTGGAAGGTAAGATCTGTG	53	156–168	4
Tap15	(TG)20	Forward TCAACCCCCTCCTTGTCTCTTC Reverse CCAGATGAGAAGCAGGATAG	53	230–264	11
Tap16	(GT)10	Forward TGCCCTGATTTAGAGAAAAC Reverse AGGAGAAGTTAGAAGGGGAA	53	199-209	7
Tap17	(AATG)5	Forward CCTGTGCAGCATTTGATAAC Reverse GGTTGACCAGTTTAATGCAG	53	142-174	6
Tap18	(CAGG)7	Forward GGACACTCAAGTGGGTCAAG Reverse AGTGTATGCTTGTCGCGC	53	168-192	5
Tap19	(TCTG)7	Forward GCTCTCTGGCTTTTACACACT Reverse GGAAAGCTGAAAAGGAGGA	53	156-192	7
Tap20	(CA)20	Forward CCATGCAATTAAGAGAAAGC Reverse CAGCTAAGGACAGGAAAATG	53	252-282	9
Tap21	(CA)22	Forward GATCCTCCTGTTTGCAGAT Reverse AGCCAAATGTTTTAGACTGAG	53	174-208	13
Tap22	(TC)29	Forward TGCCACATTTGTTCAGTCTC Reverse GGCTGAAATATTGTATCTGCA	53	274-300	5
Tap23	(CA)7	Forward AGAGTGTCAGATGTCCTGCC Reverse TGCTTTGTGTTTGAGTGTGC	53	98-120	7

The primers developed by Norton & Ashley (2004a & 2004b) returned inconsistent results. The primers often failed to amplify at the target locus, and non-specific products were frequently observed (Figure 2.6).

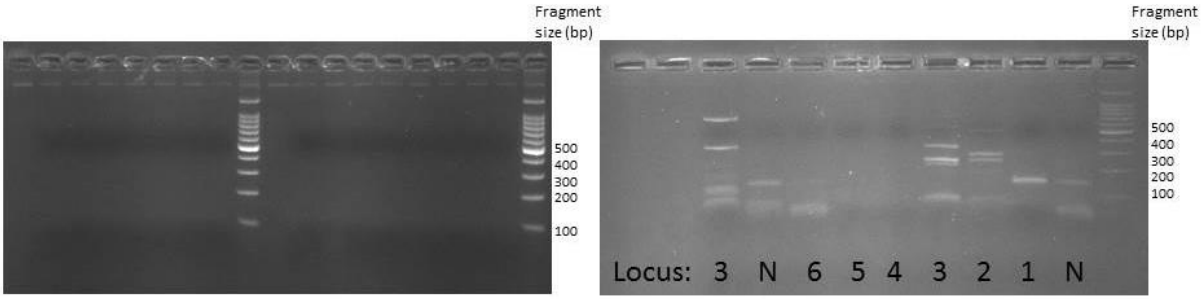


Figure 2.6: Electrophoresis gels for PCRs of Baird's tapir faecal DNA.
PCRs were performed using primers published by Norton and Ashley (2004a and 2004b) to amplify DNA from Baird's tapir faecal samples. The gels demonstrate zero amplification success (left) and non-specific banding (right).

For primers Tap7 – Tap13, amplifications were performed in a final volume of 6.60 µl containing 3 µl QIAGEN multimix, 1 µl primer (2 µM concentration of each forward and reverse primer), 0.6 µl of BSA at 0.001 mg/µl, and 2 µl DNA. PCRs were conducted using a touchdown protocol as follows: 95°C for 10 minutes; 30 cycles of 95°C for 30 seconds, annealing temperature for 30 seconds, and 72°C for 1 minute; and a final extension step of 72°C for 10 minutes. The annealing step in the touchdown programme decreased 1°C per cycle from 59 °C to 51°C at which point the remaining cycles continued with an annealing temperature of 51°C, as per the protocol outlined in Goncalves da Silva *et al*. (2009).

For primers Tap14-Tap23 amplifications were performed in a final volume of 6.60 µl containing 3 µl QIAGEN multimix, 1 µl primer (2 µM concentration of each forward and reverse primer), 0.6 µl of BSA at 0.001 mg/µl, and 2 µl DNA. PCR conditions were as follows: 95 °C for 5 minutes, 35 cycles at 94 °C for 30 seconds, 57 °C for 1 minute, 72 °C for 45 seconds followed by 15 cycles at 94 °C for 30 seconds, 53 °C for 45 seconds, 72 °C for 45 seconds and a final extension 72 °C for 10 minutes, as per the protocol outlined in Sanches *et al*. (2009).

A range of different reagent concentrations and PCR conditions were trialled with zero amplification success, judged by the appearance of appropriately-sized bands when PCR products were viewed on electrophoresis gel. A successful trial was conducted using identical PCR conditions in thermocyclers in a separate laboratory at Cardiff University (Figure 2.7). PCR conditions were then optimized using thermocyclers in the second laboratory, until PCR products appeared consistently clean.

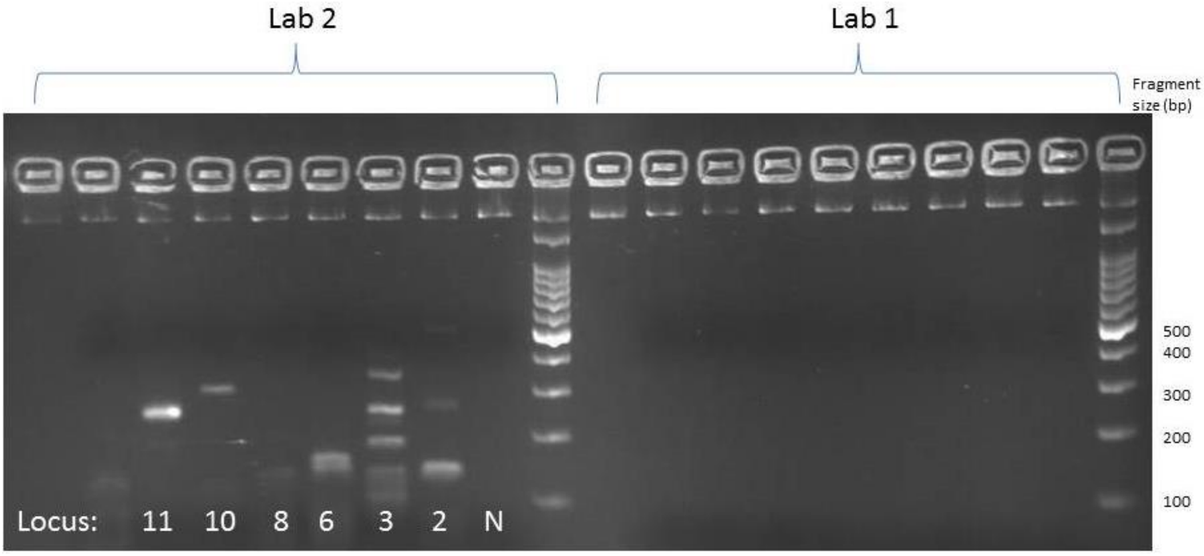


Figure 2.7: Electrophoresis gels from two identical PCRs run in two different laboratories at Cardiff University.
The gels demonstrate the difference in success between PCRs using different thermocyclers. PCRs were designed to amplify DNA extracted from Baird's tapir faeces, and were performed using primers designed by Norton and Ashley (2004a, 2004b), Goncalves da Silva *et al*. (2009), and Sanchez *et al*. (2009).

Eleven primers that consistently yielded reliable polymorphic banding patterns were selected from the initial trial of 23 primers. All of the primers developed by Norton and Ashley (2004a, 2004b) were rejected; the primers chosen for this study had previously been identified as polymorphic in *T. terrestris* (Goncalves da Silva *et al*. 2009; Sanchez *et al*. 2009). Three multiplex groups were designed (Table 2.2), and the compatibility of the primers in each multiplex group was checked using the programme Autodimer v1.0 (Vallone & Butler 2004). No compatibility issues were suggested. All further reactions were performed using the primer conditions described above.

Table 2.2: Microsatellite primer multiplex groups for Baird's tapir genotyping error rate study. Multiplex groups were chosen according to amplicon size and primer compatibility assessed using the programme Autodimer v1.0 (Vallone & Butler 2004).

Multiplex 1	**Multiplex 2**	**Multiplex 3**
Primer Tap7	Primer Tap17	Primer Tap18
Primer Tap9	Primer Tap21	Primer Tap19
Primer Tap10	Primer Tap23	Primer Tap20
Primer Tap12		
Primer Tap13		

2.4.3. Mitochondrial DNA

Analysis of genetic diversity of mitochondrial DNA has been used in non-invasive genetics studies across a wide range of taxa, and in inter- and intra-species studies of tapir (e.g. de Thoisy *et al*. 2010; Ruiz-Garcia *et al*. 2012; Cuzzuol *et al*. 2013).

2.4.3.1. Mitochondrial primer selection and PCR optimisation

The mitochondrial control region has been shown to be more polymorphic than the *cytochrome-b* gene (Tang *et al*. 2006), and is often used in preference to *cytochrome-b* for phylogenetic studies. Previous attempts to amplify the mitochondrial control region in *Tapirus* species have been unsuccessful (Manuel Ruiz-Garcia, pers. comm.). Primers designed to amplify the mitochondrial control region (Kocher *et al*. 1989) were trialled in the present study, but amplification success was zero after 16 PCRs in four separate trials using blood-extracted DNA from *T. terrestris* and DNA from a fresh faecal sample from *T. bairdii*. These primers were subsequently rejected in favour of *cytochrome-b* gene primers (Table 2.3). The study by Ruiz-Garcia *et al.* (2012) suggested that sufficient polymorphisms exist within Baird's tapir to justify the use of *cytochrome-b* for country-wide phylogenetic studies in *Tapirus* species.

Table 2.3: Conserved primers for two regions of the mitochondrial genome.
These primers were tested for utility in the amplification of DNA from Baird's tapir faecal samples. Primer pair L7 H6, designed for perissodactyls (Tougard *et al*. 2001), was successful; primer triplet L15926 L16007 H00651 designed for cross-taxa use (Kocher *et al*. 1989) was unsuccessful.

Locus	Target Region	Sequence
L7 H6	Cytochrome-b	5'-ACC AAT GAC ATG AAA AAT CAT CGT T-3' 5'-TCT CCA TTT CTG GTT TAC AAG AC-3'
L15926 L16007 H00651	Control Region	5'-TCAAAGCTTACACCAGTCTTGTAAACC-3' 5'-CCCAAAGCTAAAATTCTAA-3' 5'-TAACTGCAGAAGGCTAGGACCAAACCT-3'

Amplifications for the *cytochrome-b* gene were performed on a sub-set of blood and faecal samples with the primers L7 (5' ACC AAT GAC ATG AAA AAT CAT CGT T 3') - H6 (5' TCT CCA TTT CTG GTT TAC AAG AC 3'), designed for perissodactyls (Tougard *et al.* 2001). The PCRs were performed in 25 µl with reaction mixtures including 12.8 µl nuclease-free H_2O, 5 µl 5x GoTaq Green Buffer, 1 µl of 0.001 mg/ml BSA, 2.5 µl of 2mM MgCl2, 0.5 µl 10 µM dNTPs, 0.5 µl of each primer at 25 µM, one unit of Taq DNA polymerase (Gotaq, Promega), and 2 µl DNA from faeces. PCR reactions were carried out in a Techne Flexigene thermal cycler using the following reaction conditions: 94°C for 5 minutes, 35 cycles of 50 s at 94°C, 50 s at 53°C and 1.5 minutes at 72°C and a final extension time for 10 minutes at 72°C. All amplifications, including positive and negative controls, were checked in 2% agarose gels, employing the molecular weight marker X174 DNA digested with *Hind* III and *Hinf* I and HyperLadder IV and the gels were visualized in a Hoefer UV transiluminator.

Two pairs of internal primers (pair T1= 5' CAACATCCGAAAATCC 3' - 5' AATGTGTAGGATCCATA 3'; pair T2= 5' ATATTCTTCATCTGCCTA 3' - 5' TCGGGTGAGAATAAT '3) were designed using Sequencher (v5.2, Genecodes Corporation) and tested on a further sub-set of faecal samples to ensure compatibility with Baird's tapir faecal DNA. The newly-designed primers amplify a 746 base pair (bp) stretch of the gene. Samples were sequenced in forward and reverse to ensure accurate sequencing and assembled using Sequencher v5.2. All ambiguous bases were corrected by eye. Samples with suspected polymorphisms were re-sequenced to guarantee the validity of the polymorphism. All subsequent amplifications were performed in a 25µl volume reaction using the reaction mixture outlined above.

Chapter 3. Using genetic techniques to study Baird’s tapir populations

3.1. Abstract

The first analysis of genetic diversity in Honduran populations of Baird's tapir is presented here. Faecal samples were collected from four protected areas across the Caribbean coast of Honduras and sequenced at a 676 bp fragment of the *cytochrome-b* gene. Posterior probability, maximum likelihood and neighbour joining analyses were conducted to resolve the phylogenetic structure of the genus *Tapirus* and to determine the position of the Honduran tapirs within this phylogeny. Two Honduran haplotypes were identified, one corresponding to published sequences of Baird's tapir from Panama and the other represented by a single individual sampled in Pico Bonito National Park in northern Honduras. The implications of low genetic diversity for conservation are discussed, and we recommend that conservation strategies focus on maximising population size and connectivity to ensure the survival of the species in the short term.

In addition, efforts to characterise Baird's tapir genetic diversity using published microsatellite primers are described, and possible reasons for the failure to achieve reliable results are presented. We discuss the importance of developing a dedicated microsatellite library for use with Baird's tapir faecal samples.

Key words

Baird's tapir, Honduras, cytochrome-b, microsatellites, conservation.

3.2. Introduction

The genus *Tapirus* is the last surviving genus of the family Tapiridae, which was once found all across the Americas and Eurasia (Garcia *et al.* 2012). The four extant tapir species (*T. indicus, T. bairdii, T. terrestris, T. pinchaque*) are now confined to a much reduced distribution as a result of climatic changes and recent anthropogenically-mediated habitat loss and hunting (Garcia *et al.* 2012). The evolutionary history of the genus is well characterised (Steiner & Ryder 2011). Genetic and paleontological studies suggest that the last common ancestor between the three surviving Neotropical tapir species and the single surviving Asian species of tapir diverged approximately 17 +/- 2 MYA, with the ancestor of Baird's tapir diverging from the remaining Neotropical tapirs 10.9 +/- 5.4 MYA, and the ancestors of the lowland and mountain tapirs diverging 3.8 +/- 0.9 MYA (Steiner & Ryder 2011; Ruiz-Garcia *et al*. 2012).

All four *Tapirus* species are currently threatened with extinction from habitat loss and hunting pressure (Garcia *et al*. 2012). Of equal concern to the long-term survival of tapirs is the genetic viability of the remaining populations. Small and declining populations are often subject to the deleterious effects of inbreeding (Lacy 1997; Frankham *et al*. 2014), and may be particularly vulnerable to extinction from stochastic events. In addition, many traits relating to evolutionary and reproductive fitness, such as survival and annual reproductive success, correspond to genetic diversity (Sodhi & Ehrlich 2010; Hogg *et al*. 2006). Population genetic parameters used to characterize a species' genetic diversity include observed heterozygosity (*Ho*), defined as either the fraction of individuals in a population that are heterozygous at a given locus, or the fraction of loci that are heterozygous in an individual; and allelic diversity ($\bar{A}$), defined as the number of alleles per locus. In genetically depauperated populations, population vulnerability is exacerbated by isolation (Saccheri *et al*. 1998), but a species may persist where connectivity exists between small populations due to metapopulation or source-sink dynamics (Sodhi & Ehrlich 2010).

A species with isolated populations may not function as a metapopulation if gene flow is not possible between populations. In this case, species with a large overall population size (*Nc*) may in effect be considered as many small sub-populations, each with an effective population size (*Ne*) below the minimum viable population (MVP) size (Frankham *et al*. 2014). Current theory on MVP size suggests that an *Ne* of 100 is required to limit loss in total fitness to < 10 % over five generations, and *Ne* = 1000 is required to retain evolutionary potential in perpetuity (Frankham *et al*. 2014). Identifying genetic signatures of metapopulation dynamics is a crucial step in understanding the level of extinction threat that a species is experiencing (Whitlock & Barton 1997).

The importance of maintaining genetic diversity in tapir populations is acknowledged by the Tapir Specialist Group (TSG), and genetic factors are now incorporated in the conservation planning for all *Tapirus* species (Medici *et al*. 2011). Four of the 12 primary objectives of the Baird's Tapir Action Plan, compiled by the TSG in 2005, explicitly mention genetics, and three further objectives could only be fulfilled through the use of genetic techniques (Table 1.1; Medici *et al.* 2005).

The genetic structure of lowland tapir populations has been well characterised at both mitochondrial and genomic loci (de Thoisy *et al*. 2012; Ruiz-Garcia *et al*. 2012; Cozzuol *et al*. 2013; Pinho *et al*. 2014), and mitochondrial sequences have been used for comparative phylogenetic analysis of the *Tapirus* genus (Ruiz-Garcia *et al*. 2012; Cozzuol *et al*. 2013). The genetic structure within Baird's tapir populations is still poorly understood. Of particular interest to tapir biologists are the phylogenetic history of Baird's tapir and the current conservation status of the species in terms of its genetic potential to adapt to environmental change (Jump & Penuelas 2005).

To date, only six studies have investigated genetic diversity in Baird's tapir (Table 3.1), and no genetics studies have been performed on Baird's tapir in Honduras. This represents a significant gap in our understanding of the genetic diversity of the species and how this might affect its conservation on a regional and international level. Norton & Ashley (2004a) suggest that Baird's tapir demonstrates among the lowest levels of nuclear DNA (nDNA) heterozygosity reported in large mammals, corresponding to other genetically depauperated Neotropical mammals such as the Andean bear (*Tremarctos ornatus*) and Andean cat (*Leopardus jacobita*). Ruiz-Garcia *et al*. (2012) report that Baird's tapir mitochondrial genetic diversity levels are extremely low compared to the other Neotropical tapir species and to other Neotropical mammals.

Table 3.1: List of phylogenetic studies carried out on Baird's tapir.
The origin of the samples and the molecular regions studied are included. List provided courtesy of the Tapir Specialist Group (TSG).

Reference	Wild/Captive	Country of origin	Molecular region studied
Ashley *et al*. (1996)	Captive	Unknown	mtCOII Gene
Norman & Ashley (2000)	Captive	Unknown	mtCOII/12S ribosomal RNA Genes
Norton & Ashley (2004a)	Wild	Costa Rica, Panama	Nuclear Microsatellites
Norton & Ashley (2004b)	Captive	Captive animals derived from Belize, Costa Rica & Panama	Nuclear Microsatellites
Ruiz-Garcia *et al*. (2012)	Wild	Costa Rica, Guatemala, Mexico & Panama	mtCytochrome-b Gene
Cozzuol *et al*. (2013)	Unknown	Unknown	mtCytochrome-b Gene

Understanding how tapir move through their environment, and which environmental features have a significant effect on tapir dispersal and distribution, is a further critical area of study (Pinho *et al*. 2014). Landscape genetics is the synergy between population genetics, landscape ecology and spatial statistics, which feeds into metapopulation theory by examining and quantifying microevolutionary processes such as gene flow, genetic drift and selection across landscapes (Manel *et al.* 2003; Segelbacher *et al.* 2010). As well as enabling us to better understand and quantify the impacts of fragmentation on population dynamics in Baird's tapir, landscape genetics has the potential to address large-scale conservation issues relating to connectivity by identifying landscape features that either inhibit or facilitate migration (e.g. Coulon *et al*. 2004; Perez-Espona *et al*. 2008). Analysing the effect of a range of geographical features on Baird's tapir dispersal will enable the TSG to better understand the habitat requirements of the species, and its resilience in the face of environmental change.

In addition to phylogenetic and landscape genetics studies, non-invasive genetic sampling can be used as a surrogate for traditional capture-mark-recapture (CMR) studies in elusive species, such as Baird's tapir, where obtaining accurate estimates of population size is highly challenging. Genetic CMR studies using faeces have been used to estimate population size in a range of species including coyotes (*Canis latrans*; Kohn *et al*. 1999), forest elephants (*Loxodonta cyclotis*; Eggert *et al*. 2003) and brown bears (*Ursus arctos*; Bellemain *et al*. 2005). Petit and Valiere (2006) demonstrated that genetic CMR over one or multiple seasons yields equivalent estimates of population size to conventional CMR studies when studying a closed population without sampling heterogeneity.

This current study presents further characterisation of the evolution of the genus *Tapirus* with the addition of Honduran *T. bairdii* to phylogenetic trees for the genus, and attempts to better characterise the genetic diversity of Baird's tapir using a range of genetic markers. In addition to an analysis of genetic diversity, this study aimed to present a robust analysis of landscape and population genetics, and to perform a molecular census of the population of Baird's tapir in Cusuco National Park (PNC). This study was designed to test the hypothesis that the genetic structure of Baird's tapir in Honduras is determined in part by habitat fragmentation and isolation. The conservation implications of Baird's tapir genetic diversity are discussed in relation to the impact of isolation and fragmentation on metapopulation dynamics.

3.3. Materials and methods

3.3.1. Sample collection

Using the sampling methodology described in Chapter 2, 120 Baird's tapir faecal samples were collected during field surveys, conducted between June 2009 and August 2012, in four protected areas across the north of Honduras (Figure 3.1). Cusuco National Park (PNC) was visited on five occasions totalling 24 weeks, which is represented by the large (n=85) number of samples collected from this location. Pico Bonito National Park (PNPB) was surveyed twice, for a total of three weeks (number of samples n=5), and the proposed Sierra Rio Tinto National Park (PNSRT) and the Rio Platano Biosphere Reserve (BRP) were each surveyed once, for 11 and 15 days respectively (number of samples n=5 and n=25 respectively).

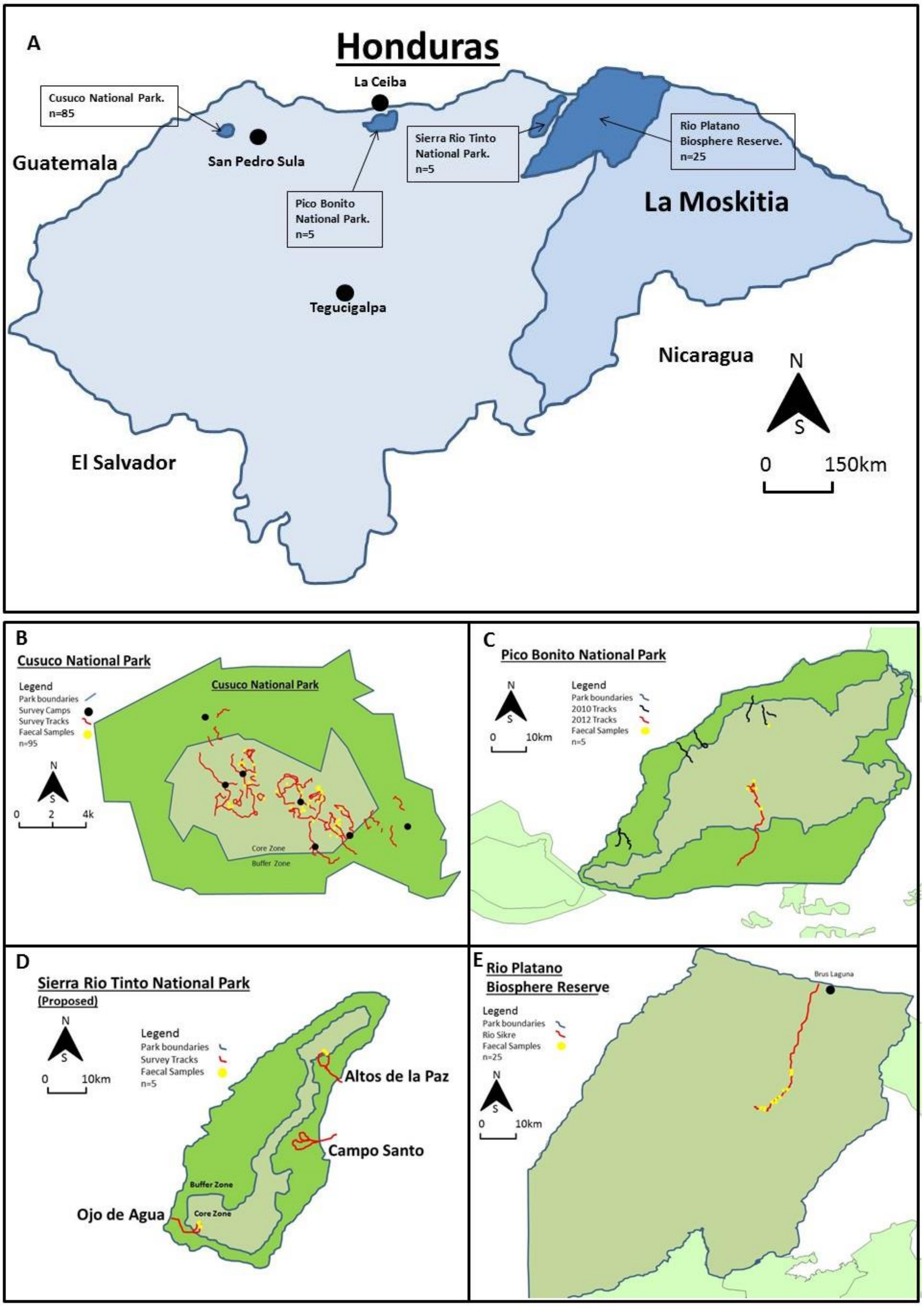


Figure 3.1: Map of faecal sampling sites in Honduras.
Sites were surveyed for Baird's tapir spoor between 2009 and 2012. A: Map of Honduras with the location of the sampling sites and corresponding number of faecal samples collected. B-E: Individual maps of each sampling site with distribution of sampling tracks (red; black for northern Pico Bonito, surveyed in 2010) and faecal samples (yellow) indicated.

3.3.1.1. Degradation experiment

To reduce the probability of collecting unviable faecal samples, a degradation experiment was conducted in PNC in June 2010 to estimate the rate of decay of faecal DNA in the sampling environment (Bertorelle *et al.* 2009). A fresh faecal bolus was placed inside a small cage so that it would not be disturbed by birds or small mammals, but was subject to natural environmental conditions. The bolus was sampled every 24 hours for 72 hours from t=0, during which time period two downpours occurred. These samples were amplified by PCR using the protocol outlined in Chapter 2.4.2.1 and visualised by gel electrophoresis to assess the difference in amplification success concentration of DNA in each sample.

3.3.2. Laboratory protocols

3.3.2.1. Microsatellite primers

DNA extracted from a selection of 12 samples (10% of the total number of samples) was chosen for a genotyping error rate study, as per Prugh *et al*. (2005). PCRs were performed using the three multiplex groups and the reaction conditions described in Chapter 2.4.2.1. PCR products were visualised on a 2% agarose gel, then loaded onto 96-well microtitre plates and sent to the Protein Phosphorylation Unit of the University of Dundee for fragment analysis using Applied Biosystems 3730 DNA analyzers. Microsatellites were scored visually using GeneMarker v1.91 (SoftGenetics, LLC, CA, USA) to identify peaks that consistently showed the same shape for alleles across samples.

3.3.2.2. Mitochondrial Primers

Two pairs of primers that prime for a 746 base pair (bp) stretch of the *cytochrome-b* gene were designed and used in PCRs following the protocol outlined in Chapter 2. A total of 28 samples were successfully sequenced for both primer pairs (T1 & T2), with a further 41 sequenced for primer pair T1 only. Before further analysis, the sequences were trimmed to remove the extremes of sequences where missing data was present. A final selection of 63 faecal sample sequences was selected for phylogenetic analysis using the trimmed sequence length of 256 bp amplified by primer pair T1; 24 faecal sample sequences were analysed using the trimmed sequence length of 398 bp amplified by primer pair T2; 25 faecal sample sequences were analysed using a total sequence length of 676 bp.

3.3.3. Phylogenetic analysis

Many modes of analysis exist for phylogenetic studies (Nei 1996). For consistency, the methods described in Ruiz-Garcia *et al.* (2012), the most comprehensive study on tapir phylogenetics to date, were used in this study. A combination of analytical methods was used to ensure consistency in assigning phylogenetic relationships between haplotypes. Bayesian inference, maximum likelihood (ML) and neighbour-joining (NJ) methods were applied to a haplotype matrix assembled using DNAsp 5.10.01 (Rozas *et al*. 2003). The number of polymorphic sites was assessed using Arlequin v3.1 (Excoffier *et al*. 2005).

The most suitable model for evolution for this DNA dataset was determined using MrModelTest v2.2 (Nylander 2004), which uses Akaike's Information Criterion (AIC) to assign an appropriate model to the data. The General Time Reversible (GTR) plus invariant sites model was selected for all three methodologies.

The Bayesian analysis of phylogenetic inference was carried out using MrBayes v3.2.0 (Ronquist & Huelsenbeck 2003). A total of 10 million Markov-chain iterations were run, disregarding the first 10% as 'burn-in'. The analysis was repeated 10 times. ML and NJ analyses were performed using PAUP* 4.10b (Swofford 2002). Support for the branching of the trees was assessed with nonparametric bootstrapping (npb; Felsenstein 1985), using 100 bootstrap replicates for both ML and NJ. A Median Joining Network (NETWORK 4.6.1.1; Bandelt *et al.* 1999) was created to assess patterns of divergence between the four *Tapirus* species.

Sequences were compared against *cytochrome-b* gene sequences for *Tapirus indicus* (n=31), *Tapirus terrestris* (n=45) and *Tapirus pinchaque* (n=3) downloaded from Genbank, and a further 11 *Tapirus bairdii* sequences, derived from tapir in Costa Rica and Panama, provided by Manuel Ruiz-Garcia (Table 3.2). Including previously published sequences for all four tapir species, a total of 115 tapir sequences were used for the phylogenetic analysis covering 676 bp of the *cytochrome-b* gene (primer pairs T1 & T2); 153 sequences for the analysis of the 256 bp stretch of the *cytochrome-b* gene (primer pair T1 only); and 114 sequences for the analysis of the 398 bp fragment amplified by primer pair T2 only. *Cytochrome-b* sequences for the domestic horse (*Equus caballus*) and white rhinoceros (*Ceratotherium simum*), other representatives of the order Perissodactyla, of which the only surviving clades are the tapirs, horses and rhinos, were downloaded from Genbank and used as outgroups (Steiner & Ryder 2011).

Table 3.2: Origin of *cytochrome-b* sequences for a phylogenetic analysis of the genus *Tapirus.* Sequences were downloaded from Genbank or provided by author. Sequences from *E. caballus and C. simum* (Steiner & Ryder 2011) were downloaded for use as outgroups in the phylogenetic analysis.

Species	Authors	Number of sequences	Genbank Accession number
T. indicus	Muangkram *et al.* (2013)	31	JX014351.1 - JX014321.1
T. terrestris	De Thoisy *et al.* (2010)	45	GQ259910.1 - GQ259954.1
T. pinchaque	De Thoisy *et al.* (2010)	3	GQ259955.1 - GQ259957.1
T. bairdii	Ruiz-Garcia *et al.* (2012)	11	Sequences provided by author.
E. caballus	Steiner & Ryder (2011)	1	JF718882.1
C. simum	Steiner & Ryder (2011)	1	JF718874.1

3.4. Results

3.4.1. Degradation study and amplification success.

Amplification success was judged by the intensity of bands on the electrophoresis gel. On the basis of the degradation study it was decided to collect samples no older than 72 hours old. After testing all the samples in the dataset, it is clear that although the success rate in amplifying DNA from samples of different ages differs considerably with age (Table 3.3), viable quantities of DNA may be obtained from a faecal bolus more than 72 hours after defecation. Amplification success was also shown to be affected by the condition of the faecal sample. Submerged samples had a low rate of amplification success, whereas samples that had been rained upon retained a high rate of success (Table 3.4).

Table 3.3: Success rate in amplifying mitochondrial (mt)DNA from Baird's tapir faecal samples of different ages.
Samples (n=120) were collected in Honduras between 2009 and 2012. Sample age was estimated by a minimum of two trained observers based upon moisture content and other environmental clues. PCR reactions were carried out at Cardiff University between 2011 and 2012.

Age (Days)	number	mtDNA success	% Success
0	33	20	60.61
1	40	27	67.50
2	19	10	52.63
3	18	7	38.89
4	1	1	100.00
5	3	0	0.00
6	3	0	0.00
10	2	0	0.00
20	1	0	0.00

Table 3.4: Success rate for DNA extraction and amplification from Baird's tapir faeces collected in different environmental conditions.
Samples (n=120) were collected in Honduras between 2009 and 2012. PCR reactions were carried out at Cardiff University between 2011 and 2012.

Sampling Conditions	number	mtDNA success	% Success
Dry	60	40	66.67
Rain	16	13	81.25
Partially submerged	23	14	60.87
Fully submerged	7	1	14.29
Unknown	14	8	57.14

3.4.2. Microsatellite analysis.

Analysis of PCRs using DNA from *T. bairdii* faecal samples showed that three primer loci (primers Tap18, Tap21 & Tap23) failed to yield any microsatellite peaks, and that peak sizes for the remaining loci differed from the published sizes (Table 3.5). The rate of success of PCRs using DNA from *T. bairdii* faecal samples, as judged by the appearance of appropriately sized bands when observing PCR products on electrophoresis gels (Figure 3.2), was 68.27%. The rate of success of the same PCRs, as judged by appropriately sized peaks observed using GeneMarker, was 17.91%. Positive PCRs, using DNA from *T. terrestris* blood, yielded 93.36% and 64.36% success when viewed on electrophoresis gels and in GeneMarker respectively (Table 3.5).

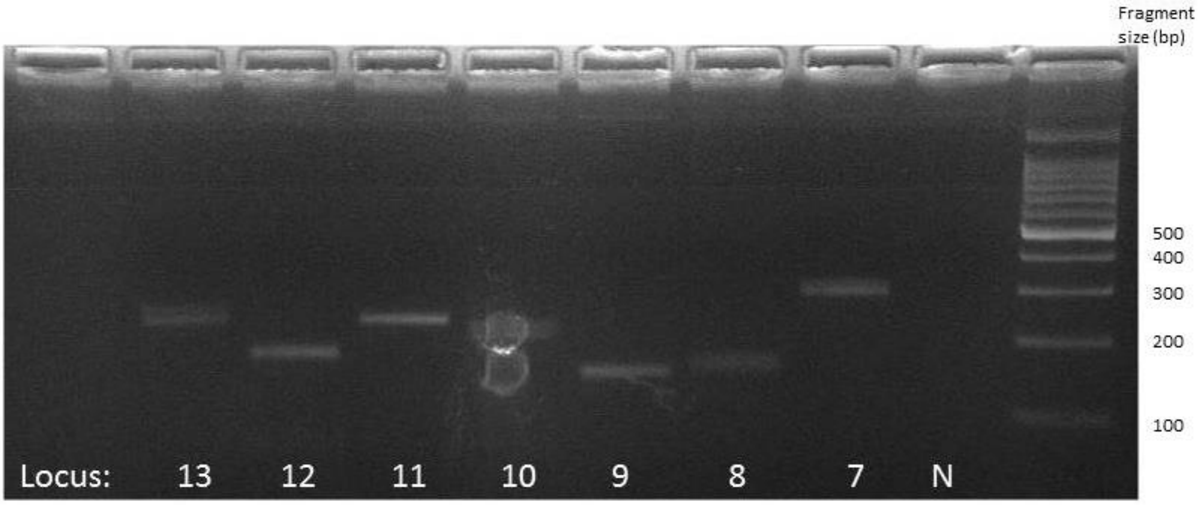


Figure 3.2: Electrophoresis gel demonstrating successful amplification with no non-specific banding. DNA was extracted from Baird's tapir faeces and amplified in PCR reactions using seven microsatellite primers designed by Goncalves da Silva *et al*. (2009).

Table 3.5: Size range of microsatellites and rate of success for PCR reactions.
Success rate (%) for the amplification of DNA from Baird's tapir faecal samples and lowland tapir blood samples is given for PCRs viewed on a gel and in GeneMarker. Primers and size range for *T. terrestris* are as published by Goncalves da Silva *et al*. (2009) and Sanchez *et al*. (2009). Size range for *T. bairdii* microsatellites are as recorded in the present study. The column 'repeats' refers to the number of repeats per primer sent for fragment analysis.

	Faecal Samples *(T. bairdii)*				Blood Samples *(T. terrestris)*			
Primer	**Repeats**	**Gel % Success**	**GeneMarker % Success**	**Micro-satellite size**	**Repeats**	**Gel % Success**	**GeneMarker % Success**	**Micro-satellite size**
Tap7	13	45	23	291-304	4	100	25	307-315
Tap9	112	100	56	143-147	4	100	100	161-171
Tap10	112	50	29	220	4	100	100	234-256
Tap12	112	56	27	177-179	4	83	100	194-202
Tap13	112	67	21	229	4	100	50	250-268
Tap17	112	68	21	154	3	100	67	142-174
Tap18	102	63	0	N/A	3	100	33	168-192
Tap19	112	71	17	187-189	3	100	100	156-192
Tap20	102	70	3	274	3	75	33	252-282
Tap21	112	74	0	N/A	2	86	50	174-208
Tap23	112	87	0	N/A	2	83	50	98-120
	Mean	**68.27**	**17.91**		**Mean**	**93.36**	**64.36**	

PCRs were repeated up to 112 times per locus. Many microsatellite peaks were found to be un-scoreable due to variation in the size or pattern of the peaks between replicates, and extremes of peak intensity, varying by over a factor of 10 between replicates in some cases. Later repeats showed a variable peak pattern that suggested the fragment analysis process was corrupted, or that the samples had degraded over time (Figure 3.3).

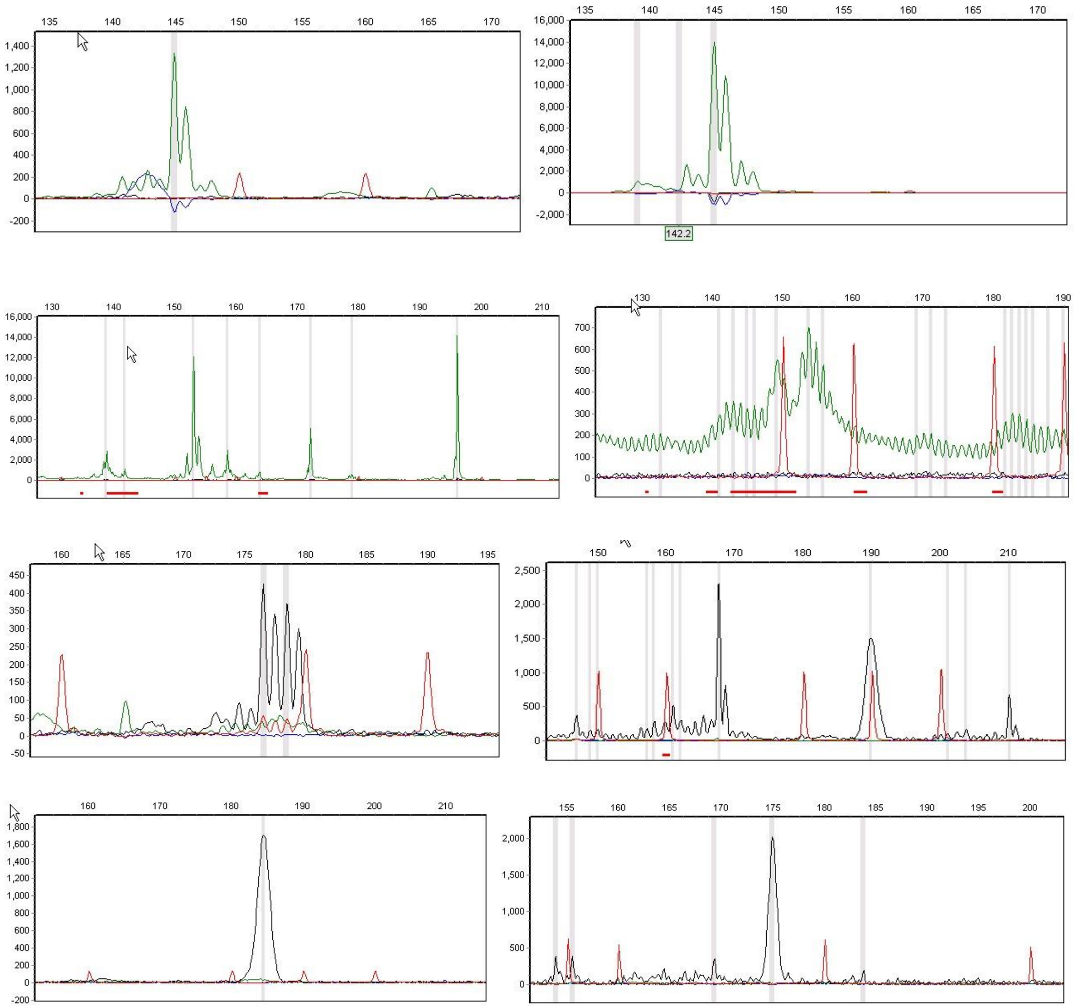


Figure 3.3: Microsatellite peaks viewed in Genemarker.
Electropherograms showing microsatellite peaking patterns for a single *T. bairdii* faecal sample, collected in Cusuco National Park (PNC), Honduras, at two different microsatellite loci: locus Tap9 (green, published size range 161-171 base pairs) and locus Tap12 (black, published size range 194-202 base pairs), demonstrating inconsistent peak pattern, intensity and score. Microsatellite loci are from Goncalves da Silva *et al*. (2009).

Consensus genotypes were calculated after eight repeats of 12 samples across all 11 loci (Table 3.6). Observed heterozygosity (*Ho*) ranged from 0 - 0.75 per locus (mean = 0.09), and from 0 - 0.33 per sample (mean = 0.08). Allelic diversity ($\bar{A}$) ranged from 0 - 3 per locus, with a mean of 1.63 alleles per locus. Three loci failed to yield any genotypes and only four loci were polymorphic. The overall rate of genotyping success was very low, with a mean number of loci successfully genotyped per sample of 3.83. A full table of genotypes including all eight repeats is included in the supplementary materials (Supplementary Table 3.7).

Table 3.6: Consensus genotypes for 12 Baird's tapir faecal samples.
Samples were collected in Honduras. Genotypes were assigned using 11 microsatellite loci after eight repetitions. Sample D was collected in Pico Bonito National Park, 2012. Samples O & S were collected in Cusuco National Park (PNC), 2012. Sample 93 was collected in PNC, 2011. Samples 1, 2, 15, 38, 56 & 57 were collected in PNC, 2010. Sample 58 was collected in Pico Bonito National Park (PNPB), 2010. Sample 62 was collected in the Rio Platano Biosphere Reserve (BRP), 2011. *Ho*: Observed heterozygosity per sample or per locus. *Ā*: allele frequency per locus. Microsatellite loci are as published by Goncalves da Silva *et al*. (2009) and Sanchez *et al*. (2009).

		Locus (consensus size)											
		7	**9**	**10**	**12**	**13**	**17**	**18**	**19**	**20**	**21**	**23**	***Ho***
Sample (consensus size)	**D**	(0)	(147)	(220)	(0)	(229)	(154)	(0)	(0)	(0)	(0)	(0)	**0**
	O	(291)	(145/145)	(220)	(177/179)	(229)	(154)	(0)	(0)	(0)	(0)	(0)	**0.33**
	S	(0)	(145)	(220)	(177)	(229)	(154)	(0)	(189)	(274)	(0)	(0)	**0**
	1	(0)	(0)	(220)	(0)	(0)	(154)	(0)	(0)	(0)	(0)	(0)	**0**
	2	(0)	(0)	(0)	(0)	(0)	(154)	(0)	(0)	(0)	(0)	(0)	**0**
	15	(304)	(147)	(0)	(0)	(0)	(154)	(0)	(0)	(0)	(0)	(0)	**0**
	38	(291)	(145)	(220)	(177/179)	(229)	(154)	(0)	(0)	(0)	(0)	(0)	**0.17**
	56	(0)	(0)	(0)	(0)	(0)	(154)	(0)	(0)	(0)	(0)	(0)	**0**
	57	(0)	(145/147)	(220)	(0)	(0)	(154)	(0)	(0)	(0)	(0)	(0)	**0.33**
	58	(0)	(143)	(220)	(177/179)	(229)	(154)	(0)	(187)	(0)	(0)	(0)	**0.17**
	62	(0)	(143)	(220)	(0)	(229)	(154)	(0)	(187)	(274)	(0)	(0)	**0**
	93	(0)	(0)	(0)	(0)	(0)	(154)	(0)	(0)	(0)	(0)	(0)	**0**
Ho		**0**	**0.25**	**0**	**0.75**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	
Ā		**2**	**3**	**1**	**2**	**1**	**1**	**0**	**2**	**1**	**0**	**0**	

Due to the inconsistent and unscoreable microsatellite peaks achieved early in the study, the low genotyping success rate and the clear degradation of the quality of peaks viewed in the electropherograms later in the study, further analysis of the microsatellite diversity of Baird's tapir in Honduras was unfeasible, and this aspect of the project was discontinued.

3.4.3. Mitochondrial DNA Analysis

From the final dataset of 153 sequences analysed at the 256 bp segment of the *cytochrome-b* gene amplified by primer pair T1, DNAsp and Arlequin identified 17 tapir haplotypes, with all of the Honduran tapir corresponding to a single haplotype.

For the analysis of the 398 bp segment (n=114) amplified by primer pair T2, a total of 26 *Tapirus* haplotypes were identified. The three *T. pinchaque* samples segregated into two haplotypes and the 45 *T. terrestris* samples into 19 haplotypes. *T. bairdii* clustered into three haplotypes: two Panamanian individuals comprised one haplotype, one single Baird's tapir from PNPB in Honduras comprised the second, and the remainder of the samples from Honduras, Costa Rica and Panama (n=32) comprised the third.

No further analysis was conducted on sequences amplified by a single primer pair only.

3.4.3.1. Final haplotype study

From the final dataset of 115 sequences using the 676 bp segment of the *cytochrome-b* gene, 33 tapir haplotypes were identified by DNAsp and Arlequin comprising a total of 146 substitutions including 133 transitions, 13 transversions, and a final total of 140 polymorphic sites. Two haplotypes corresponded to *T. indicus*, two to *T. pinchaque*, four to *T. bairdii* including the two haplotypes from the Honduran dataset, and the remaining 33 to *T. terrestris*, 16 of which were singletons.

3.4.3.2. Phylogenetic Analysis

The four *Tapirus* clades were identified and strongly supported by the ML and NJ analyses from Paup and the posterior probability value from MrBayes (Figure 3.4). Within *T. bairdii* four haplotypes were identified, one (n=2) comprising solely Panamanian samples, one (n=3) comprising solely Costa Rican samples, one (n=1) comprising one single Honduran sample, differing by a single polymorphism from the final haplotype (n=30), comprising the remaining Panamanian and Honduran samples. Ruiz-Garcia *et al*. (2012) reported 11 Baird's tapir haplotypes, which translated to only three in the present study due to the reduced-coverage of the primers (total length 676 bp compared to 890 bp for the Ruiz-Garcia study).

Bayesian clustering into species clades demonstrated very high posterior probability values (Figure 3.4). The divisions between *T. bairdii* haplotypes were less well supported: Bayesian posterior probability (((30,31,0.29),32,0.59),33,1); ML ((30,31,32,59%),33,100%); NJ ((30,31,32,65%),33,100%).

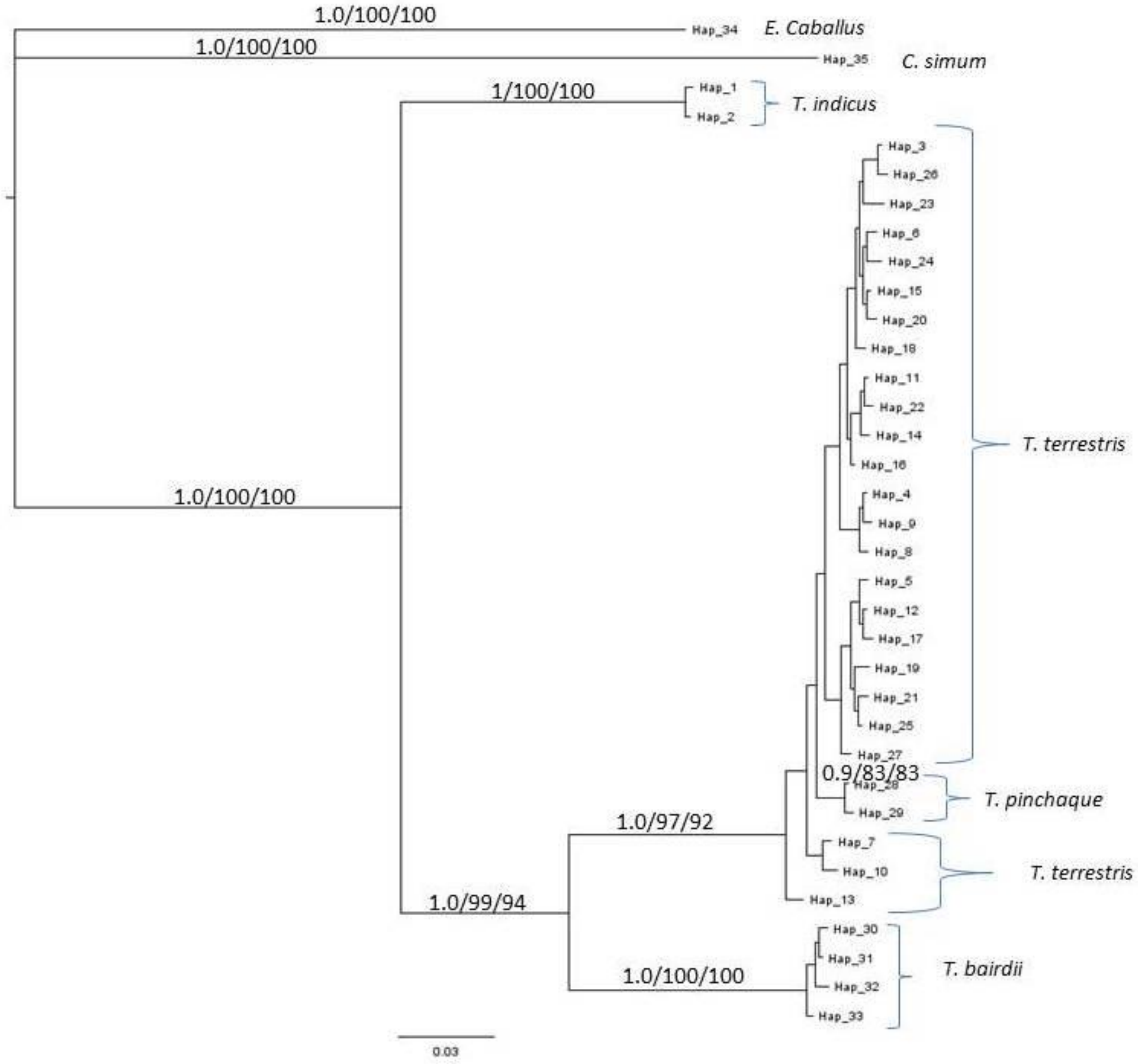


Figure 3.4: Bayesian tree of *Tapirus* haplotypes based on *cytochrome-b* sequences.
Equus caballus was used as an outgroup, and *Ceratotherium simum* as a representative of the remaining extant Perissodactyla lineage. Scores above branches in respective order are: posterior probabilities, nonparametric bootstrap (npb) support values (%) for maximum likelihood (100 replicates), and npb support values (%) for neighbour-joining (100 replicates) for each genus/species clade.

The median-joining network (Figure 3.5) shows strong resolution of the four species clades and confirms the haplotype segregation suggested by Mr Bayes, Arlequin, DNAsp and PAUP.

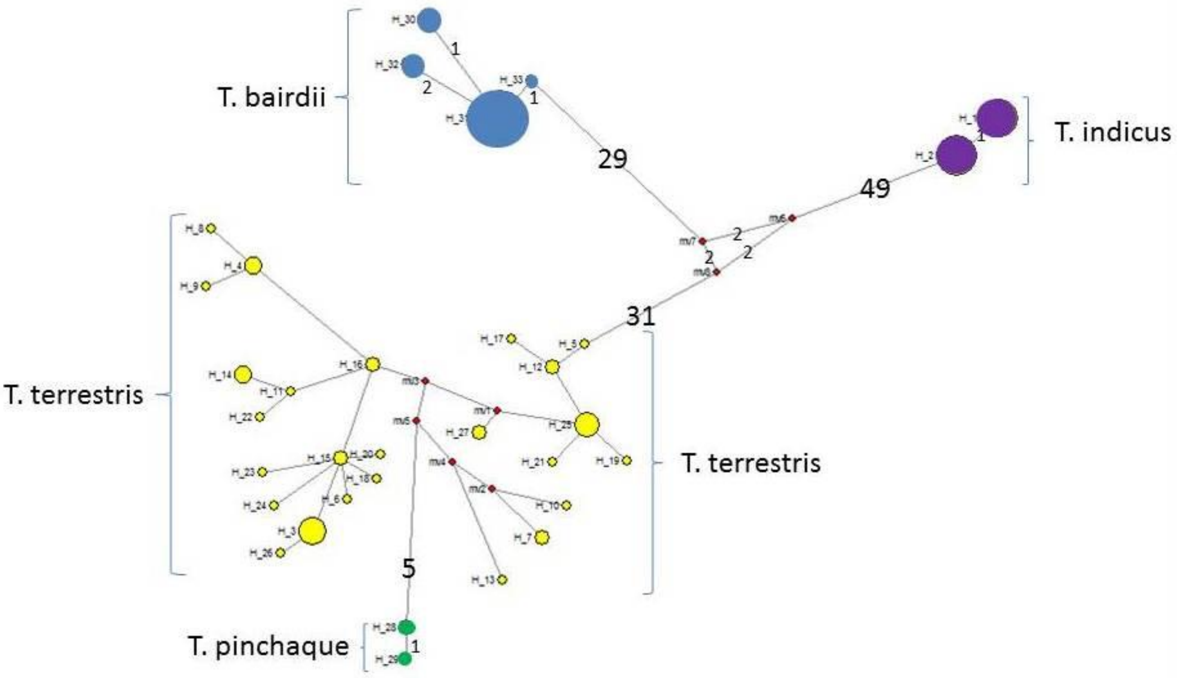


Figure 3.5: Median-Joining Network for haplotypes identified in the four *Tapirus* species.
Key: *T. indicus* (purple), *T. terrestris* (yellow), *T. pinchaque* (green), and *T. bairdii* (blue). Hypothetical connecting haplotypes not identified in the present study are shown in red. The number of mutations between significant clades is shown on each branch.

3.5. Discussion

3.5.1. Amplification success

Sample age has been demonstrated to have an effect on the reliability of genotyping studies (Pigott 2004). In the present study, DNA was successfully amplified from a sample estimated to be 96 hours old. The probability of obtaining viable quantities of DNA from a faecal sample is higher if working with mitochondrial DNA (mtDNA), since mtDNA exists in higher copy numbers per cell than nuclear DNA (Taberlet *et al*. 1999).

Environmental conditions may also affect the probability of successfully amplifying DNA from faecal samples (Piggott 2004). From the present study, exposure to rain over a time-frame of 72 hours or less did not have the negative effect on DNA quality that would have been expected (Piggott 2004). Only fully-submerged boli had a very low rate success rate (Table 3.4). Naranjo (1995, 2009) reported that tapirs may preferentially defecate in water, with a study in Corcovado National Park, Costa Rica, discovering 94% (n=136) of samples in or near water bodies. In the present study, out of 107 samples where precise location data were recorded, 50 (46.73%) were discovered either in or next to a permanent or seasonal water body.

3.5.2. Microsatellite Analysis

Of the 11 loci analysed, three (Tap18, Tap21 & Tap23) failed to yield any scoreable genotypes and only four (Tap7, Tap9, Tap12 & Tap19) were found to be polymorphic in *T. bairdii*, which is insufficient to perform any statistical analysis of population genetic structure.

The *Ho* and *Ā* values calculated here (*Ho* = 0 - 0.75 per locus, mean = 0.09, *Ā* = 0 - 3 alleles per locus, mean = 1.63 alleles per locus) are lower than those previously reported for Baird's tapir (*Ho*=0.39, *Ā*=3.8; Norton & Ashley, 2004a) and many times lower than the figures previously published for these loci (*Ho* = 0.34-0.88, mean=0.68, *Ā*=5.35 alleles per locus [Goncalves da Silva *et al.* 2009]; *Ho* = 0.35-0.93, mean=0.64, *Ā*= 7.4 alleles per locus [Sanchez *et* al. 2004]), which were calculated using high-quality tissue samples from *T. terrestris*. A lower rate of polymorphism is expected when primers designed for one species are used on a related species (Gonzalez-Martinez *et al*. 2004), but the rates achieved here are too low to be informative. The two samples from the Rio Platano Biosphere Reserve (samples 58 and 62) were the only samples to be scored as homozygous for a 143 bp allele at locus Tap9, which suggests that some regional structure may be identifiable in Baird's tapir in Honduras, if a sufficient number of samples can be successfully genotyped.

Due to the well-documented limitations of working with faecal DNA, a low amplification success rate across the entire dataset when using microsatellite primers was anticipated. Given the high success rate demonstrated by the visualisation of appropriately sized bands after gel electrophoresis (68.27%), albeit from the freshest samples in the dataset, it was hoped that it would be possible to genotype a sufficiently large proportion of the dataset to draw statistically robust conclusions about the genetic status of Baird's tapir in Honduras. The reasons for the failure to achieve consistent results from the microsatellite analysis could be many, but prime candidate explanations would be the low quality of DNA yielded from faecal samples, and the evolutionary distance between lowland tapir – for which the primers were developed – and Baird's tapir. The last common ancestor between lowland and Baird's tapir lived approximately 10.9 MYA (Ruiz-Garcia *et* al. 2012), which could be sufficient to cause primer slippage due to a lack of primer site specificity (Fisher *et al*. 1996). The species are cladistically well-resolved at the *cytochrome-b* gene (Figure 3.5; 1.0 posterior probability support, 99% ML, 94% NJ support) and it is likely that they are considerably more divergent at highly variable microsatellite sites, which could cause primer slippage at the binding site, exacerbated by using poor-quality faecal DNA (Boston *et al*. 2012). This explanation is slightly unsatisfactory however, as the primers yielded relatively good results (68.27% success) when observing bands on an electrophoresis gel. The disparity in the success rate achieved with the gels and that achieved when the fragments were analysed (17.91% gave peaks, of frequently variable shape) suggests that the problem may have occurred during the analysis stage, rather than the amplification stage of the process. The corruption of the peak pattern and the appearance of significantly different microsatellite scores in later attempts to analyse the samples (Figure 3.3), suggests that the samples may have degraded over time. The samples were shown to be sufficiently intact to amplify and analyse part of the *cytochrome-b* gene, though mtDNA exists in higher copy numbers per cell than nuclear DNA (Taberlet *et al*. 1999), and is much easier to amplify.

The potential for cross-amplification between taxa has been thoroughly reviewed (Barbara *et al*. 2007). Mammals may have a 40% chance of cross-amplification between families, as much as 50% in the Bovidae (Maudet *et al*. 2003), 72% in the Sciuridae (Gunn *et al*. 2005) and 90% in the Felidae (Williamson *et al*. 2002), and between-genera transfer success is much higher, although amplicon size may differ between species when using non-specific primers (Gonzalez-Martinez *et al*. 2004). Rates of molecular evolution have been shown to vary greatly between species (Zhang & Hewitt 2003), but have been suggested to be low in the Tapiridae (Trifonov *et al*. 2008), which would suggest a strong potential for cross-amplification success between species.

In lieu of estimating Baird’s tapir population sizes using genetic CMR, population estimates using non-molecular techniques are presented in Chapter 6. Hypotheses relating to landscape genetics, nDNA genetic diversity and the feasibility of genetic CMR in Baird’s tapir remain untestable at this time. The failure to achieve usable results from the published microsatellite primers is, however, significant in itself. Although other laboratories may achieve different results, caution should be applied when using any of the existing published primers for a Baird’s tapir genetics project, particularly with faecal DNA. It is clearly important to develop a microsatellite library for use with Baird’s tapir faeces as a resource for future tapir researchers.

3.5.3. Mitochondrial Analysis

The results obtained here are consistent with previous studies in suggesting that Baird’s tapir displays very low levels of genetic diversity in comparison with other mammals, including other members of the genus *Tapirus* (de Thoisy *et al*. 2010; Ruiz-Garcia *et al*. 2012). This study only identified one polymorphism in a single individual from 69 samples from which sequences were successfully derived, collected from the far northwest to the far northeast of Honduras, and eight polymorphisms in samples from Honduras to Panama, suggesting a remarkable level of homogeneity in the species’ mitochondrial genome.

The low genetic diversity in Baird’s tapir means that defining management units using population genetic parameters (Moritz 1994; Palsboll *et al*. 2007) is problematic. In a species such as Baird’s tapir, where evidence of population genetic divergence is limited, other determinants of population structure should be prioritised in defining management units, such as geographical isolation (Schwartz *et al*. 2007). In a landscape as highly fragmented as Honduras, each individual population in each protected area could be seen as a separate management unit in itself, and efforts should be made to preserve all individuals within all remaining populations to maximise the genetic diversity of the species.

3.5.4. Conservation implications of Baird’s tapir genetic diversity

In the face of rapidly changing habitat due to both anthropogenic pressures and climate change, the low genetic diversity exhibited by Baird’s tapir is a valid conservation concern, as low genetic diversity may confer low adaptive potential (Frankham 1995). The pattern we have identified in Baird’s tapir is similar to that in *T. indicus*, which has a similar species range size, though a smaller overall population. Muangkram *et al*. (2013) suggest that there may be as few as seven *cytochrome-b* haplotypes in the remaining population of *T. indicus*, from a total of 19 polymorphic sites, even less than the 11 *T. bairdii* haplotypes identified by Ruiz-Garcia *et al*. (2012) and the additional *T. bairdii* haplotype added by this study. This level of genetic uniformity suggests that both species are likely to have poor adaptive potential (Frankham *et al*. 1995).

Although insufficient data were recovered to analyse population structure, signatures of structure were identified in both the mtDNA and microsatellite analyses. The identification of a single mtDNA polymorphism represented in one site in Honduras is an example of how mutations, which contribute to the genetic diversity of the species, can arise or be maintained in isolated populations (Sodhi & Ehrlich 2010), and demonstrates the importance of maintaining or re-establishing connectivity with other populations, to promote the maintenance of rare alleles through metapopulation dynamic processes (Saccheri *et al*. 1998).

Insufficient data were obtained to identify signatures of gene flow between the four populations of Baird’s tapir sampled, but the human geography of Honduras, and current rates of deforestation, suggest that the tapir populations in PNC, PNPB and PNSRT are isolated from each other and from the large wilderness area of the BRP, with little or no chance of migration existing between these protected areas (Hansen *et al*. 2013). If true, the remaining populations of Baird’s tapir in Honduras will not function as a metapopulation (Sodhi & Ehrlich 2010), and these small, isolated populations will be more susceptible to extinction through stochastic processes (Saccheri *et al*. 1998; Franklin *et al*. 2014), without the possibility of re-colonisation. The potential isolation of the remaining populations of Baird’s tapir in Honduras, and the implications of isolation for the conservation of the species, are more completely treated in Chapters 4 and 6.

From these data, there is too little genetic diversity to identify signatures of population bottlenecks or expansions. Some assumptions can, however, be made. Previous studies have identified that Baird’s tapir have among the lowest levels of nDNA and mtDNA diversity recorded in Neotropical mammals (Norton & Ashley 2004; Ruiz-Garcia *et al*. 2012), which is supported by the addition of only a single new haplotype from this Honduran study. These findings, although too incomplete to provide a complete signature of demographic history, do suggest a history of isolation, genetic drift and bottlenecks, which is likely to be exacerbated by ongoing hunting and deforestation across the species’ range (Garcia *et al*. 2012; Ruiz-Garcia *et al*. 2012).

Given the extremely low genetic diversity currently identified in Baird’s tapir, conservation strategies that aim to maintain genetic diversity should focus on maximising the size of tapir populations where possible. This should include both the protection of existing large populations, which may retain the majority of the genetic diversity of the species, and the protection of smaller, isolated populations, where genetic drift or other stochastic processes may have introduced novel genes to the population (Pardo *et al*. 2005). The identification of suitable management units for conservation, based upon the genetic signature of tapir metapopulations, should be considered a priority for tapir conservationists.

In Chapter 6 of this thesis I will estimate the size of the remaining Baird's tapir populations in Honduras, and discuss these estimates in relation to current theory on minimum viable population (MVP) sizes. No species of mammal has been shown to be unaffected by inbreeding, and MVP estimates are greatly affected by the effects of inbreeding and the loss of genetic diversity (Frankham *et al*. 2014). Although we do not have a complete picture of the resilience of Baird's tapir populations to low genetic diversity, we can assume that they are as susceptible to the deleterious effects of inbreeding and genetic drift as other mammals (Lacy 1997), and that MVP sizes for Baird's tapir are likely to be larger than for more out-bred species (Frankham *et al*. 2014).

The findings presented here reinforce the importance of using a combination of genetic markers to best characterise genetic diversity in threatened species, where diversity at a limited number of genes might be uninformative or misleadingly low (Wan *et al*. 2004). The identification of variable genomic markers for Baird's tapir continues to be a priority for researchers, to better understand the phylogenetic history of the species and the conservation implications of this history. By exploring other genetic markers, it will be possible to identify whether any real genetic diversity exists in Baird's tapir, and if not, conservation strategies must be designed to maximise what genetic diversity remains, by preventing further population loss, and by maintaining or re-establishing connectivity between existing populations.

3.6. Supplementary materials

Supplementary Table 3.7: Microsatellite scores and consensus scores for Baird's tapir faecal samples.
Samples were collected in Honduras between 2009 and 2012. Scores are given per sample/per locus. Microsatellites were scored by visual assessment of chromatograms in GeneMarker v1.91. Loci are as published by Goncalves da Silva *et al*. (2009) and Sanches *et al*. (2009).

		Locus / microsatellite score										
Sample	**Repeat**	7	9	10	12	13	17	18	19	20	21	23
d	1	0	147	0	0	0	0	0	0	0	0	0
d	2	0	147	219	177	228	0	0	0	0	0	0
d	3	0	147	0	0	0	0	0	0	0	0	0
d	4	0	143/145	0	0	0	0	0	0	0	0	0
d	5	0	0	220	0	0	154	0	0	0	0	0
d	6	0	147	0	0	229	154	0	0	0	0	0
d	7	0	147	0	0	0	154	0	0	0	0	0
d	8	0	0	0	0	0	0	0	0	0	0	0
Consensus		**0**	**147**	**220**	**0**	**229**	**154**	**0**	**0**	**0**	**0**	**0**
o	1	0	143/145	219	177/179	228	153	0	0	0	0	0
o	2	0	0	0	0	0	0	0	0	0	0	0
o	3	291	143/145	219	177/179	228	153	0	0	0	0	0
o	4	291	143/145	219	177	228	153	0	0	0	0	0
o	5	0	143/145	0	0	0	0	0	0	0	0	0
o	6	0	143/145	0	0	0	0	0	0	0	0	0
o	7	0	143/145	220	177/179	229	154	0	0	0	0	0
o	8	0	143/145	220	0	229	154	0	0	0	0	0
Consensus		**291**	**143/145**	**220**	**177/179**	**229**	**154**	**0**	**0**	**0**	**0**	**0**
s	1	0	145	219	177	0	153	0	189	274	0	0
s	2	0	143/145	0	0	228	153	0	189	274	0	0
s	3	0	145/147	219	0	0	153	0	0	0	0	0
s	4	0	145	219	177	228	153	0	0	0	0	0
s	5	0	145	220	0	0	154	0	189	274	0	0
s	6	0	145	0	0	229	154	0	0	0	0	0
s	7	0	145/147	0	0	0	154	0	0	0	0	0
s	8	0	145	220	0	229	154	0	0	0	0	0
Consensus		**0**	**145**	**220**	**177**	**229**	**154**	**0**	**189**	**274**	**0**	**0**
1	1	0	0	219	0	0	0	0	0	0	0	0
1	2	0	0	0	0	0	153	0	0	0	0	0
1	3	0	0	0	0	0	0	0	0	0	0	0
1	4	0	143/145	219	0	0	0	0	0	0	0	0
1	5	0	0	0	0	0	154	0	0	0	0	0
1	6	0	0	0	0	0	154	0	0	0	0	0
1	7	0	0	220	0	0	0	0	0	0	0	0
1	8	0	145	0	0	0	0	0	0	0	0	0
Consensus		**0**	**0**	**220**	**0**	**0**	**154**	**0**	**0**	**0**	**0**	**0**

2	1	0	145	0	0	0	0	0	0	0	0	0
2	2	0	0	0	0	0	153	0	0	0	0	0
2	3	0	147	0	0	0	153	0	0	0	0	0
2	4	0	145/147	0	0	0	153	0	0	0	0	0
2	5	0	145	220	0	0	0	0	0	0	0	0
2	6	0	0	0	0	0	154	0	0	0	0	0
2	7	0	147	0	0	0	154	0	0	0	0	0
2	8	0	145/147	0	0	0	154	0	0	0	0	0
Consensus		**0**	**0**	**0**	**0**	**0**	**154**	**0**	**0**	**0**	**0**	**0**
15	1	0	0	0	0	0	153	0	0	0	0	0
15	2	304	0	0	0	0	153	0	0	0	0	0
15	3	304	147	0	0	0	153	0	0	0	0	0
15	4	0	0	0	0	0	153	0	0	0	0	0
15	5	0	0	0	0	0	0	0	0	0	0	0
15	6	0	0	0	0	0	154	0	0	0	0	0
15	7	0	147	0	0	0	154	0	0	0	0	0
15	8	0	0	0	0	0	154	0	0	0	0	0
Consensus		**304**	**147**	**0**	**0**	**0**	**154**	**0**	**0**	**0**	**0**	**0**
38	1	291	145	0	177/179	228	154	0	0	0	0	0
38	2	0	145	219	0	228	154	0	0	0	0	0
38	3	291	145	219	177/179	228	154	0	0	0	0	0
38	4	0	145	0	177	0	154	0	0	0	0	0
38	5	291	145	220	177/179	229	154	0	0	0	0	0
38	6	0	145	220	0	229	154	0	0	0	0	0
38	7	291	145	220	177/179	229	154	0	0	0	0	0
38	8	0	145	0	177	0	154	0	0	0	0	0
Consensus		**291**	**145**	**220**	**177/179**	**229**	**154**	**0**	**0**	**0**	**0**	**0**
56	1	0	0	0	0	0	154	0	0	0	0	0
56	2	0	147	0	0	0	0	0	0	0	0	0
56	3	0	143/145	0	0	0	0	0	0	0	0	0
56	4	0	0	0	0	0	154	0	0	0	0	0
56	5	0	0	0	0	0	154	0	0	0	0	0
56	6	0	147	0	0	0	0	0	0	0	0	0
56	7	0	0	0	0	0	0	0	0	0	0	0
56	8	0	0	0	0	0	154	0	0	0	0	0
Consensus		**0**	**0**	**0**	**0**	**0**	**154**	**0**	**0**	**0**	**0**	**0**

57	1	0	143	0	0	0	154	0	0	0	0	0
57	2	0	0	0	0	0	154	0	0	0	0	0
57	3	0	145/147	219	0	0	154	0	0	0	0	0
57	4	0	0	219	0	0	154	0	0	270	0	0
57	5	0	0	0	0	0	154	0	0	0	0	0
57	6	0	0	0	0	0	154	0	0	0	0	0
57	7	0	0	0	0	0	154	0	0	0	0	0
57	8	0	145/147	220	0	0	154	0	0	0	0	0
Consensus		**0**	**145/147**	**220**	**0**	**0**	**154**	**0**	**0**	**0**	**0**	**0**
58	1	291	143	219	177/179	228	154	0	187	0	0	0
58	2	0	143	219	0	228	154	0	187	0	0	0
58	3	0	143	219	179	228	154	0	187	0	0	0
58	4	0	143	219	0	228	154	0	187	274	0	0
58	5	0	143	220	177/179	229	154	0	187	0	0	0
58	6	0	143	220	177/179	229	154	0	187	0	0	0
58	7	0	143	220	0	229	154	0	187	0	0	0
58	8	0	143	220	177/179	229	154	0	187	0	0	0
Consensus		**0**	**143**	**220**	**177/179**	**229**	**154**	**0**	**187**	**0**	**0**	**0**
62	1	0	143	219	0	0	154	0	187	274	0	0
62	2	0	143	0	0	228	154	0	0	0	0	0
62	3	0	143	219	0	228	154	0	0	0	0	0
62	4	0	143	0	0	0	154	0	0	0	0	0
62	5	0	143	220	0	0	0	0	187	274	0	0
62	6	0	143	0	0	229	154	0	0	0	0	0
62	7	0	143	220	0	229	154	0	187	270/274	0	0
62	8	0	143	0	0	0	154	0	0	0	0	0
Consensus		**0**	**143**	**220**	**0**	**229**	**154**	**0**	**187**	**274**	**0**	**0**
93	1	0	147	0	0	0	0	0	0	0	0	0
93	2	0	0	0	0	0	154	0	0	0	0	0
93	3	0	0	0	0	0	0	0	0	0	0	0
93	4	0	145/147	0	0	0	0	0	0	0	0	0
93	5	0	147	0	0	0	0	0	0	0	0	0
93	6	0	0	0	0	0	154	0	0	0	0	0
93	7	0	0	0	0	0	0	0	0	0	0	0
93	8	0	145/147	0	0	0	0	0	0	0	0	0
Consensus		**0**	**0**	**0**	**0**	**0**	**154**	**0**	**0**	**0**	**0**	**0**

Chapter 4. Using Patch Occupancy Analysis to Assess the Distribution and Conservation Status of Baird's Tapir in Honduras

4.1. Abstract

Surveys for Baird's tapir spoor were carried out in four protected areas in Honduras between December 2010 and June 2012. Rates of occupancy for the core and buffer zone of each protected area were calculated using the software PRESENCE v5.1 (Hines 2010), and the impact of a range of environmental variables on the distribution of Baird's tapir was analysed with a general linear model. Baird's tapir occupancy was found to be broadly positively associated with Euclidian distance from the Park boundary and Park size, and negatively associated with deforestation rates and Park edge:area ratio. Baird's tapir distribution in Honduras seems to depend upon a complex array of environmental and anthropogenic factors, and the conservation of the species in Honduras will depend upon the maintenance of sufficiently large and inaccessible refuges that can help drive source-sink metapopulation dynamics.

Key words

Baird's tapir, Honduras, occupancy, detectability, PRESENCE, deforestation, source-sink metapopulation dynamics, conservation.

4.2. Introduction

Large-scale monitoring programmes of threatened species are widely used to inform species-level conservation management plans (Hines *et al*. 2010; Sodhi & Ehrlich 2010). Conservation decision-making relies upon the best available data relating to the biology and conservation status of the target species, including fundamental parameters such as population trajectory, distribution and abundance, along with an assessment of the extrinsic threats to the species (Sodhi & Ehrlich 2010).

Estimating the distribution and abundance of rare or elusive species can be particularly challenging in terms of time and/or resources, due to inherent difficulties in observing such species in the wild (Mackenzie *et al*. 2002). When obtaining indices of absolute abundance is impractical, indices of relative abundance may provide a suitable surrogate (Mackenzie *et al*. 2003). Occupancy, the proportion of the landscape occupied by a target species, is a useful population parameter for the study of spatial and metapopulation dynamics (Mackenzie *et al*. 2003). In addition, it has been used as a proxy for population size in programmes monitoring populations of threatened large mammals such as Royal Bengal tiger (*Panthera tigris*; Hines *et al*. 2010) and jaguar (*Panthera onca*; Zeller *et al*. 2011), where estimates of absolute abundance are not possible.

Occupancy estimates for a target species can be generated by sampling a proportion of its habitat and recording whether the species was present or absent from the survey site. In practice, estimates of presence/absence are actually estimates of detection/non-detection (Donovan & Alldredge 2007), as occupancy estimates are constrained by the detection probability of the target species (Mackenzie *et al* 2002). Detection probability can be calculated from the pattern of detection and non-detection over repeat sampling occasions, and is incorporated into the formulae used to estimate occupancy. Error in detection can be minimised by repeatedly sampling a study site, either temporally, by re-visiting the study site, or spatially, by sampling multiple points within the survey area (Mackenzie *et al*. 2006).

The presence of a target species may be intrinsically linked to environmental characteristics such as habitat type or the availability of specific resources, creating a heterogeneous distribution of the species across the landscape (Sodhi & Ehrlich 2010). Identifying and quantifying the contribution of different environmental features to determining the distribution of a species can help conservation practitioners by identifying areas that are unlikely to be occupied, or by highlighting areas to invest in for the conservation of the target species (Wilson *et al*. 2011).

The distribution of many species is known to be determined by anthropogenic as well as natural features; human-caused habitat loss and fragmentation are seen as the greatest threats to biodiversity today (Sodhi & Ehrlich 2010). Hunted species may be disproportionately affected by habitat loss and fragmentation due to concomitant increases in hunting rates (Carillo *et al*. 2000). This has been suggested to be the case for Baird's tapir, the largest terrestrial mammal in the Neotropics, which is now confined to a small fraction of its original range (Garcia *et al*. 2012). Periodic assessments of Baird's tapir populations from 1982-1996 classified the species as vulnerable according to criteria for the IUCN Red List of Endangered Species. This was changed to endangered in 2002 as a result of external threats from habitat loss and fragmentation, poaching, and the inferred negative effects of population fragmentation and demographic contraction (Castellanos *et al.* 2008).

Although the distribution of Baird's tapir is imprecisely defined across its entire range, they are known to inhabit diverse habitats from sea level to 3620 m ASL (Garcia *et al*. 2012). This flexibility, coupled with the species' broad diet (Naranjo 2009; Garcia *et al*. 2012) and evidence that they are heavily hunted in populated areas (Castellanos *et al*. 2008; Dunn *et al*. 2012), suggests that Baird's tapir occupancy is likely to be limited primarily by anthropogenic factors. Honduras is an ideal candidate country to investigate the determinants of Baird's tapir occupancy, as anthropogenic and environmental pressures on tapir distribution vary greatly across the complex Honduran landscape.

Due to ongoing deforestation across their range, the majority of habitat available to Baird's tapir exists within Central America's network of protected areas (Naranjo 2009; Garcia *et al*. 2012). Approximately 60 protected areas in Honduras are considered to contain habitat that could have once supported Baird's tapir populations. Tapir have only been confirmed to inhabit 11 out of 15 protected areas that have been comprehensively surveyed either by members of the Tapir Specialist Group (TSG) or by the NGO Panthera, and are thought unlikely to survive in any more than five of the remaining sites (N. Estrada, pers. comm.).

Closer examination of the structure of the Honduran protected area network suggests that Baird's tapir occupancy is restricted to four regions, as several protected areas overlap (Figure 4.1). The four regions are: Cusuco National Park (PNC); the Nombre de Dios Cordillera (comprising the Texiguat Wildlife Refuge, Pico Bonito National Park (PNPB) and

Nombre de Dios National Park); The Sierras Region (comprising the proposed Sierra Rio Tinto National Park (PNSRT), El Carbon National Park, and Sierra de Agalta National Park); and the group of protected areas in La Moskitia (comprising the Rio Platano Biosphere Reserve (BRP), Patuca National Park, Tawahka Asangni Biosphere, Rus Rus Biological Reserve, Warunta National Park, and Mocoron National Park).

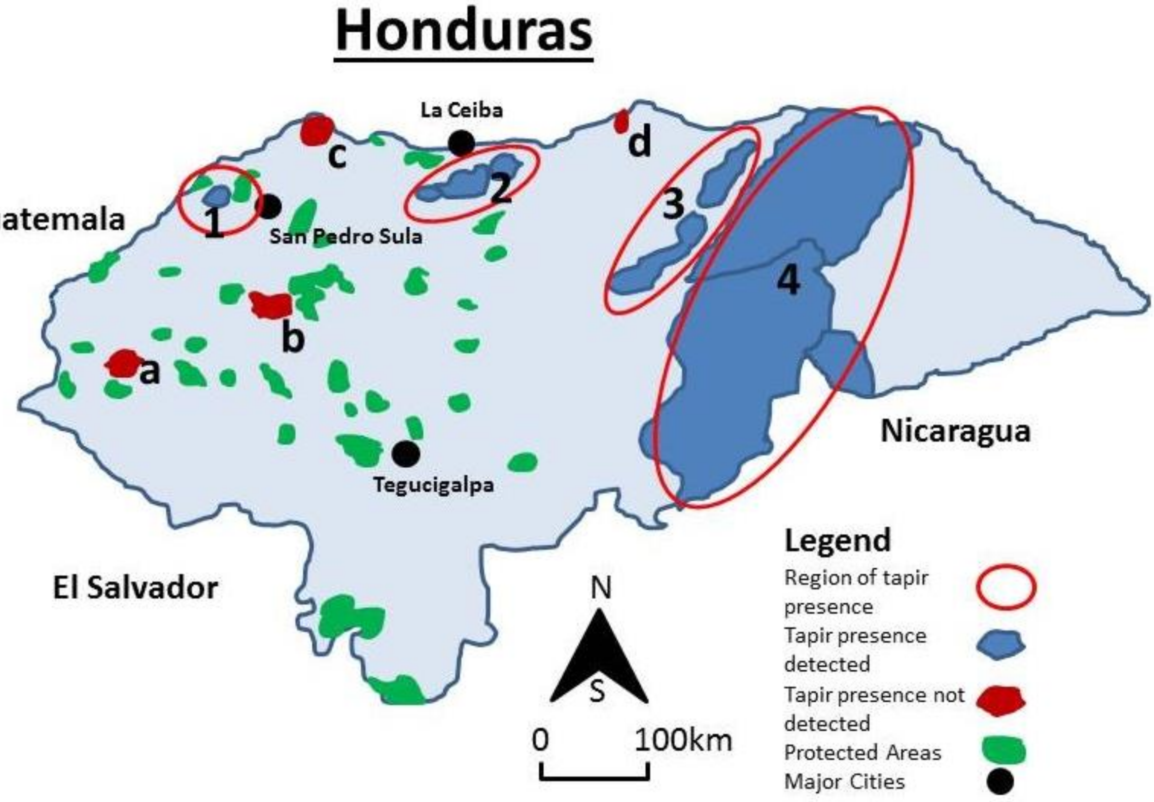


Figure 4.1: Map of the protected areas of Honduras including the remaining regions of tapir occupancy.
1-4: The four regions where Baird's tapir have been confirmed to be present. 1: Cusuco National Park (PNC); 2: Nombre de Dios Cordillera Region; 3: Sierras Region; 4: La Moskitia Region). a-d: The four protected areas where surveys have not detected tapir presence. a: Celaque National Park; b: Cerro Azul Meambar National Park; c: Jeannette Kawas National Park; d: Capiro y Calentura National Park.

We hypothesised that Baird's tapir distribution and abundance is mediated by human activity on a local and national scale, and that the likelihood of encountering tapir spoor would be lower in areas, such as the Park buffer zones, that are either easily accessible by people (Naranjo & Bodmer 2007), or that are remote and yet experiencing elevated levels of deforestation and hunting (Flesher & Ley 1996). We also hypothesised that Baird's tapir occupancy was correlated with distance to the Park boundary and with Park size, and that occupancy would be higher in those Parks with a low ratio of Park edge:area and a high ratio of core zone size:buffer zone size. To test these hypotheses and obtain a broader view of

Baird's tapir distribution and conservation, surveys for the presence of tapir spoor were carried out in four protected areas, one in each of the remaining regions of tapir occupancy in Honduras.

The results presented here are used to inform the population census and population and habitat viability analysis in Chapter 6, and may be applicable for studies on other species of large mammal experiencing similar threats.

4.3. Methodology

4.3.1. Sampling methodology

The general sampling methodology for the observation of tapir spoor (sightings, prints, faeces, evidence of foraging), and the site-specific methodology for each sampling site are as described in Chapter 2. Data from five sampling sites, two in PNPB and one in each of the remaining three protected areas are presented. For the analysis, only data collected between December 2010 and June 2012 were included, to mitigate local changes in occupancy and detectability that might occur outside of that time period and affect the analysis. Data from PNC in all years except 2011 were excluded from this analysis. Data from PNC includes all surveys (on- and off-transect; Chapter 2) conducted in 2011. The temporal analysis of tapir occupancy in PNC is presented in Chapter 5. Survey effort in each site was standardised where possible (Table 4.1). The highly challenging environment meant that there were differences in survey effort between sites. Potential biases in these data were accounted for by standardising data by survey effort and assessing occupancy by landscape unit. Nevertheless there may be residual biases resulting from these differences in survey effort.

In PNC, PNPB and PNSRT all surveys for tapir spoor were conducted on foot by a minimum of two trained observers. Observers walked at approximately 1 km/hour along pre-existing trails, or, where no trails existed, along ridges and streams, depending upon the terrain. In the BRP surveys were conducted by boat, using the Rio Sikre as a single survey line. Tapir spoor was observed on the river banks, and counted by four trained observers when traversing the river at a speed of approximately 5km/hour. All encounters with spoor (Figure 4.2) were recorded on a Garmin Vista HCX GPS unit for use with the programmes Garmin Basecamp v4.1 and ArcGIS 10.1.

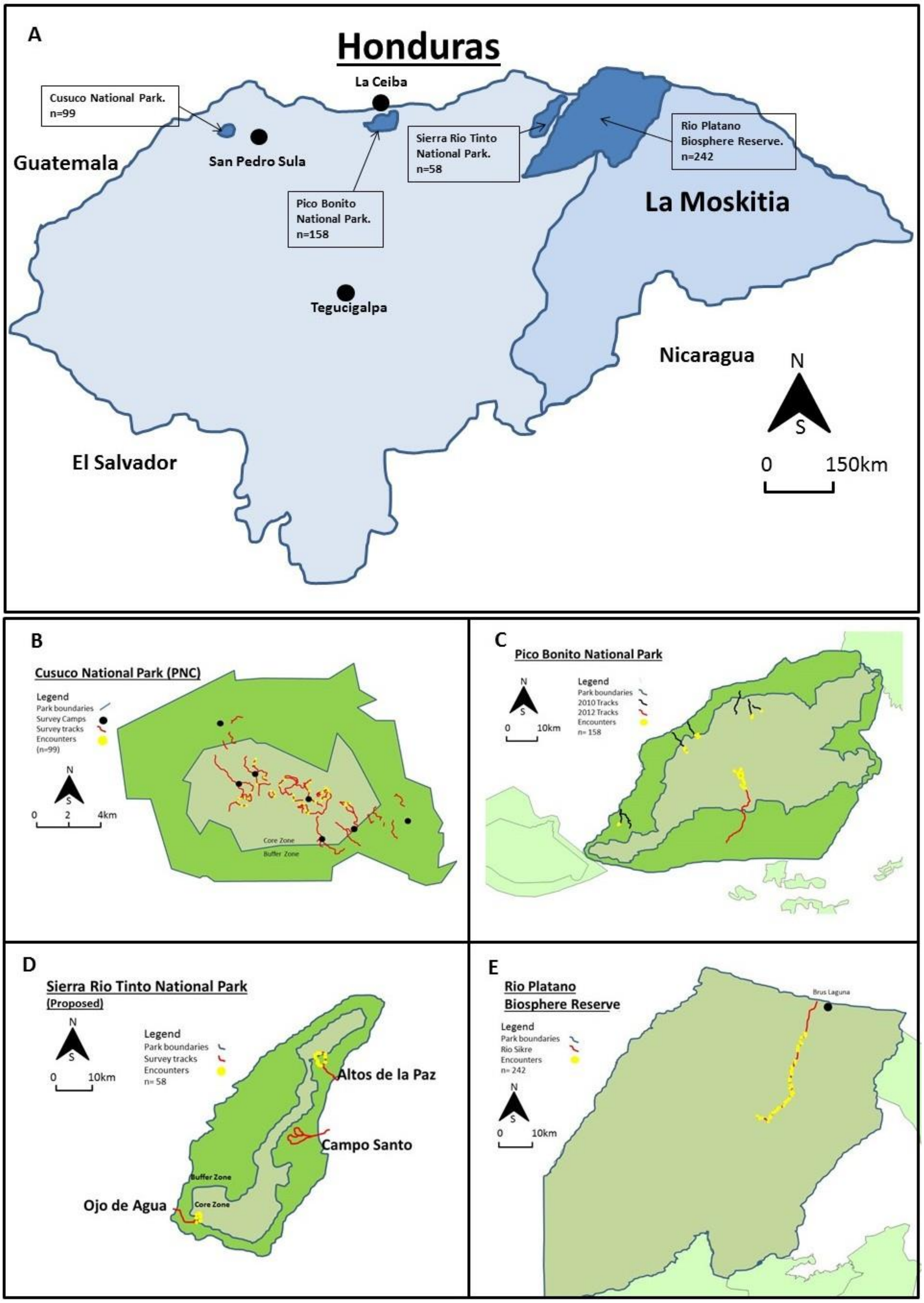


Figure 4.2: Map of sampling sites in Honduras.

Sites were surveyed for Baird's tapir spoor between December 2010 and June 2012. A: Map of Honduras with the location of the sampling sites and corresponding number of encounters with tapir spoor. B-E: Individual maps of each sampling site with distribution of sampling tracks (red; black for northern Pico Bonito, surveyed in 2010) and encounters with tapir spoor (yellow) indicated.

4.3.2. Data Analysis

Spatial analyses were performed in ArcGIS 10.1 using a map of the protected areas of Honduras provided by the Honduran Institute of Conservation and Forestry (ICF). Precise information about the location of the core zone in PNSRT and the BRP is not available. The core zone for PNSRT was re-drawn based upon maps provided by N. Estrada, Honduran Representative of the Tapir Specialist Group (TSG). The core zone for the BRP was not drawn, though it is known that the Rio Sikre sits entirely within the core zone of the Reserve.

4.3.2.1. Patch occupancy analysis

Each sampling site was divided into landscape units (LSUs) or 'sites' for the analysis. Sites for occupancy studies were chosen at a scale appropriate for the biology of the study species and the nature of the landscape (O'Connell *et* al. 2006; Hines *et al*. 2010). Sites of 1 km x 1 km were chosen as tapir have an estimated annual home range of approximately 1 km^2 (Medici *et al*. 2005). Sampling sites in each study site were randomly selected *post hoc* to ensure independence of data in the analysis. Using ArcGIS 10.1 a 1 km x 1 km grid was placed over a digital map of each study area using the 'create fishnet' tool, and all of the LSUs in which surveys for tapir spoor were conducted were selected. 50% of the original selection was chosen using the 'random points' tool in ArcGIS to include in the analysis. Spatial replicates were created within the LSUs by overlaying a 100 m x 100 m grid and selecting those squares in which surveys had been undertaken. Individual sampling units were, in turn, chosen by randomly selecting 50% of the original selection, and this subset of sampling units were then analysed to produce an unbiased estimate of the proportion of sites that are occupied.

The programme PRESENCE v5.1 (Hines 2010), and methodologies outlined by Mackenzie *et al*. (2002) were used to estimate site occupancy (*Psi*) and detection probability (*p*) for Baird's tapir in all five sampling sites, using a single season analysis that included the categorical variables 'site' (representing each of the five study sites) and 'core'. Core was defined as a binary depending whether LSUs were inside or outside of the Park core zone. All LSUs in the BRP were located within the core zone of the Park. All models were ranked according to AIC (Johnson & Omland 2004).

In addition to calculating rates of occupancy, the effect of Euclidian distance from the Park boundary, and of deforestation levels were analysed to assess their impact on the probability of encountering tapir spoor across the five sampling sites. For each sampling site, the Euclidian distance from the Park boundary to the centre of each 1 km x 1 km LSU sampled was calculated using the spatial analyst tool in ArcGIS 10.1. Data on forest loss in 30 m x 30 m squares between the years 2000 and 2012, and on forest cover in the year 2000 were from Hansen *et al*. (2013). These authors did not differentiate between types of forest loss, but the majority is assumed to be anthropogenic. The effect of deforestation on tapir occupancy was assessed using the spatial analyst tool in ArcGIS 10.1 by comparing the number of 30 m x 30 m squares that were deforested within each LSU where tapir spoor was encountered, with those LSUs where tapir spoor was not encountered. The effect of these variables was analysed with a general linear model using the statistical package R 3.03. The model was controlled for effort, by including the number of 100 m x 100 m squares walked per LSU; for sampling site; and for whether the LSU was in the core zone or the buffer zone of each Park. The interaction of all the covariates was also included in the model.

The effect of Park size, and the effect of the ratio of core size:buffer size, and Park edge:area on *Psi* were also tested with a linear correlation test using the statistical software package PAST (Hammer *et al*. 2001).

4.4. Results

4.4.1. Spoor surveys

Across the five sampling sites, a mean encounter rate of 1.58 tracks/km (range 0.71-2.97; Table 4.1) was recorded.

Table 4.1: Results of Baird's tapir surveys in Honduras.
Surveys for Baird's tapir spoor were carried out in five study sites in four protected areas in Honduras surveyed between December 2010 and June 2012. Survey effort and the encounter rate with Baird's tapir spoor are included. Protected areas are: Cusuco National Park (PNC), Pico Bonito National Park (PNPB; surveyed in 2010 [north] and 2012 [south]), proposed Sierra Rio Tinto National Park (PNSRT), Rio Platano Biosphere Reserve (BRP).

Location/year	**Survey days**	**Total survey track length (km)**	**Number of encounters with tapir spoor**	**Frequency of encounter/km**
PNC 2011	40	72.9	99	1.36
PNPB North 2010	8	52.2	37	0.71
PNPB South 2012	10	54.97	119	2.16
PNSRT 2011	8	57	58	1.02
BRP 2011	15	94.1	242	2.57

4.4.2. Five Sites Occupancy Estimates

A single season model was run using the software PRESENCE 5.1 (Hines 2010) to explain the distribution of spoor and to estimate *Psi* and *p* for Baird's tapir in the core zone and buffer zone of the five study sites. The strongest model suggests that both *Psi* and *p* are constrained by 'core', and that *p* varied significantly across the five sites. The top five models were similarly supported by the data (Delta AIC <2), so caution must be applied before assigning a rank to these models. All non-null models significantly out-performed the null model, suggesting that *Psi* and *p* vary between the five study sites, and there is an actual impact of 'core' on both *Psi* and *p* (Table 4.2).

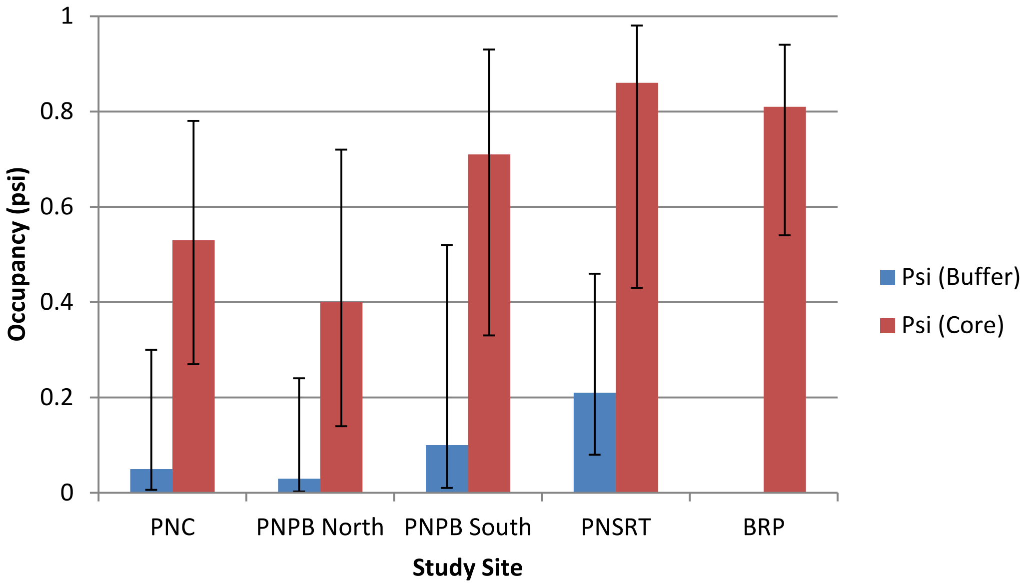
Table 4.2: Model selection statistics for Baird's tapir distribution in Honduras.
The programme PRESENCE 5.1 (Hines 2010) was used to compare competing models to describe Baird's tapir occupancy (*Psi*) and detectability (*p*) across five sampling sites in Honduras. Covariates used were 'site', representing the five study sites; and 'core', representing the core zone of each study site. Models are ranked by AIC.

Model	**Delta AIC**	**AIC Weight**	**-2 Log Likelihood**	**No. Parameters**
Psi(core),p(site,core)	0.00	0.2493	572.72	9
Psi(.),p(core)	0.26	0.2189	584.98	3
Psi(core),p(core)	0.65	0.1801	583.37	4
Psi(.),p(site,core)	0.85	0.1630	575.57	8
Psi(site),p(core)	1.60	0.1120	576.32	8
Psi(site,core),p(core)	2.68	0.0653	575.40	9
Psi(site,core),p(site,core)	7.11	0.0071	569.83	14
Psi(core),p(.)	8.80	0.0031	593.52	3
Psi(.),p(.) [Null model]	33.38	0.000	620.10	2

The models *Psi(site),p(.)* and *Psi(),p(site)* were used to estimate *Psi* and *p* in each study site (Table 4.3). The models *Psi(site,core),p(.)* and *Psi(),p(site,core)* were used to estimate *Psi* and *p* for the core zone and buffer zone of each study site separately (Figure 4.3). Detectability, and to a lesser extent occupancy varied significantly depending upon whether surveys were undertaken in the core zone of the buffer zone of each Park. Occupancy was strongly correlated (r = 0.90, p = 0.036) with rate of encounter across the five sampling sites.

Table 4.3: Estimates of Baird's tapir occupancy in five sampling sites in Honduras. Occupancy (*Psi*) and detectability (*p*) were estimated using a single season analysis in the software PRESENCE 5.1 (Hines 2010) from spoor encounter data collected during surveys of four protected areas (Cusuco National Park, Pico Bonito National Park [surveyed in 2010 (north) and 2012 (south)], Sierra Rio Tinto National Park, Rio Platano Biosphere Reserve) in Honduras between December 2010 and June 2012. Estimates presented here are taken from models *Psi*(site),*p*(.) and *Psi*(.),*p*(site).

Study Site	**Occupancy (*Psi*)**	**95% CI**	**Detectability (*p*)**	**95% CI**
Cusuco National Park	0.35	0.17-0.59	0.24	0.17-0.35
Pico Bonito National Park North (2010)	0.26	0.10-0.54	0.10	0.05-0.22
Pico Bonito National Park South (2012)	0.54	0.25-0.81	0.38	0.25-0.53
Sierra Rio Tinto National Park	0.37	0.19-0.60	0.30	0.19-0.44
Rio Platano Biosphere Reserve	0.81	0.54-0.94	0.33	0.25-0.42



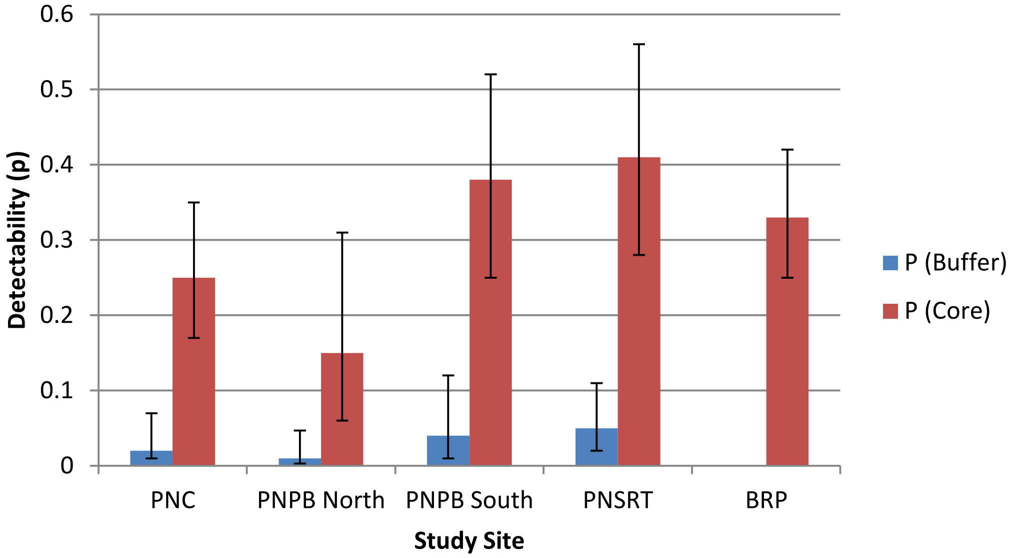


Figure 4.3: Baird's tapir occupancy and detectability in five sampling sites in Honduras. Occupancy (*Psi*) and detectability (*p*) were estimated using the programme PRESENCE 5.1 (Hines 2010), from detection data collected in five sampling sites in four protected areas (Cusuco National Park [PNC], Pico Bonito National Park [PNPB; two separate sites, surveyed in 2010 and 2012], Sierra Rio Tinto National Park [PNSRT], Rio Platano Biosphere Reserve [BRP]) between December 2010 and June 2012. Estimates presented here are taken from models *Psi(site,core),p(.) and Psi(.),p(site,core)*.

4.4.3. Determinants of tapir occupancy

The probability of encountering tapir spoor differed significantly between the five sites, and the distribution of spoor was partially explained by both deforestation and Euclidian distance from the Park boundary (Figure 4.4). Survey effort (the proportion of each LSU surveyed) had a non-negligible effect on the probability of encountering tapir spoor (t = 3.462, p = 0.063), so the model was statistically controlled for effort.

Across the five sampling sites, there was a significant effect of Euclidian distance on the probability of encountering tapir spoor (LSUs further from the Park boundary had a higher probability of being occupied by tapir; t = 38.025, p <0.001), although the extent to which distance had an impact varied between the five sites. This effect was reduced when only analysing LSUs in the core zone of each Park. Deforestation was significantly higher in LSUs where tapir were not encountered than those where they were encountered (t = 15.09, p = 0.035), though the size of this effect varied considerably between sites. This effect was also reduced when only taking into account LSUs in the core zone of the five study sites.

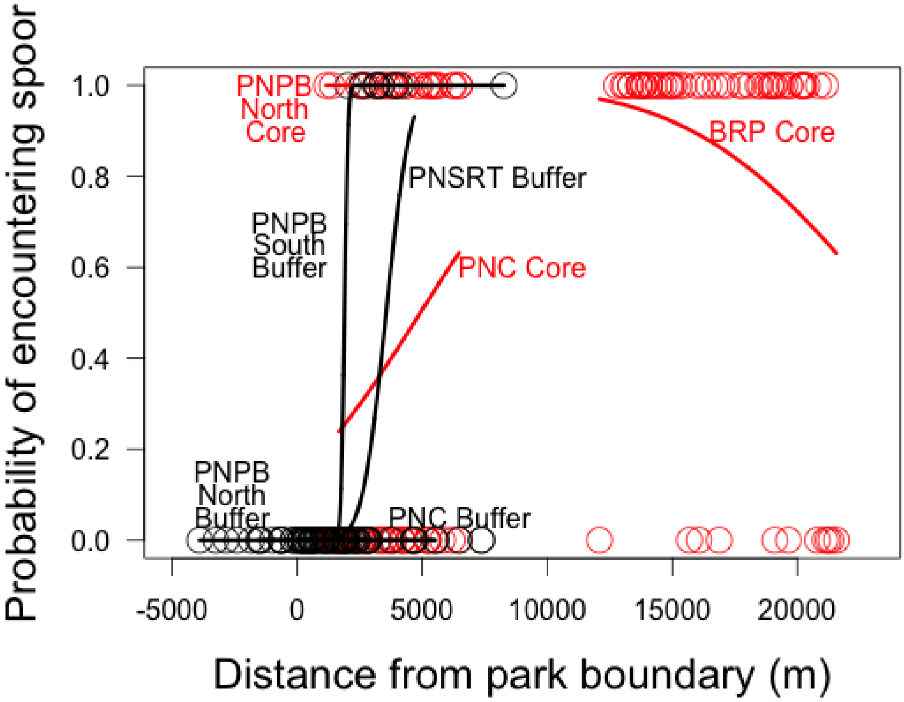


Figure 4.4: GLM of Baird's tapir distribution in Honduras.
The GLM assessed the effect of the level of deforestation and the distance from the Park boundary to each 1 km^2 landscape unit (LSU) surveyed for Baird's tapir spoor in the core zone and buffer zone* of five sampling sites in four protected areas (Cusuco National Park [PNC], Pico Bonito National Park [PNPB, surveyed in 2010 (north) and 2012 (south)], Sierra Rio Tinto National Park [PNSRT], and the Rio Platano Biosphere Reserve [BRP]) in Honduras.
Lines represent the probability of encountering tapir spoor depending upon distance from Park boundary.
Circles represent 1km^2 LSUs in which tapir were present (probability of encountering tapir = 1) or absent (probability of encountering tapir = 0). LSUs in black relate to surveys undertaken in the buffer zone of the sampling sites. LSUs in red relate to surveys undertaken in the core zone of the sampling sites.
*The buffer zone of the BRP was not surveyed.

Using the overall occupancy estimates for each protected area (Table 4.4), including the mean occupancy for the two study sites in PNPB (*Psi* = 0.40, range 0.26 – 0.54), occupancy was strongly correlated with Park size (r >0.99, p <0.001; Figure 4.5; the correlation remains with the removal of the data point for BRP: r >0.99, p = 0.004), and negatively correlated with the edge:area ratio (r = -0.80, p = 0.20). Occupancy in the core zone and buffer zone of each Park was also positively correlated with core zone size (r = 0.46, p = 0.54) and buffer zone size (r = 0.72, p = 0.48), and overall occupancy was positively correlated with the core zone size:buffer zone size ratio (r = 0.90 p = 0.10).

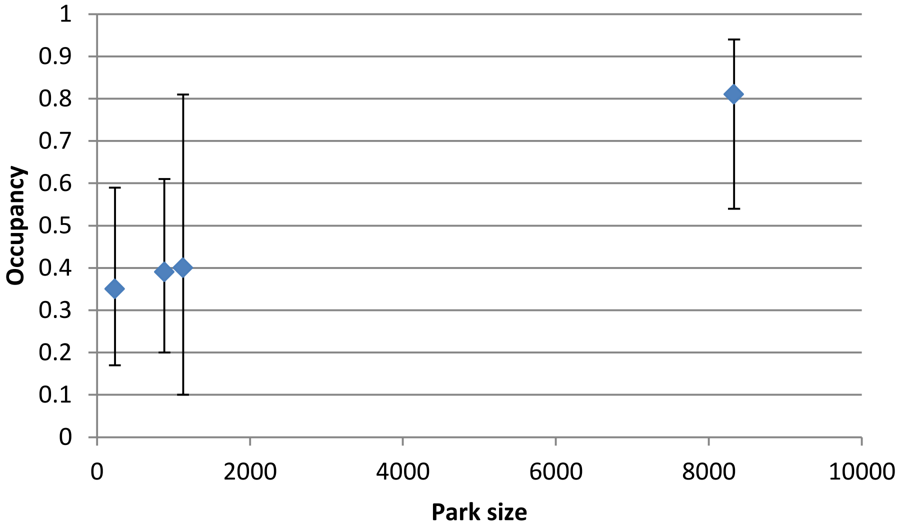


Figure 4.5: The relationship between Park size and Baird's tapir occupancy in Honduras.
Occupancy was estimated using the programme PRESENCE 5.1 (Hines 2010), from detection data collected in four protected areas (Cusuco National Park [PNC], Pico Bonito National Park [PNPB], Sierra Rio Tinto National Park [PNSRT], Rio Platano Biosphere Reserve [BRP]) between December 2010 and June 2012.

4.5. Discussion

4.5.1. Occupancy estimates

Baird's tapir were found to be present in all four protected areas surveyed and this is the first time that the species has been recorded in PNSRT. Occupancy has not been previously estimated in Baird's tapir, though three previous studies calculated the average encounter rate with tapir spoor in Honduras, with a mean value between the three studies of 0.85 tracks/km (range 0.21-2.22; Demmer & Overman 2001; N. Estrada pers. comm.). *Psi* was highly correlated with encounter rate (r = 0.90, p = 0.036), and the encounter rates reported here (0.71-2.57 encounters/km) fell within the boundaries of previous estimates (Naranjo 2009). The PRESENCE models demonstrate that *Psi* and *p* vary between the five sampling sites, and between the core zone and buffer zone of the protected areas.

The data support the conclusion that *Psi* is higher in the core zone than the buffer zone in all of the sampling sites. Tapir were recorded in the buffer zones of PNPB (*Psi* = 0.03 in 2010 and 0.10 in 2012) and PNSRT (*Psi* = 0.21), but were absent from the buffer zone of PNC. Due to overlapping confidence limits for *Psi* in the core and buffer zones of all five study sites, a cautious interpretation of the occupancy estimates is advised. Estimates of *p* are more strongly supported by the data, and confidence limits do not overlap for any of the study sites (Figure 4.3). There was also a positive correlation (r = 0.90) between *Psi* and the core zone size:buffer zone size ratio of the sampling sites, though this result must be treated with caution due to the weak statistical significance of the result (p = 0.10) as a result of the small sample size in the present study, and due to the effect of BRP, which has a core zone size:buffer zone size ratio almost twice as large as the next largest site.

The high estimate of *Psi* in the core zone of PNSRT (0.86) may be due to a sampling bias resulting from sampling a small proportion of the core zone (Seaman *et al*. 1999). To improve the accuracy of the occupancy estimates, a greater proportion of each reserve should be surveyed, with surveys conducted in the core zone and buffer zone of each site to account for the likely difference in occupancy rates between the zones. Only PNC was surveyed comprehensively, with 26% of the Park surveyed over the duration of this study (31.4% of the core, 11.4% of the buffer). BRP was the least comprehensively surveyed site with only 1.6% of the core, and 0% of the buffer incorporated in surveys; whereas PNPB and PNSRT both had 6% coverage (5.1% core, 6.9% buffer in PNPB; 2.6% core, 6.6% buffer in PNSRT). Previous studies have demonstrated that increased sampling effort significantly improves estimates of large mammal abundance from spoor encounter data (Funston *et al*. 2010). Although a larger proportion of the BRP needs to be surveyed to verify the estimates presented here, the high estimate of *Psi* in the BRP (*Psi* = 0.81) is testament to the importance of this area as a refuge and potential source population for Baird’s tapir.

4.5.2. The importance of accessibility on occupancy

The observation that the probability of encountering tapir spoor is strongly correlated with ‘core’ and with Euclidian distance from Park boundary is highly intuitive. The heterogeneous distribution of hunted species in a landscape has been shown to correspond with accessibility (e.g. Blom *et al*. 2005). Anecdotes from local people suggest that tapir are commonly hunted in all of the protected areas surveyed, and carcasses were discovered in PNC and the BRP. Hunting levels of Baird's tapir in north eastern Honduras have been shown to be much higher near to permanent human habitation (Dunn *et al*. 2012), and a gradient in mammal abundance proportional to hunting intensity has been identified in many other species (e.g. Muchaal & Ngandjui 1999; Wright *et al*. 2000; Hill *et al*. 2003).

The effect of Euclidian distance on the probability of encountering tapir spoor differed between the five sites, but was particularly strong in the buffer zones of the three protected areas where buffer zones were also surveyed (PNC, PNPB, PNSRT), where the probability of encountering tapir spoor is very low when close to the Park boundary. Inside the core zone Euclidian distance had a smaller effect on tapir presence (and was negatively correlated with tapir presence in the core zone of the BRP, between 12 km and 20 km from the Park boundary), which suggests that the effect of distance is non-linear. The correlation between Park size and occupancy (Figure 4.5) is consistent with the hypothesis that occupancy is affected by both distance to boundary and by Park size, and although the small sample size requires that this correlation is viewed with caution, it is likely that this result reflects actual trends. Larger refuges provide a buffer for hunting, as larger areas are harder to penetrate enabling them to preserve source-sink metapopulation dynamics (Naranjo & Bodmer 2007).

Higgs and Usher (1980) demonstrated that many small Parks are more effective at preserving biodiversity (measured as the number of species) than few larger Parks. Although this may apply to overall species abundance, when looking at the conservation of taxa with large home ranges such as tapir, the reverse may be true (Cardillo *et al*. 2005), and it has been shown that large mammal species abundance demonstrates a strong positive correlation with reserve size (Chiarello 1998). In reserves with high levels of hunting, as in Honduras, extinction rates for large mammals have been found to be far higher than would be predicted simply based upon the size of the reserve (Brashares *et al*. 2001).

As hunting pressure is highest around reserve boundaries (Brashares *et al*. 2001), large reserves such as the BRP - which is eight times larger than the next largest study site - with lower edge:area ratios, are more likely to harbour sustainable populations of at-risk species such as Baird's tapir. Smaller Parks have a larger boundary:volume ratio and are more intrinsically susceptible to edge effects. In this study, *Psi* was negatively correlated with edge:area ratio ($r = -0.80$), and although the statistical significance of this result was weak ($p = 0.2$) due to the small sample size, it is not unreasonable to assume that this correlation would remain over a larger sample of reserves. The linking of protected areas in Honduras, as in the Nombre de Dios Region, the Sierras Region and La Moskitia (Figure 4.1), has greatly reduced the amount of ‘boundary’ relative to the overall size of the protected areas. Woodroffe and Ginsberg (1998) demonstrated that the major source of mortality in 10 species of large carnivores that they analysed was ‘conflict’ with people near reserve boundaries. One of the factors they identified for this was that larger species tend to be wider ranging, so are more likely to be exposed to threats around reserve borders. Maximising the size of the protected areas that retain Baird’s tapir populations, and allowing corridors of movement between them may help to preserve metapopulation dynamic processes and reduce mortality from edge effects (Sodhi & Ehrlich 2010). A more complete treatment of the sustainability of the regions of Baird’s tapir occupancy is presented in Chapter 6.

4.5.3. Deforestation and tapir occupancy

In the absence of accurate data on human activity and hunting levels inside Honduran protected areas, deforestation is a useful potential indicator of anthropogenic pressure. Detection of tapir spoor was negatively correlated with deforestation across the whole dataset. When only analysing LSUs inside the core zones of each protected area, where deforestation levels are much lower (Figure 6.2), deforestation was a poor predictor of detection. This suggests that in areas with low levels of deforestation other environmental variables, such as hunting and resource availability, may be determining their distribution, as has been observed in other endangered forest-dwelling mammals (Kinnaird *et al*. 2003; Brodie *et al*. 2014).

Populations of tropical forest-dwelling mammals may be able to withstand low levels of deforestation such as selective logging (Johns 1985; Brodie *et al*. 2014), and a preference for foraging in or near forest clearings has been identified in some species, such as forest buffalo (*Syncerus caffer*; Blake 2002) and Maxwell's duiker (*Cephalophus monticola*; Fimbel 1994). Although Baird’s tapir have been shown to preferentially occupy intact forest over altered landscapes such as agriculture and pasture (Tejeda-Cruz *et* al. 2009; Medici 2010; Garcia *et al.* 2012), they have also been shown to demonstrate little preference for avoiding forest edges (Kinnaird *et al*. 2003; Dunn *et al*. 2012), and may preferentially forage in secondary forest or other disturbed habitats due to the abundance of young shoots and other foods at browsing height (Fragoso 1991; Naranjo & Bodmer 2007; Dunn *et al*. 2012; Garcia *et al*. 2012).

The five study sites differ in the extent to which deforestation influenced the probability of encountering tapir, which is probably attributable to different levels of hunting associated with the deforestation in each site (Carillo *et al*. 2000). The highest amount of deforestation in any cell where tapir spoor was encountered was 33.21%. This cell, on the edge of the core zone in Altos de la Paz in PNSRT, had only been deforested in the two weeks prior to being surveyed, and it is unlikely that hunting pressure had yet penetrated the forest here. The absence of tapir from the buffer zone of PNC is probably a result of elevated levels of hunting, agriculture and pasture use, caused by many years of human occupation and modification (Medici 2010; Garcia *et al*. 2012).

The results from PNPB north and PNPB south enable a comparison between spatially separate areas in the same Park under different levels of anthropogenic stress. The LSUs surveyed in the north of the Park (surveyed in 2010) have undergone six times the deforestation since the year 2000 as those in the south of the Park (surveyed in 2012), and the communities in the north are known to frequently hunt tapir. No hunting trails were encountered during surveys in the south, whereas several hunting trails were encountered in the north. The difference in *Psi* estimates between these two areas (0.26 in 2010, 0.54 in 2012) is likely to arise from the disparity in hunting rates between the two sampling sites, though without access to accurate data on hunting levels it is hard to separate the effects of hunting from deforestation and other variables (Brodie *et al*. 2014). Naranjo and Bodmer (2007) recorded a great disparity in the population density of Baird's tapir in proximate sampling sites under differing levels of hunting pressure in Mexico (0.05 tapir/km^2 in hunted, and 0.20 tapir/km^2 in relatively unhunted sites). The potential impact of the skewed population distribution in PNPB on Baird's tapir metapopulation dynamics is more fully explored in Chapter 6.

Where Baird's tapir have been previously reported to frequent secondary or disturbed forests in protected areas, human presence and activity has usually been slight (Naranjo & Bodmer 2007; Tejeda-Cruz *et al*. 2009; Naranjo 2009). Dunn *et al*. (2012), however, reported high levels of Baird's tapir activity near to agricultural areas in villages that regularly hunt tapir in La Moskitia. This may be a result of tapir living at sufficiently high population densities in the region to enable source-sink metapopulation dynamic processes to maintain populations under high levels of hunting pressure, which would be extirpated in the absence of immigration (Hanski & Simberloff 1997).

4.5.4. Conclusions

The results presented here support the hypothesis that Baird's tapir distribution is affected by human activities on both a local and national scale. The observation that deforestation levels did not predict tapir presence in the core zones of the survey sites suggests that where deforestation and human activity occur at relatively low levels, small increases in deforestation may not be the primary determinant of tapir occupancy in the absence of hunting pressure (Brodie *et al*. 2014). The increase in hunting intensity that may accompany deforestation (Carillo *et al*. 2000) is likely to be a predictor of tapir presence and occupancy rates, particularly in areas where low tapir population densities do not allow for source-sink metapopulation dynamics to replenish depopulated areas (Naranjo & Bodmer 2007; Sodhi & Ehrlich 2010). These findings support evidence from previous studies that deforestation *per se* does not appear to be the defining factor in determining tapir distribution, which depends upon a suite of variables relating to habitat suitability and levels of anthropogenic disturbance (Naranjo & Bodmer 2007; Naranjo 2009; Garcia *et al.* 2012). Environmental variables such as deforestation, accessibility, reserve size and reserve shape may be suggestive of tapir occupancy provided certain other habitat requirements are fulfilled, such as spatial requirements and sufficient fluvial features (Garcia *et al*. 2012), but the actual probability of Baird's tapir being present in any given area is both context and situation specific.

Chapter 5. Transect networks, observation bias and unintended conservation consequences for Baird’s tapir

5.1. Abstract

We report the consequences of creating a novel biodiversity monitoring transect network on the efficiency of monitoring population trends in Baird's tapir and its potential consequences for conservation. Using the software PRESENCE 5.1 (Hines 2010), we identify a change in the distribution of Baird's tapir in Cusuco National Park (PNC), Honduras, during the seven years since a novel transect network was created in the Park, and infer a population decline in that time, related to an increase in accessibility and hunting pressure on the population. The results presented here suggest that detectability of Baird's tapir spoor on the transect network is negatively correlated with accessibility and has declined significantly since 2006. Baird's tapir occupancy is higher in areas of PNC not bisected by the transect network, and the probability of encountering tapir spoor increases with distance up to 700 m from the nearest transect. We suggest that transect networks can alter the population dynamics and distribution of threatened species in areas where transect networks are created, running counter to the generally held view that research stations in tropical ecosystems provide proxy protection to the ecological communities under study in adjacent areas. This situation may be common, and conservation practitioners working with vulnerable taxa may consider other options or modified transect designs before creating a novel transect network.

Key words

Baird's tapir, Honduras, PRESENCE, transect, extinction, survey bias.

5.2. Introduction

The use of survey transects for forest access and biological monitoring (henceforth referred to as transects) in conservation has become standard practice for researchers wishing to conduct standardised, repeatable surveys, as they are accepted to enable robust statistical analysis of population trends over time (Anderson *et al*. 1979). Surveys using such transects assume that the sample population (those individuals within the overall population whose home ranges overlap the transect network) is representative of the overall population, and that the sampling methodology does not alter the distribution of organisms in the survey area for the duration of the monitoring period.

Transects may be created specifically for the purpose of biodiversity monitoring, or may take advantage of existing networks of trails, such as those used by hunters (e.g. Muchaal & Ngandjui 1999). Transects may be particularly useful for monitoring species that live in inaccessible regions such as dense forests, where survey capability is inhibited by landscape features.

The distribution of many species is affected by proximity to or association with humans or human activity (e.g. Hewison *et al*. 2001; Brodie *et al.* 2014). The variability in the response of different species to human activity is one of the confounding factors in developing conservation strategies for affected areas (Gardner *et al*. 2009). Some taxa are characterised as being particularly sensitive to anthropogenic disturbance, such that human activity may become one of the dominant factors in determining their distribution. Hunting activities in particular often have a detrimental effect on certain species by reducing population numbers (Reyna-Hurtado & Tanner 2005; Buij *et al*. 2007). The presence of researchers, rangers and other conservation personnel inside protected areas has been shown to have a positive effect on biodiversity by discouraging poachers and other offenders, and by providing economic benefits for local communities (Laurance 2013). The benefits to biodiversity may be particularly strong for threatened and overharvested species, and poaching has been shown to decrease towards the centre of research areas (Campbell *et al*. 2011).

Commercially valuable species, such as those targeted by hunters or animal traders, are often particularly threatened by human disturbance, and the heterogeneous distribution of hunted species in a landscape has been shown to correspond to gradients in hunting intensity, which in turn correlate with accessibility (e.g. Muchaal & Ngandjui 1999; Wright *et* *al*. 2000; Hill *et al*. 2003). Life history traits may, however, also play an equally important role as hunting in determining which species withstand changes in land use practices (Brashares 2003); classical k-selected species are often disproportionally affected by hunting (Bodmer *et al*. 2002).

The impact of road infrastructure on the stability of ecosystems has been well documented (Trombulak & Frissell 2001). By facilitating access into previously remote areas, roads have been shown to increase levels of hunting, deforestation and other disruptive behaviours, with particularly negative effects on certain vulnerable taxa (McLellan & Shackleton 1988; Nelson & Hellerstein 1997; Foley *et al*. 2005). Roads can also cause population fragmentation by acting as an ecological barrier (Dyer *et al*. 2002; Laurance *et al*. 2009). Isolated populations, unable or unwilling to cross roads, are then susceptible to the effects of inbreeding, edge effects and other ecological pressures (Laurance *et al*. 2009; Brodie *et al*. 2014).

Many studies have used transect survey methodologies to assess the impact of proximity to roads or settlements on the distribution of mammal species in protected areas (e.g. Blom *et al*. 2005; Buij *et al*. 2007); or to assess the influence of different rates of hunting on species distribution and habitat preference (e.g. Reyna-Hurtado & Tanner 2005 for Baird's tapir and other Neotropical ungulates). None of these studies investigated the impact of transects *per se* on either rates of hunting or on the distribution of hunted species in the landscape.

Baird's tapir is the largest terrestrial mammal in the Neotropics. It is a popular hunted species across its range and has traditionally been a source of bushmeat for indigenous peoples (Dunn *et al.* 2012). It is currently classified as endangered, with hunting and habitat loss cited as the primary reasons for its perilous conservation status (Castellanos *et al.* 2008). The population of Baird's tapir in Honduras is estimated to be around 500 individuals, and rapid conversion of forest to agricultural land combined with unregulated hunting by poachers have precipitated a critical conservation situation for this iconic species (Castellanos *et al.* 2008; McCann *et al*. 2012).

The results of an analysis of Baird's tapir occupancy in Cusuco National Park (PNC) in Honduras are presented. Encounter data gathered between 2006 and 2013 were compared to assess whether occupancy and detectability have changed over time, and whether any change in the above parameters can be attributed to new environmental pressures that have been introduced by the creation and maintenance of a network of transects used for biodiversity surveys.

This study was designed to test the hypothesis that tapir occupancy is affected by the ease of human access into tapir habitat; and to compare on- and off-transect estimates of occupancy and detection probability from PNC to test the hypothesis that the transect data may not be representative of wider occupancy and detectability. In addition, annual deforestation levels in PNC are compared to an adjacent site with no transect network to test the hypothesis that the transect network has contributed to an increase in deforestation in PNC.

5.3. Methods

The general sampling methodology for the observation of tapir spoor (sightings, prints, faeces, evidence of foraging) is as described in Chapter 2.

5.3.1. Study site

The study was carried out in PNC, in the Merendon Mountains near the major conurbation of San Pedro Sula. The Park consists of a core zone in which all deforestation, hunting and inhabitation is nominally prohibited, and a buffer zone in which communities are allowed to develop, and people are allowed to expand agricultural interests and collect firewood. Satellite imagery and ground observations confirm that the buffer zone of PNC is considerably more degraded than the core zone, and continues to experience rapid conversion into pasture and plantations (Hansen *et al*. 2013; Chapter 6).

To maintain a standardised sampling regime, seven sites were chosen and (where relevant) partially cleared by researchers from the science tourism company Operation Wallacea, to serve as camps for researchers operating in PNC from June-August each year. Four or five transects were established, radiating away from each camp up to a distance of 3km (Figure 5.1). Three separate paths were also established to access the core-zone camps from villages located in the buffer-zone. The transects, completed in 2006, were cut along existing lines of weakness, utilising animal trails, access paths used by hunters, or following obvious landscape features such as ridges and streams; and significantly facilitated access into the core zone of the Park.

5.3.2. Transect surveys

Line transect surveys for tapir spoor were carried out in 2006 and then annually from 2008 to 2013, with each transect walked twice per sampling season. Additional surveys were undertaken off-transect during years 2011 to 2013. Off-transect surveys were designed to survey regions of the Park not intersected by the transect network, and were conducted to test whether the sample population is representative of the overall population. Transects and off-transect tracks were walked, by a minimum of two trained observers, at approximately 1.5 km/hour, and all encounters with tapir spoor were recorded. The location of all tracks, dung and other signs of tapir presence were recorded using a GPS (Garmin Vista HCX GPS) for use with the programmes Garmin Basecamp v4.1 and ArcGIS 10.1. The "overall population" refers to all the tapir in PNC. The "sample population" here refers to tapir with territories that overlap the transect network.

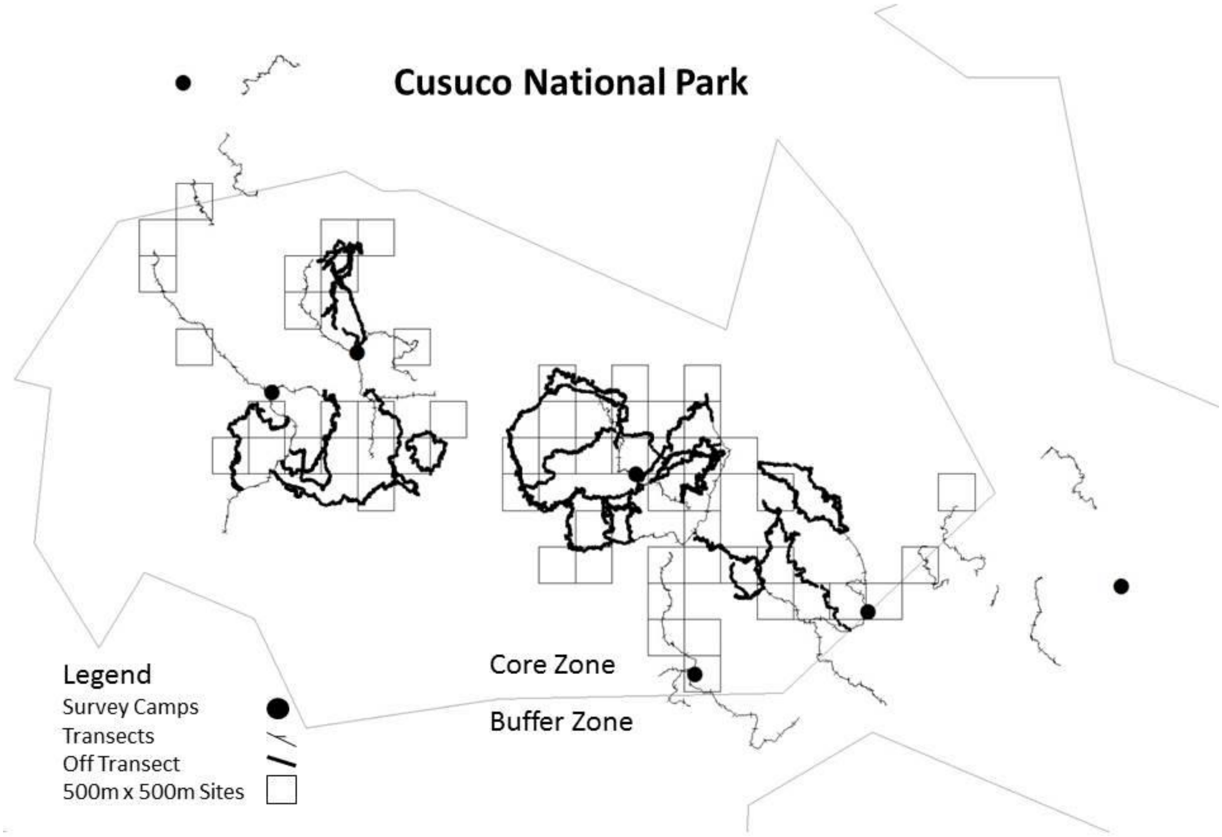


Figure 5.1: Sampling sites in Cusuco National Park, Honduras.
Tracks, both on- and off-transect, were used to survey for Baird's tapir spoor between 2006 and 2013. Also shown is the final selection of 500 m x 500 m sites chosen for the patch occupancy analysis with the programme Presence 5.1 (Hines 2010).

Survey tracks were broken down into distinct sites or 'Landscape Units' (LSUs) for patch occupancy analysis. To deal with potential spatial autocorrelation in the analysis, a random sampling strategy was employed for selection of LSUs. Using the 'create fishnet' tool in ArcGIS 10.1 a 500 m x 500 m grid was overlaid on a digital map of the Park. Baird's tapir have an estimated annual home range of 1 km^2, a smaller grid was used to reflect the likelihood of tapir utilising a smaller home range over the duration of each survey season, as has been reported previously (Foerster & Vaughan 2002). All the grid squares that intersected transect or off-transect tracks were selected. 50% of these grid squares were randomly selected using the 'random points' tool in ArcGIS 10.1 to include as LSUs or 'sites' in the analysis. A total of 28 on-transect and 26 off-transect LSUs were included in the analysis. Spatial replicates were created within each LSU by creating a 100 m x 100 m grid and selecting a random sample of 50% of those grid squares in which surveys had been conducted. Transects outside of the core zone were excluded from the analysis as tapir occupancy was not recorded in the highly modified buffer zone during the study period.

5.3.3. Data Analysis

5.3.3.1. Deforestation

Data on forest loss in 30 m x 30 m squares between the years 2000 and 2012, and on forest cover in the year 2000 were downloaded from Hansen *et al*. (2013). Annual deforestation levels in PNC and the adjacent Water Protection Area (WPA; where no transect network exists) were compared with a general linear model (GLM) using the statistical package R 3.03 to test whether changes in the levels of deforestation in PNC could be attributed to the creation of the transect network in PNC in 2006, or whether they are better explained by background levels of deforestation.

5.3.3.2. Patch occupancy analysis

Tapir encounter data were analysed using PRESENCE 5.1 (Hines 2010), using the parameters as per Mackenzie *et al*. (2002) to estimate values for occupancy (*Psi*) and detectability (*p*). Multi-season models were used to test for changes in *Psi* and *p* on the transect network between 2006 and 2013, and to compare estimates of *Psi* and *p* from on- and off-transect surveys between 2011 and 2013. Model selection was based on AIC (Johnson & Omland 2004).

The effect of accessibility on the distribution of tapir spoor was explored by adding the covariate ‘access’ to the analyses. Ease of access was represented as a binary covariate with each LSU being identified as either ‘easy’ (along a well-defined transect in close proximity to human habitation, n [number of sites] =18), or ‘hard’ (along a poorly maintained and remote transect, n=20). An additional covariate ‘off transect’ (not located along any known trail) was included in the further analysis that incorporated off transect surveys for years 2011 to 2013. The effect of the covariate ‘off transect’ on the parameter *eps* (the probability of an LSU that was occupied in one year being unoccupied in the next year; Mackenzie *et al*. 2002) was also investigated.

5.3.3.3. Spoor encounter frequency distribution analysis

Using the ‘create fishnet’ tool in ArcGIS 10.1, all of the 100 m x 100 m squares in which surveys had been conducted (on- and off-transect) between the years 2011 and 2013 were mapped. Using the spatial analyst tool in ArcGIS 10.1, the Euclidian distance was calculated from the nearest transect to each of the 100 m x 100 m squares in which surveys had been conducted (2011 n= 517; 2012 n=797; 2013 n=431), and to each of the 100 m x 100 m squares in which tapir spoor was encountered during the same period (2011 n=80; 2012 n=173; 2013 n=30). The frequency distributions of Euclidian distances between the nearest transect and the squares walked vs the squares where tapir spoor was encountered were plotted and the similarity of these distributions was analysed using a two-sample Kolmogorov-Smirnov test using the statistics package R 3.03. The number of squares in which surveys were conducted was used to represent effort, and the frequency distribution of encounters/effort was plotted against distance from transect to observe the change in the probability of encountering tapir depending on distance. The assumptions of the plot were controlled for effort by calculating the confidence intervals of the Poisson distribution of encounters.

5.4. Results

5.4.1. Encounter data

A total of 674 encounters with tapir spoor were recorded during on-transect surveys, and 329 during the off-transect surveys at mean encounter rates per kilometre of 3.05 and 3.18, respectively. The basic encounter rate on the transects decreased markedly between 2010 and 2011 (Table 5.1).

Table 5.1: Trends in encounters with Baird's tapir spoor in Cusuco National Park (PNC), Honduras. Data was collected during field surveys between 2006 and 2013 using a maintained transect network. Data from the buffer zone of PNC, where tapir were not recorded, are excluded. Off-transect encounter data are presented for years 2011-2013, when additional off-transect surveys were conducted.

Year	Encounters		Distance Walked (km)		Encounter Rate per km	
	On transect	Off transect	On transect	Off transect	On transect	Off transect
2006	162	N/A	32.6	N/A	4.97	N/A
2008	149	N/A	32.6	N/A	4.57	N/A
2009	163	N/A	32.6	N/A	5.00	N/A
2010	151	N/A	31.5	N/A	4.79	N/A
2011	22	77	30.7	25.00	0.72	3.08
2012	27	252	30.7	76.85	0.88	3.28
2013	13	26	30.7	20.87	0.42	1.25

5.4.2. Deforestation

Forest loss in PNC and the adjacent WPA demonstrated a linear increase between 2000 and 2012 (Figure 5.2). The GLM showed that annual fluctuations in the rate of forest loss in PNC are correlated with coincident fluctuations in the WPA (intercept t = 3.570, p = 0.0051, WPA t = 3.570, p= 0.0051). The correlation was less well supported by the data when year was included in the model (intercept t = 3.698, p = 0.0049; WPA t = -1.965, p = 0.081; year t value = -2.027, p = 0.0733).

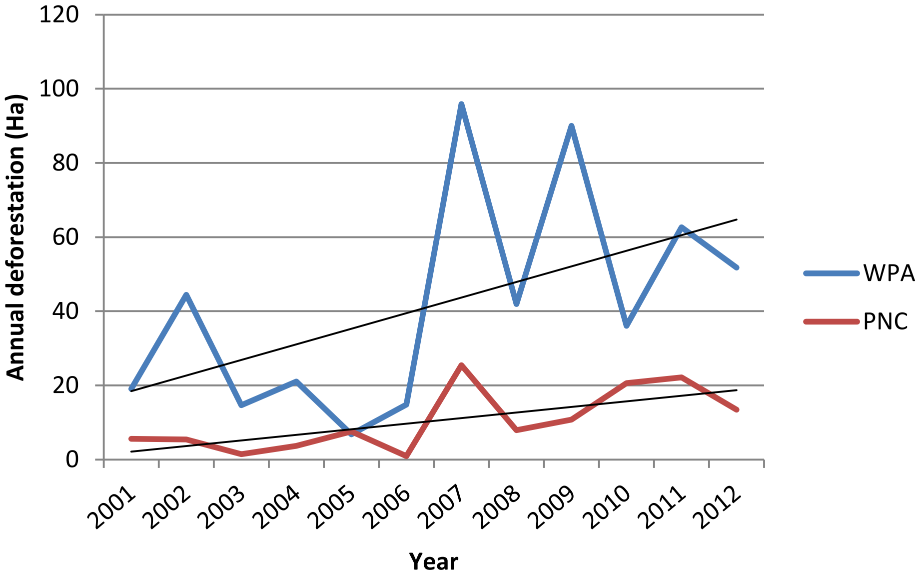


Figure 5.2: Annual deforestation levels in Cusuco National Park (PNC) and the adjacent Water Protection Area (WPA) in Honduras.
Forest loss data (in hectares) were derived from Hansen *et al*. (2013) and calculated using the Spatial Analyst tool in ArcGIS 10.1.

5.4.3. On-transect analysis from 2006-2013

Multi-season analyses were used to compare the distribution of tapir along the transect network between years 2006 and 2013. The best fitting models from the PRESENCE analysis suggested the distribution of encounters with tapir spoor is best explained by annual changes in *p* and on the accessibility of the LSUs (Table 5.2). The models show that *Psi* remained relatively constant, though demonstrating a negative trend over time, whereas detectability declined significantly over the duration of the study period. All models suggested that *p* was significantly higher along hard-to-access transects. *Psi* was also higher on hard-to-access transects, though not significantly so (Figure 5.3).

Table 5.2: Model selection statistics for Baird's tapir distribution along a network of transects in Cusuco National Park (PNC), Honduras.

The programme PRESENCE 5.1 (Hines 2010) was used to compare competing models to describe Baird's tapir occupancy (*Psi*) and detectability (*p*) along the transect network in PNC between 2006 and 2013. Covariates used were 'year', representing the seven years of survey data; and 'access', representing the ease of access (easy or hard) of each Landscape Unit (LSU) in the model. Models are ranked by AIC.

Model	**Delta AIC**	**AIC Weight**	**-2 Log Likelihood**	**No. Parameters**
Psi,gamma(.),eps(.)p(year,access)	0.00	0.8651	292.52	11
Psi,gamma(.)eps(access),p(year,access)	7.03	0.0257	299.55	11
Psi,gamma(year)eps(.),p(year,access)	7.34	0.0220	289.86	16
Psi(year),gamma(.),p(year,access)	13.51	0.0010	296.03	16
Psi,gamma(.),eps(.),p(access)	14.14	0.0007	320.66	4
Psi,gamma(.),eps(.),p(year)	19.36	0.0001	313.88	10
Psi,gamma(.),eps(.),p(.)[Null model]	35.44	0.0000	341.96	4

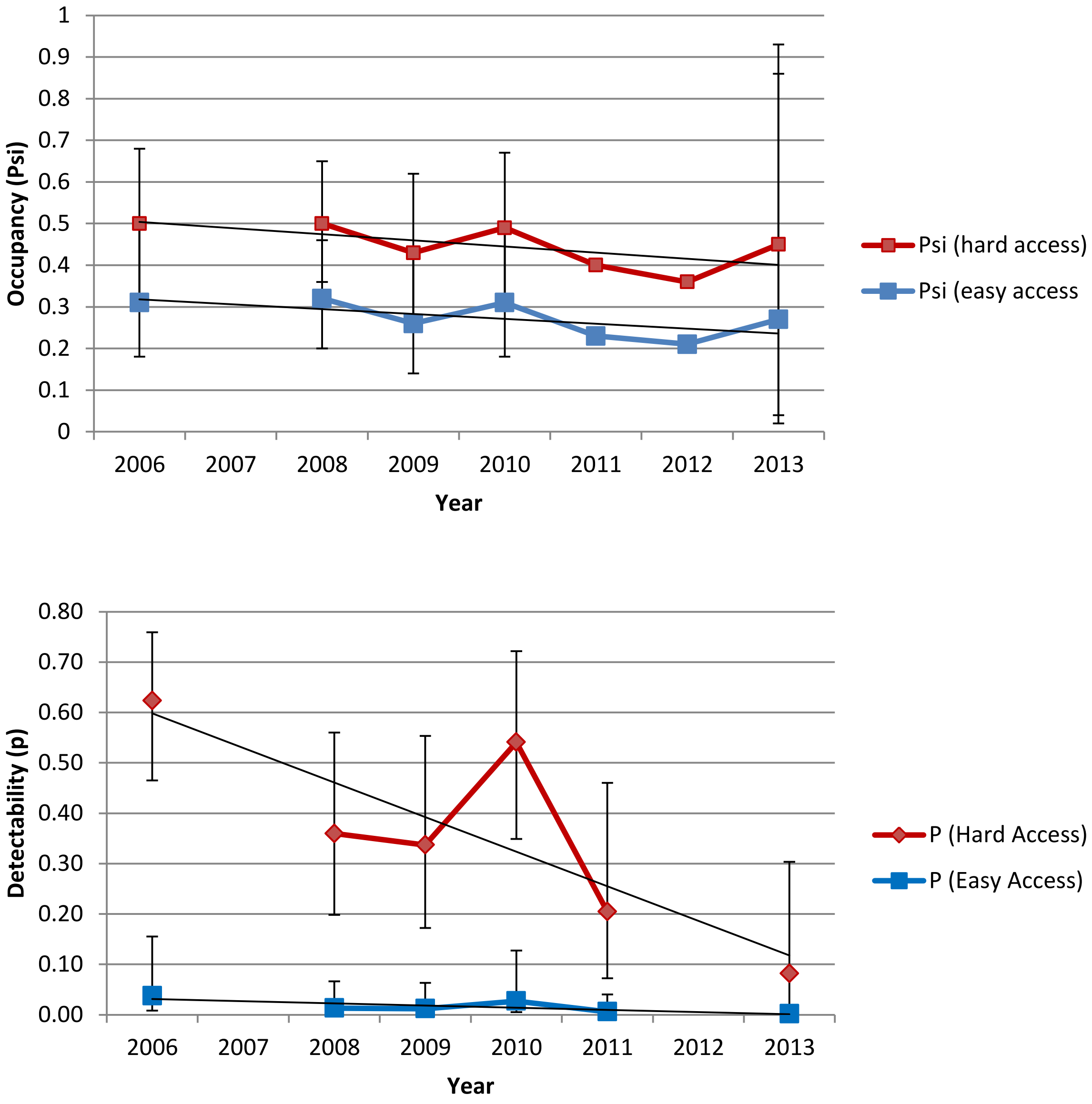


Figure 5.3: Baird's tapir occupancy (*Psi*; top) and detectability (*p*; bottom) in Cusuco National Park (PNC), Honduras.

Data are from the best fitting models for these data: [*Psi*: *psi(year,access),gamma(.),p(.)*; *p*: *psi,gamma(.),eps(.),p(year,access)*]. Data were collected during annual transect surveys for tapir spoor in PNC between 2006 and 2013.

5.4.4. On- versus off-transect analyses from 2011-2013

A second set of analyses were run to investigate whether the probability of an LSU being occupied by tapir changed depending upon whether or not it was intersected by a transect for years 2011, 2012 and 2013, where off-transect data also exist. Of the 54 LSUs analysed (28 on-transect and 26 off-transect), 30 were occupied at some point between 2011 and 2013, and of these 21 (70%) were off-transect.

The best fitting multi-season models from the PRESENCE analysis suggested that the data are best explained by a change in *Psi* depending upon the covariate 'off transect' (i.e. that any given site has a higher probability of being occupied if it is off transect). The models also identified a change in *p* between years. Models that held *Psi* constant while altering *p* according to whether LSUs were off-transect were rejected (Table 5.3; see supplementary materials for detailed model statistics for *Psi* and *p* of the best fitting models). Models that included the parameter *eps(off transect)* identified that occupied on-transect LSUs were more likely to be unoccupied in subsequent years than were occupied off-transect LSUs, though not significantly so, and these models did not rank highly.

Table 5.3: Model selection statistics for Baird's tapir distribution in Cusuco National Park (PNC), Honduras.

The programme PRESENCE 5.1 (Hines 2010) was used to compare competing models to describe Baird's tapir occupancy (*Psi*) and detectability (*p*) in PNC between 2011 and 2013, using tapir spoor encounter data from on-transect and off-transect surveys. Covariates used were 'year', representing the three years of survey data, and 'off transect', representing whether each Landscape Unit (LSU) in the model was located on or off the existing transect network in PNC. Models are ranked by AIC.

Model	**Delta AIC**	**AIC Weight**	**-2 Log Likelihood**	**No. Parameters**
Psi(off transect),gamma(.),p(year)	0.00	0.4124	435.39	5
Psi(off transect),gamma(year),p(year)	1.68	0.1780	435.07	6
Psi(off transect),gamma(.),p(year, off transect)	1.93	0.1571	435.32	6
Psi(off transect),gamma(.),p(.)	2.99	0.0925	442.38	3
Psi(year, off transect),gamma(.),p(year)	4.10	0.0531	433.49	8
Psi(year, off transect),gamma(.),p(.)	5.04	0.0332	438.43	6
Psi(off transect),gamma(off transect),p(year)	6.68	0.0146	442.07	5
Psi,gamma(.),eps(off transect),p(year)	11.69	0.0012	445.08	6
Psi(.),gamma(.),p(year)	12.72	0.0007	448.11	5
Psi,gamma(.),eps(.),p(year)	14.67	0.0003	448.06	6
Psi,gamma(.)eps(.),p(year, off transect)	15.06	0.0002	446.45	7
Psi(year),gamma(.),p(year)	16.62	0.0001	448.01	7
Psi(.),gamma(.),p(.)[Null model]	17.00	0.0002	456.39	3

The frequency distribution of 100 m x 100 m squares walked (effort) vs squares where tapir were encountered supports the hypothesis that Baird's tapir display a heterogeneous distribution in PNC. The probability of encountering tapir increases with distance from transect until approximately 700 m from the nearest transect (Figure 5.4). The results of the two-sample Kolmogorov-Smirnov test were highly significant for all years individually (2011: D = 0.3753, $p < 0.0001$; 2012: D = 0.2064, $p < 0.0001$; 2013: D = 0.3097, p = 0.0092), and combined (D = 0.3189, $p < 0.0001$).

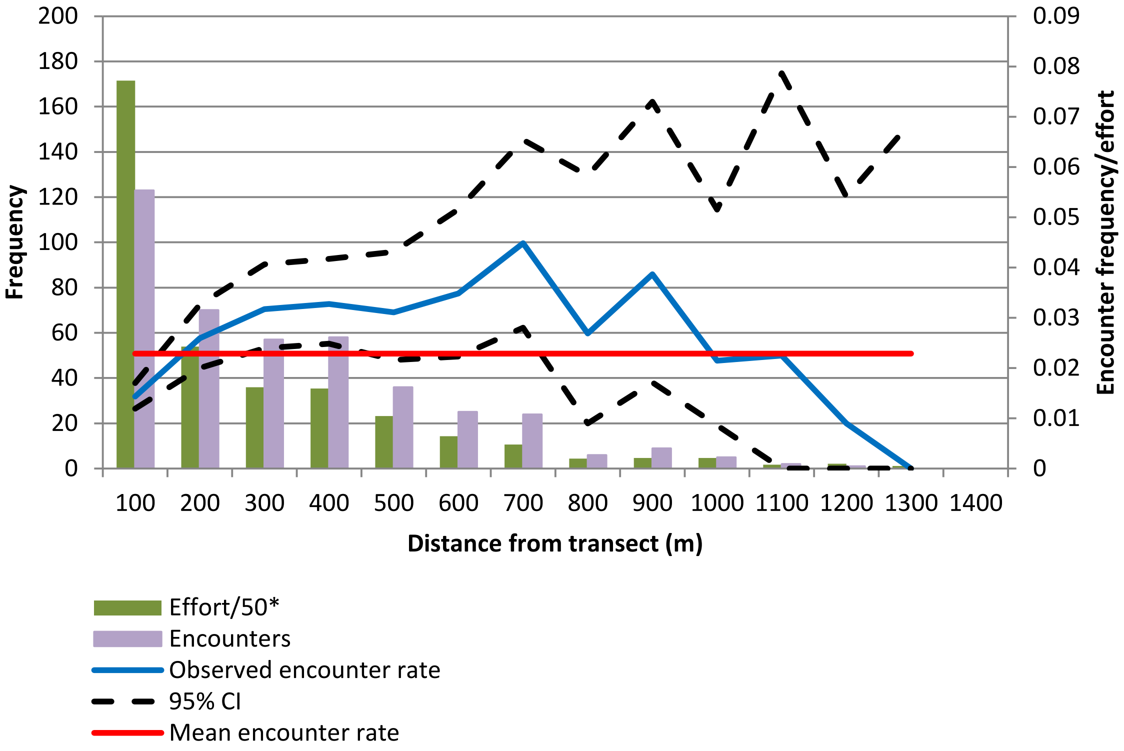


Figure 5.4: Frequency distribution of surveys for Baird's tapir spoor in Cusuco National Park (PNC), Honduras.

Data was collected during on- and off-transect surveys for Baird's tapir spoor in PNC between 2011 and 2013. The primary vertical axis plots the distribution of the Euclidian distance from the nearest transect to each 100 m x 100 m square surveyed (effort/50) and to each square where Baird's tapir were encountered (encounters). *The actual number for 'effort' was divided by 50 so it could be plotted on the same axis as 'encounters'.

The secondary vertical axis plots the observed encounter rate (encounter frequency/effort) against the mean encounter rate across all surveys.

5.5. Discussion

5.5.1. Changes in occupancy and detectability

On-transect analyses of Baird's tapir encounter data suggest that tapir distribution is correlated with human accessibility, and that both *Psi* and *p* have changed over the duration of the survey period. The on-transect vs off-transect analyses demonstrate that tapir are heterogeneously distributed across the Park and are less likely to be encountered in areas associated with the transect network. Due to this distribution bias, population trends in the sample population (as defined by those individuals whose home ranges overlap the transect network) may not accurately reflect trends in the overall population.

Data from on- and off-transect surveys between 2011 and 2013 demonstrate that the frequency of encounters with tapir spoor increases with distance from the transect network up to a distance of approximately 700 m. The decline in encounter rate after 700 m is likely to be a product of the low survey effort in the more remote areas (Funston *et al*. 2010). With the addition of only a single extra encounter per 100 m increment in distance from transect after 700 m, the encounter rate levels off, and the pronounced decline in encounter rate is no longer observed.

In light of the observed distribution bias, it is important to determine whether the results presented here represent a change in the distribution of tapir in PNC, or a change in the distribution and population density of the species across the whole Park. Different species may display alternative responses to anthropogenic pressure. African elephants (*Loxodonta africana*) may modify their foraging patterns depending upon fluctuating levels of disturbance in their habitat, to preferentially forage in areas with low levels of disturbance. Black rhinoceros (*Diceros bicornis*) do not preferentially occupy less-disturbed areas, and reductions in population density may represent overhunting rather than changes in behaviour (Leader-Williams *et al*. 1990). Tapir have not been recorded to demonstrate a strong preference for areas of low human activity *per se* (Fragoso 1991; Kinnaird *et al*. 2003; Naranjo & Bodmer 2007; Dunn *et al*. 2012), but are known to be susceptible to hunting pressure (Brooks *et al*. 1997; Naranjo & Bodmer 2007; Naranjo 2009). This might suggest that the distribution bias observed here is more likely to be attributed to the loss of individuals with home ranges that overlap the transect network rather than to a behavioural change.

If the distribution bias represented a behavioural change whereby tapir were preferentially avoiding the transect network, off-transect surveys should record high encounter rates with tapir spoor, as the tapir would, in theory, be living at higher population densities if the same size population was utilising a smaller percentage of the Park. Unnaturally high population densities due to reductions in habitat availability have been recorded in orang-utan (*Pongo pygmaeus*), which may live at high densities in small remaining pockets of gallery forest (Bruford *et al*. 2010). Rates of encounter with tapir during off-transect surveys were lower than during on-transect surveys between 2006 and 2010 (Table 5.1), and estimates of *p* during off-transect surveys were lower than for hard-to-access transects between 2006 and 2010 (Supplementary Tables 5.4 & 5.5). This would suggest that the population has experienced a change in density in addition to a change in distribution.

Many species are affected by human disturbance (e.g. Wright *et al.* 2000; Fernandez-Juricic 2000), and large mammals in particular have been shown to experience a raised extinction threat in the presence of human activity (Cardillo *et al*. 2005). The results presented here infer a net decline in tapir population density, concentrated in areas where human accessibility is least constrained, and support the hypothesis that tapir distribution can be modified by human activity. It is not clear, however, whether tapir are preferentially avoiding the transect network, or whether hunting has selectively removed the majority of the individuals whose home ranges overlapped the transect network, and that these areas have yet to be recolonized.

A hunting-related decline is not unexpected as hunting is one of the leading causes of population decline in large mammals (Ceballos & Ehrlich 2002), and mammal abundance in different regions has been shown to correlate closely with hunting, with the greatest disparity in abundance occurring in those species that are hunted for food, which is the case for Baird's tapir (Carrillo *et al*. 2000). Extinction risk is exacerbated in large mammals due to intrinsic factors such as low rates of reproduction, making them even more vulnerable to hunting and other environmental pressures (Bodmer *et al.* 2002; Cardillo *et al.* 2005).

Deforestation in the core zone of PNC has increased linearly since the year 2000. The finding that changes in the amount of deforestation in the core zone of PNC correlate with coincident changes in the adjacent WPA suggests that the observed increase in deforestation in the core zone of PNC in 2007, directly after the creation of the transect network, might be explained by other social factors not analysed here, rather than by loggers using the transect network. No transect network exists in the WPA where an equivalent spike in deforestation was also recorded.

The observation that deforestation in the core zone of PNC rose independently of the creation of the transect network (Figure 5.2) raises the possibility that an increase in hunting may also have occurred at the same time, had the transect network not been created, since poaching and illegal deforestation often occur in coincidence (Sodhi & Ehrlich 2010). Interviews with local hunters (Chapter 6) have, however, attributed elevated hunting rates to the improved access into the core zone of PNC caused by the creation of the new transect network. The testimony of local hunters and the observation of hunters using the transect network over the duration of this study, makes it is reasonable to suggest that the creation of the transect network, in addition to modifying the distribution of tapir in PNC, has contributed in part to a decline in population density by making it easier for hunters to enter the core zone of the Park.

5.5.2. Implications for conservation planning when designing surveys in novel locations.

Robust biological and socio-economic data are essential for effective conservation planning (Loiselle *et al*. 2003; Polasky 2008), but the means by which data are collected in the field should be carefully evaluated with regard to their wider impacts on wildlife, as well as their methodological robustness. Here, it has been demonstrated how it would be easy to misidentify an area as undergoing a demographic crash when actually what might have happened is a change in distribution, which could lead to the misallocation of resources for conservation when those resources would be better spent in a different area (Loiselle *et al*. 2003). This research suggests that off-transect surveys of randomly chosen sites within a study area should be undertaken periodically to assess whether the sample population is genuinely representative of the overall population in those taxa where vulnerability to human disturbance is suspected. It may be discovered that in certain cases the use of transects to monitor population trends is inappropriate, and a different sampling regime is required instead.

In this instance, the change in *Psi* and *p* was found to be due in part to an actual population decline, and the observation that creating a novel network of transects in PNC is correlated with a decline in population density in Baird's tapir should come as a warning signal to other researchers working with vulnerable taxa. The assumption that research centres confer a conservation benefit to biodiversity is widely accepted and has been supported empirically (Campbell *et al*. 2011; Laurance 2013). The evidence from the present study shows that research activity may not always confer protection, and may have unintended negative consequences for the conservation of certain taxa.

On the basis of these findings, the potential consequences of installing a transect network in certain environments, given the potential impact on vulnerable taxa, should be routinely evaluated prior to the establishment of such a network. Road building has been linked to increases in bushmeat hunting by improving access to hunting grounds (McLellan & Shackleton 1988; Nelson & Hellerstein 1997; Foley *et al*. 2005), and it is not unreasonable to propose that the creation of transects may have a similar effect under certain circumstances. On this basis, creating transects that might increase unwanted human activity should be avoided in favour of other survey methodologies, such as off-transect surveys using patch occupancy analysis, which are increasingly feasible given advances in GPS technologies (Buij *et al*. 2007).

This cautionary note is clearly context-specific and may only apply to certain sites and certain vulnerable taxa. The example reported here could be interpreted as a ‘worst case scenario’. Baird’s tapir, as the largest terrestrial mammal in Central America, are a highly prized target for poachers, and due to their low recruitment rate are likely to suffer local extirpations more rapidly than other species (Cardillo *et al.* 2005; Medici *et al.* 2005).

In conclusion, without careful planning, creating a novel system of transects can obscure efforts to monitor population trends in certain target species by creating a distribution bias, and may increase the extinction risk to vulnerable taxa and habitats by facilitating human access to the study site. To our knowledge, this is the first time a population decline has been attributed in part to the creation of a novel transect network, a phenomenon that has otherwise been regularly observed in the creation of roads. The example of PNC should serve as a warning to other conservation practitioners that proper planning is crucial in guaranteeing the long-term viability of novel study sites and the validity of survey methodologies, and that a range of methodologies should be considered in order to maximise the efficacy of data collection and minimise the negative collateral effects on biodiversity.

5.6. Supplementary Materials

Supplementary Table 5.4: Model selection statistics for Baird's tapir occupancy (*Psi*) and detectability (*p*) on the survey transect network in Cusuco National Park (PNC), Honduras.
The covariate 'access' relates to ease of access to the survey site (easy or hard). Estimates for *Psi* and *p/p*(access) are derived from transect surveys for Baird's tapir spoor in PNC between 2006 and 2013.

Model	Year	Psi	95% Conf. Interval	Access	P/P (access)	95% Conf. Interval
Psi,gamma(.),eps(.)p(year,access)	2006	0.6502	0.4025 - 0.8369	Easy	0.0376	0.0082 - 0.1555
				Hard	0.6234	0.4648 - 0.7594
	2008	0.5589	0.3624 - 0.7554	Easy	0.0131	0.0025 - 0.0664
				Hard	0.3596	0.1984 - 0.5601
	2009	0.4982	0.2785 - 0.7179	Easy	0.0119	0.0021 - 0.0630
				Hard	0.3370	0.1724 - 0.5535
	2010	0.4578	0.2095 - 0.7061	Easy	0.0271	0.0053 - 0.1274
				Hard	0.5410	0.3489 - 0.7217
	2011	0.4310	0.1589 - 0.7031	Easy	0.0060	0.0009 - 0.0401
				Hard	0.2047	0.0722 - 0.4601
	2012	0.4131	0.1225 - 0.7038	Easy	0.0000	0.0000 - 0.0000
				Hard	0.0000	0.0000 - 0.0000
	2013	0.4013	0.0963 - 0.7063	Easy	0.0021	0.0002 - 0.0191
				Hard	0.0818	0.0179 - 0.3036

Supplementary Table 5.5: Model statistics for Baird's tapir occupancy (*Psi*) and detectability (*p*) in Cusuco National Park (PNC), Honduras.
The covariate 'off transect' relates to whether surveys were conducted outside the existing survey transect network in PNC. Estimates for *Psi/Psi*(off transect) and *p/p*(off transect) are derived from on- and off-transect surveys between 2011 and 2013.

Model	Year	Psi/Psi(off transect)	95% C.I.	On/Off Transect	P/P(off transect)	95% C.I.
Psi(off transect),gamma(.),p(year)	2011	0.5000	0.5000 - 0.5000	On	0.2359	0.1622 - 0.3299
		0.9679	0.1331 - 0.9998	Off		
	2012	0.5000	0.5000 - 0.5000	On	0.3130	0.2415 - 0.3946
		0.9679	0.1331 - 0.9998	Off		
	2013	0.5000	0.5000 - 0.5000	On	0.1248	0.0542 - 0.2621
		0.9679	0.1331 - 0.9998	Off		
Psi(off transect), gamma(year),p(year)	2011	0.5000	0.5000 - 0.5000	On	0.2344	0.1618 - 0.3270
		0.9759	0.0510 - 1.0000	Off		
	2012	0.5000	0.5000 - 0.5000	On	0.3101	0.2385 - 0.3920
		0.9759	0.0510 - 1.0000	Off		
	2013	0.5000	0.5000 - 0.5000	On	0.1224	0.0523 - 0.2608
		0.9759	0.0510 - 1.0000	Off		
Psi(off transect), gamma(.),p(year, off transect)	2011	0.5000	0.5000 - 0.5000	On	0.2267	0.1380 - 0.3494
		0.9636	0.1775 - 0.9997	Off	0.2415	0.1592 - 0.3488
	2012	0.5000	0.5000 - 0.5000	On	0.3000	0.1936 - 0.4335
		0.9636	0.1775 - 0.9997	Off	0.3176	0.2395 - 0.4076
	2013	0.5000	0.5000 - 0.5000	On	0.1218	0.0518 - 0.2608
		0.9636	0.1775 - 0.9997	Off	0.1310	0.0527 - 0.2899

Psi(off transect),gamma(.),p(.)	2011	0.5000 0.5000 - 0.5000	On	0.2645 0.2172 - 0.3179
		1.0000 0.0000 - 1.0000	Off	
	2012	0.5000 0.5000 - 0.5000	On	0.2645 0.2172 - 0.3179
		1.0000 0.0000 - 1.0000	Off	
	2013	0.5000 0.5000 - 0.5000	On	0.2645 0.2172 - 0.3179
		1.0000 0.0000 - 1.0000	Off	
Psi(year, off transect), gamma(.),p(year)	2011	0.3807 0.1959 - 0.6079	On	0.2411 0.1646 - 0.3387
		0.9544 0.3185 - 0.9989	Off	
	2012	0.3495 0.3495 - 0.3495	On	0.3243 0.2462 - 0.4137
		0.9482 0.2220 - 0.9991	Off	
	2013	0.3663 0.3663 - 0.3663	On	0.1342 0.0579 - 0.2808
		0.9517 0.2349 - 0.9992	Off	
Psi(year, off transect),gamma(.),p(.)	2011	0.3557 0.1749 - 0.5898	On	0.2757 0.2208 - 0.3383
		0.9880 0.0000 - 1.0000	Off	
	2012	0.3578 0.1846 - 0.5782	On	0.2757 0.2208 - 0.3383
		0.9881 0.0000 - 1.0000	Off	
	2013	0.2545 0.0948 - 0.5266	On	0.2757 0.2208 - 0.3383
		0.9808 0.0000 - 1.0000	Off	
Psi(off transect), gamma(off transect),p(year)	2011	0.5000 0.5000 - 0.5000	On	0.2389 0.1584 - 0.3436
		0.8898 0.6025 - 0.9773	Off	
	2012	0.5000 0.5000 - 0.5000	On	0.3366 0.2610 - 0.4216
		0.8898 0.6025 - 0.9773	Off	
	2013	0.5000 0.5000 - 0.5000	On	0.1046 0.0441 - 0.2285
		0.8898 0.6025 - 0.9773	Off	
Psi,gamma(.), eps(off transect),p(year)	2011	0.7366 0.4243 - 0.9139		0.2092 0.1268 - 0.3253
	2012	0.4172 0.3244 - 0.5100		0.3480 0.2714 - 0.4335
	2013	0.3168 0.1460 - 0.4875		0.1534 0.0600 - 0.3395
Psi(.),gamma(.),p(year)	2011	0.5942 0.4358 - 0.7352		0.2586 0.1721 - 0.3693
	2012	0.5942 0.4358 - 0.7352		0.3423 0.2662 - 0.4275
	2013	0.5942 0.4358 - 0.7352		0.1211 0.0508 - 0.2617
Psi,gamma(.),eps(.),p(year)	2011	0.6098 0.3924 - 0.7909		0.2541 0.1623 - 0.3746
	2012	0.5886 0.4259 - 0.7512		0.3441 0.2669 - 0.4305
	2013	0.5743 0.3361 - 0.8125		0.1253 0.0494 - 0.2832

Psi,gamma(.) eps(.),p(year, off transect)	2011	0.6352 0.3856 - 0.8285	On	0.2068 0.1117 - 0.3509
			Off	0.2858 0.1758 - 0.4290
	2012	0.6127 0.4364 - 0.7889	On	0.2719 0.1628 - 0.4177
			Off	0.3644 0.2801 - 0.4579
	2013	0.5979 0.3393 - 0.8566	On	0.1078 0.0403 - 0.2578
			Off	0.1564 0.0591 - 0.3535
Psi(year),gamma(.),p(year)	2011	0.6077 0.4180 - 0.7696		0.2549 0.1636 - 0.3744
	2012	0.5873 0.5873 - 0.5873		0.3444 0.2701 - 0.4272
	2013	0.6588 0.6588 - 0.6588		0.1107 0.0483 - 0.2339
Psi(.),gamma(.),p(.)[Null model]	2011	0.5601 0.4070 - 0.7025		0.2971 0.2381 - 0.3637
	2012	0.5601 0.4070 - 0.7025		0.2971 0.2381 - 0.3637
	2013	0.5601 0.4070 - 0.7025		0.2971 0.2381 - 0.3637
Psi(year), gamma(.),p(year, off transect)	2011	0.6116 0.4016 - 0.7870	On	0.2115 0.1173 - 0.3511
			Off	0.2932 0.1854 - 0.4305
	2012	0.5992 0.5992 - 0.5992	On	0.2737 0.1705 - 0.4086
			Off	0.3682 0.2857 - 0.4591
	2013	0.7027 0.7027 - 0.7027	On	0.0915 0.0390 - 0.1999
			Off	0.1348 0.0548 - 0.2951
Psi(year),gamma(.),p(.)	2011	0.5974 0.4081 - 0.7615		0.2969 0.2376 - 0.3638
	2012	0.5972 0.4181 - 0.7537		0.2969 0.2376 - 0.3638
	2013	0.3582 0.1496 - 0.6391		0.2969 0.2376 - 0.3638
Psi,gamma(.),eps(.),p(.) [Null model]	2011	0.6027 0.4079 - 0.7696		0.2973 0.2385 - 0.3638
	2012	0.5479 0.3913 - 0.7046		0.2973 0.2385 - 0.3638
	2013	0.5107 0.3098 - 0.7116		0.2973 0.2385 - 0.3638
Psi(year),gamma(.),p(off transect)	2011	0.5305 0.3682 - 0.6867	On	0.5000 0.5000 - 0.5000
			Off	0.3442 0.2713 - 0.4253
	2012	0.5193 0.3771 - 0.6585	On	0.5000 0.5000 - 0.5000
			Off	0.3442 0.2713 - 0.4253
	2013	0.3087 0.1354 - 0.5599	On	0.5000 0.5000 - 0.5000
			Off	0.3442 0.2713 - 0.4253

Chapter 6. Baird’s tapir in Honduras: a population and habitat viability analysis of a species under threat of local extinction

This chapter extends McCann *et al*. (2012), which presented a population and habitat viability analysis (PHVA) of the population of Baird's tapir in Cusuco National Park, supported by patch occupancy analysis of encounter data from the Park. In this chapter, I have removed the original patch occupancy analysis, which is instead covered in Chapter 5; and have added analyses of the remaining areas of Baird's tapir occupancy in Honduras to more directly address questions about the conservation status of the species across the whole country. The original paper is attached as Appendix 1.

6.1. Abstract

Despite conservation efforts, accelerating levels of hunting and habitat loss continue to pose a threat to Baird's tapir in Honduras. Here we calculated the potential population size of Baird's tapir in the 13 protected areas where tapir are expected to remain based upon the amount of available habitat and on density estimates from four protected areas, and simulated the effects of different hunting levels on the future dynamics of the Honduran metapopulation using the stochastic simulation software VORTEX. We calculate that the Honduran metapopulation may number 2800-4585 adult Baird's tapir, and suggest that the current IUCN estimate of >500 individuals is a considerable underestimate. We identify that high levels of deforestation and human activity may isolate many of the protected areas where tapir remain, and suggest that immediate intervention is required to maintain connectivity between these protected areas to prevent the imminent extirpation of the species from large parts of its national range.

Key words: PHVA, VORTEX, Baird's tapir, Honduras

6.2. Introduction

Understanding the extinction threat faced by populations of endangered species is crucial to their conservation (Lindenmayer *et al*. 1993). The ability to predict future population outcomes given a range of environmental parameters and scenarios has become a key factor in the creation of many conservation programmes (Akcakaya & Sjogren-Gulve 2000). Central to this concept is the use of Population and Habitat Viability Assessment (PHVA), which incorporates stochastic population modelling to estimate the vulnerability of small populations and to simulate the possible future dynamics of these populations based upon a range of assumptions about future intrinsic and extrinsic factors (Lacy 1993).

PHVA can be used for a wide range of applications, including comparing management options for threatened populations, estimating minimum viable population (MVP) sizes for short- or long-term survival, and establishing sustainable hunting limits (Sodhi & Ehrlich 2010). Although much of the literature on the use of PHVA is theoretical, this approach has been crucial in developing conservation management plans for wild populations of threatened species including the Bornean orang-utan (*Pongo pygmaeus*; Bruford *et al*. 2010), Sumatran rhino (*Dicerorhinus sumatrensis*; Maguire *et al*. 1987), and African wild dog (*Lycan pictus*; Bach *et al*. 2010).

Although this technique may be applied to any species, PHVA is of greatest value for understanding the threats faced by rare k-selected species, and in estimating the level of management required to mitigate these threats (Lindenmayer *et al*. 1993). In addition to demographic threats, small or declining populations are further threatened by the deleterious effects of low genetic diversity, which can reduce adaptive potential and reproductive fitness (Saccheri *et al*. 1998; Spielman *et al*. 2004). This may be exacerbated by habitat fragmentation, which inhibits migration and constrains the potentially stabilising effect of metapopulation dynamics (Sodhi & Ehrlich 2010). Habitat fragmentation and the suite of factors - including hunting - that accompany it, is acknowledged as one of the greatest threats facing many species of large mammal, including Central American species such as white lipped peccary (*Tayassu pecari*; Peres 2001), jaguar (*Panthera* onca; Haag *et al*. 2010) and Baird’s tapir (*Tapirus bairdii*; Garcia *et al*. 2012).

Baird’s tapir is the largest terrestrial mammal in the Neotropics, and despite national laws that prohibit hunting, and international regulations that prohibit trade in body parts, Baird’s tapir populations continue to decline due to poaching and habitat loss across their range

(Castellanos *et al*. 2008; Naranjo 2009; Dunn *et al*. 2012). PHVAs have previously been conducted to assess the viability of Baird's tapir across its entire range (Medici *et al*. 2005; Barongi *et al.* 2006), but never on discrete populations of Baird's tapir, known to be experiencing a current extinction threat.

Baird's tapir is thought to be restricted to four regions in Honduras, comprising 13 protected areas (Chapter 4). The previous chapter presented evidence that the Baird's tapir population in Cusuco National Park (PNC) is experiencing a rapid population decline, attributable to the ongoing poaching of tapir inside the Park. The high levels of deforestation occurring inside each of these 13 protected areas (Hansen *et al*. 2013) would suggest that it is likely that tapir populations in the other regions of tapir occupancy are experiencing similar threats to the population in PNC, and some of these populations may not be viable in the short to medium-term.

Population viability is related in part to the size of the remaining population (Allendorf & Luikhart 2007; Frankham 2014), as population size is correlated to demographic stability (Lande 1993), genetic diversity and mean population fitness (Frankham 2012). Accurate estimates of abundance are an important starting point for population trajectory simulations. Previous studies have attempted to estimate the population density and to infer population size in Baird's tapir populations in Mexico, Belize, Costa Rica and Panama (range: 0.05-1.6 tapirs/km^2, mean: 0.43 tapirs/km^2, calculated from the mean values of nine studies). The abundance of tapir in northeastern Honduras has been estimated by multiplying the amount of available habitat by previously published population density figures from Mexico and Belize (Flesher 1999) and its validity is therefore hampered by this assumption. Additional unpublished studies from Honduras have estimated the amount of remaining tapir habitat, and calculated the number of encounters with tapir spoor per km in three protected areas (0.21 encounters/km, Sierra de Agalta National Park; 0.77 encounters/km, Rus-Rus Biological Reserve; 0.55 encounters/km, Tawahka Asangni Biosphere; N. Estrada pers. comm.). Estimating the relative - or absolute - size of tapir populations in Honduras, based upon encounter data generated in the field as well as the amount of available habitat, is an important advance in understanding the conservation status of the species.

Modelling future population trajectories for Baird's tapir in Honduras under different assumptions could help inform the development of an effective conservation strategy for the species across its range. The objective of this study was to carry out predictive modelling on the remaining populations of Baird's tapir in Honduras, based on population estimates derived from five study sites in four protected areas. In addition, an attempt was made to parameterize and quantify the effect of uncontrolled hunting where hunting levels are known, and to calculate sustainable rates of harvest for the four regions of Baird's tapir occupancy remaining in Honduras, to assess the viability of those populations in the long term.

6.3. Methodology

6.3.1. Sampling methodology

The sampling methodology was as previously described (Chapter 2).

6.3.2. Remaining tapir habitat

The amount of available habitat within each Park was calculated using the Spatial Analyst tool in ArcGIS 10.1. Data on forest loss in 30 m x 30 m squares between the years 2000 and 2012, and on forest cover in the year 2000 were downloaded from Hansen *et al*. (2013). Any square with >50% forest cover in 2000, or that did not undergo deforestation between 2000 and 2012, was considered to be inhabitable by Baird's tapir. Any square with <50% forest cover in 2000, or that underwent deforestation of any form between 2000 and 2012 was considered uninhabitable.

The forest cover data do not differentiate between plantation and natural forest, so a proportion of the forested cells are likely to represent plantations in frequent use, or other types of forest that are unlikely to be inhabitable by tapir. It is therefore likely that the amount of available habit is overestimated.

6.3.3. Population estimates for the study sites

Accurate estimates of population density, based upon encounter rates, require a distance metric to be included in the calculations (Buckland *et al*. 1993). No live tapir were encountered during the course of the present study, and due to the steep and densely forested nature of much of the habitat in the study sites (Cusuco National Park: PNC; Pico Bonito National Park: PNPB; Sierra Rio Tinto National Park: PNSRT, Rio Platano Biosphere Reserve: BRP), it was impractical to record distance-metric data for spoor encounters. Instead, approximate population density was inferred from occupancy estimates generated from spoor encounter data using the programme PRESENCE 5.1 (Hines 2010; Chapter 4). Estimates were checked to ensure that they fall within reasonable boundaries by reference to previously published studies on tapir population density (Naranjo 2009).

Population estimates were generated for both the core zone and buffer zone of each study site by assuming that population density correlates 1:1 with occupancy, whereby each occupied 1 km^2 landscape unit (LSU) represents one tapir (Chapter 4). Estimates were calculated by multiplying the occupancy estimate by the amount of available habitat for the core zone and buffer zone of each Park. For PNPB, occupancy estimates from 2010 were used to estimate occupancy for the whole Park, as deforestation levels in the areas surveyed in 2010 (1.86% deforestation) more closely resemble those for the whole Park (1.31% deforestation) than do those from the areas surveyed in 2012 (0.25% deforestation).

Estimates for the BRP, where only the core zone was surveyed, were complicated by the fact that a map of the core zone boundary is not available. Deforestation data downloaded from Hansen *et al*. (2013) show that deforestation in the BRP is concentrated along the outer edge of the Reserve. As it is not currently possible to confirm exactly how much deforestation occurred in the core zone and buffer zone of the BRP separately, it was assumed that the deforestation occurred equally in both areas to calculate the remaining habitat in each area, although it is probable that deforestation is likely to have been higher in the buffer zone. Occupancy rates for the core zone of the BRP were estimated as per Chapter 4. Occupancy rates for the buffer zone were estimated by assuming a similar rate as that of the buffer zone in PNC, which has lost a similar proportion of forest (5.08%) since 2000 as the BRP (5.88%; Table 6.2).

6.3.4. Population estimates for the regions of tapir occupancy

Population estimates for nine un-surveyed protected areas in the three regions of tapir occupancy (Nombre de Dios Cordillera; The Sierras Region; La Moskitia) were calculated using the same methodology as described above. The amount of available habitat in the un-surveyed protected areas was calculated using data downloaded from Hansen *et al* (2013) as previously described. Occupancy rates were estimated based upon those of the adjacent study site. Occupancy levels for the three un-surveyed protected areas with a core zone and buffer zone were based upon those of the adjacent study site and adjusted depending on the amount of forest loss in the core zone and buffer zone of each protected area since 2000. All un-surveyed protected areas with no core zone were assumed to have a continuous distribution of tapir at a rate of occupancy equivalent to the buffer zone of the neighbouring study site.

The key assumption of this method is that the number of tapir at a particular site follows a Poisson probability distribution (Royle & Nichols 2003) whereby tapir are evenly distributed in their habitat, and that occupancy of an LSU does not impact the probability of occupancy in any other LSU. Although this method of extrapolation has been used previously, for Baird's tapir in Mexico (Naranjo 2009) and Honduras (Flesher 1999), it is unlikely that wild populations of Baird's tapir follow such a regular distribution (Chapter 5), so the population estimates presented here are likely to be higher than reality for some regions.

6.3.5. Stochastic modelling

VORTEX 10 (Lacy 2014) was used to simulate population projections for Baird's tapir populations in the four study sites, and for the remaining three regions of tapir occupancy in Honduras. Life history parameters, including fecundity and mortality rates, were based on a combination of published and unpublished data on Baird's tapir and the closely related lowland tapir (*Tapirus terrestris*; Medici *et al.* 2007; Medici 2010; Gatti *et al*. 2011), and on two previous PVA studies conducted on Baird's tapir (Barongi *et al.* 1994; Medici *et al*. 2005; Table 6.1). Mortality is assumed to increase between ages 1-2 and 2-3 as young tapir migrate out of their natal territory and are consequently exposed to greater risks. The breeding system was changed from monogamous (as per the 2005 PVA) to polygynous for the purposes of this study. Tapirs are not known to mate exclusively and the use of a monogamous breeding system in VORTEX could introduce inaccuracy into the model, especially in terms of projecting genetic diversity.

Scenarios assessing the effect of a range of hunting levels were simulated for each of the four protected areas surveyed, and for the three regions of tapir occupancy. Inbreeding depression was included in all scenarios, given the high probability that, with the exception of the population in the BRP and La Moskitia, the remaining populations are effectively isolated and experiencing zero or limited migration. A lethal equivalents estimate of 6.29 was used, as per O'Grady *et al*. (2006).

Initial population (Pi) sizes were calculated using the methodology described above. Two Pi values were used in PHVAs of each protected area. The 'maximum' Pi was derived from the occupancy value for each study site. The 'minimum' Pi was derived from the lower 95%

confidence interval value of occupancy for each study site. Carrying capacity (K) estimates were calculated by assuming an even distribution of one tapir per km^2 across the remaining available habitat in each protected area. One thousand iterations were run for each hunting scenario over a timeframe of 100 years, which is the equivalent of approximately 10 generations (Castellanos *et al*. 2008).

The aim of this modelling exercise was to create a series of population projections under different hunting pressures to calculate the level at which harvesting becomes unsustainable, leading to the extinction of the tapir populations in each of the study sites; and the level of harvesting at which the populations are able to maintain growth under normal stochastic processes. Population sustainability is difficult to define. Previous studies have assumed PE <0.05 to be an acceptable level of risk for a population (e.g. Wielgus 2002; Mattsson *et al*. 2008). In this study, the hunting rates at which both PE >0.05 and negative stochastic population growth was reached were calculated. The sustainable hunting rate as per Naranjo & Bodmer (2007; hunting rate <0.2 x annual productivity) was also calculated, and the effect of a range of hunting rates on genetic diversity (heterozygosity expected under Hardy-Weinberg) was investigated. In addition, the PNC population was modelled under the current estimated hunting rate of 2 tapir per year (Table 6.4), and the PNSRT population was modelled under a hunting rate of 10 tapir per year, estimated for the adjacent Sierra de Agalta National Park in 2007 (Table 6.4; N. Estrada pers. comm.). Hunting was modelled to occur evenly between the sexes and to only affect adults.

Table 6.1: Baird's tapir life history parameters used for input into the program VORTEX 10. Input data largely derived from Medici *et al*. (2005).
*Inbreeding depression default value of 6.29 for Lethal Equivalents is the combined mean effect of inbreeding on fecundity and first year survival (O'Grady *et al*. 2006).
**Data on density dependent reproduction in Baird's tapir do not exist. Consequently this mechanism was not used in the PHVA models.

Parameter	**Baseline Value**
Number of populations	1
Inbreeding depression Lethal Equivalents	6.29*
Breeding system	Polygynous
Age of first reproduction (♀/♂)	3/3
Maximum age of reproduction	20
Annual % adult females reproducing	45
Density dependent reproduction?	No**
Maximum litter size	1
Offspring gender ratio	50:50
% adult males in breeding pool	100
% mortality from age 0-1 (♀/♂) (SD)	10/10 (2.5)
% mortality from age 1-2 (♀/♂) (SD)	15/15 (3)
% mortality from age 2-3 (♀/♂) (SD)	20/25 (5/7)
% mortality from age above 3 (♀/♂) (SD)	7/7 (2)
Catastrophe	No
Harvest	Various rates of harvest.
Supplementation	No

6.4. Results

6.4.1. Population density and census estimates

Occupancy estimates for the core zone and buffer zone of each study site were taken from the model *psi(park,core),p(.)* (Chapter 4), and used to derive occupancy and population estimates for the remaining un-surveyed protected areas (Table 6.2). Under the assumption that occupancy = population density, the population densities calculated from the study sites in the present study (0.03 tapirs/km^2 – 0.86 tapirs/km^2) were in line with previous estimates for the species (range 0.05-1.6 tapirs/km^2, mean 0.43 tapirs/km^2, see above; Naranjo 2009). The population estimates for PNC, which suggest a remaining population between 22 and 49, correspond with the estimate of 25 individuals based on interviews with local hunters and guides. Different environmental pressures in the remaining protected areas may lead to high variation in the actual population density in those regions (Naranjo & Bodmer 2007).

Table 6.2: Forest loss and Baird's tapir occupancy estimates in 13 protected areas in Honduras. Forest loss data and amount of available habitat were derived from Hansen *et al*. (2013) and calculated using the Spatial Analyst tool in ArcGIS 10.1. Occupancy estimates in the four study sites: Cusuco National Park, Pico Bonito National Park, Sierra Rio Tinto National Park and the Rio Platano Biosphere Reserve, were generated from spoor encounter data using the programme Presence v5.1 (Hines *et al*. 2010; Chapter 4). Occupancy estimates in the remaining nine Protected Areas were generated based upon occupancy in the adjacent study site, and modified depending upon deforestation rates. Population estimates for all protected areas were calculated by multiplying the occupancy estimate by the amount of available habitat for the core zone and buffer zone of each protected area.
*Buffer zone location not available. Buffer zone occupancy was estimated to be the same as for Cusuco National Park due to similar rates of deforestation in these reserves.
#Core zone occupancy same as buffer zone occupancy due to similar rates of deforestation in each zone.
+Core zone occupancy estimate same as buffer zone estimate for the adjacent study site (Rio Platano Biosphere Reserve) due to high levels of deforestation.
++Buffer zone occupancy estimate at base level due to very high rates of deforestation.

Study Site/Region	% Remaining forest lost since year 2000 Total/Core/Buffer	Available habitat (km^2) Core/ Buffer	Core zone Occupancy/ 95% CI	Buffer zone Occupancy/ 95% CI	Estimated population Lower/Upper/K
Cusuco National Park	3.91 / 1.65 / 5.08	75 / 138	0.53 / 0.27-0.78	0.05 / 0.01-0.30	21/ 46 / 213
Pico Bonito National Park	1.36 / 0.15 / 2.83	558 / 444	0.40 / 0.14-0.72	0.03 / <0.01-0.24	79/236/ 1002
Sierra Rio Tinto National Park	10.43 / 5.75 / 12.08	210 / 559	0.86 / 0.43-0.98	0.21 / 0.08-0.46	135/298/769
Rio Platano Biosphere Reserve	5.88	4317/2148	0.81 / 0.54-0.94	0.05 / 0.01-0.30*	2344/3604/6466
Nombre de Dios National Park	4.10	237	N/A	0.03/ <0.01-0.24	1 / 7 / 237
Texiguat Wildlife Refuge	3.51 / 3.26 / 3.82	172/142	0.03/0.00-0.24#	0.03 / <0.01-0.24	1 / 9 / 314
Sierra de Agalta National Park	2.19 / 1.02 / 3.68	377/286	0.86/0.43-0.98	0.21 / 0.08-0.46	153 / 325 / 663

Carbon National Park	4.38	327	N/A	0.21 / 0.08-0.46	26 / 69 / 327
Tawahka Asangni Biosphere	9.72	2225	N/A	0.05 / 0.01-0.30	13 / 111 / 2225
Patuca National Park	15.29 / 8.17 / 21.78	1565/1463	0.05/0.01-0.30^{+}	0.01 / <0.01-0.29^{++}	15 / 83 / 3028
Rus Rus National Park	5.52	707	N/A	0.05 / 0.01-0.30	4 / 35 / 707
Warunta National Park	1.30	640	N/A	0.05 / 0.01-0.30	4 / 32 / 640
Mocoron National Park	1.44	564	N/A	0.05 / 0.01-0.30	3 / 28 / 564

The sum of population estimates from each protected area was used to derive estimates for the three regions of tapir occupancy, and for the whole of Honduras (comprising the three regions plus PNC; Table 6.3).

Table 6.3: Population estimates for Baird's tapir in Honduras (excluding Cusuco National Park). Estimates were generated by summing the population estimates for the 13 protected areas that comprise the four regions of tapir occupancy in Honduras (Table 6.2).

Region of Tapir Occupancy	**Estimated population Lower / Upper / K**
Nombre de Dios Cordillera Region	81 / 253 / 1553
The Sierras Region	314 / 692 / 1759
La Moskitia Region	2384 / 3894 / 13066
Honduras	2800 / 4585 / 16591

6.4.2. Estimating hunting rates in PNC.

Interviews with local guides were carried out in June 2012 to assess the level of hunting in PNC over the previous years, and updated in 2013 (Table 6.4). The guides suggested that the average harvest rate was approximately two individuals every three years before 2006. Eleven tapir were poached in 2006, which was locally attributed to hunters using the newly-created transect network to access remote parts of the Park. The hunting returned to previous levels in 2007. Between 2010 and 2011 the hunting rate increased again, which coincided with an increase in deforestation from the previous two years (Figure 5.2; Hansen *et al*. 2013), which was locally attributed to the activity of illegal coffee growers. Hunting rates have been previously estimated for three other protected areas in Honduras (Table 6.4).

Table 6.4: Approximate hunting rates and population estimates for Baird's tapir in four protected areas in Honduras.
Estimates for Cusuco National Park were made by local guides and hunters during interviews in June 2012, repeated in June 2013. Population estimates allow for some births and natural deaths, hence do not follow exactly from the number of tapir hunted per year.
Estimates for the remaining protected areas were made by local guides and hunters during interviews conducted by colleagues in communities adjacent to the protected areas. Estimates for Tawahka Asangni Biosphere were made in 2001 (Demmer & Overman 2001). Estimates for Patuca National Park were made in 2002 (Dunn *et al*. 2012). Estimates for Sierra de Agalta National Park were made in 2007 (N. Estrada pers. comm.).

Protected Area	**Year**	**Tapir hunted**	**Possible Population**
Cusuco National Park	Pre-2006	<1/year	>60
	2006	11	50
	2007	0	40
	2008	1	40
	2009	1	40
	2010	2	38
	2011	8	30
	2012	4	27
	2013	2	25
Tawahka Asangni Biosphere	2001	7	N/A
Patuca National Park	2002	13	N/A
Sierra de Agalta National Park	2007	10	N/A

6.4.3. Stochastic modelling

6.4.3.1. Modelling Sustainable hunting levels for the four study sites

Despite the inclusion of inbreeding depression, all populations recovered to K, with zero chance of extinction, when hunting was modelled to cease immediately (Table 6.5).

When considering population sustainability to mean PE <0.05, all populations are sustainable under an annual hunting rate of approximately 0.10 +/- 0.05 of the overall population size (range 0.05-0.12), or 0.47 +/- 0.27 of the population productivity (range 0.2-0.52). Negative population growth (mean r) was achieved when hunting reached approximately 0.15 +/- 0.01 of the overall population size (range 0.14-0.16), or 0.68 +/- 0.08 of the population productivity (range 0.60-0.72). Using a unified harvest Model (Robinson &

Redford 1994), Naranjo & Bodmer (2007) suggested that population growth can only be maintained if hunting remained below 0.2 of annual productivity.

The PNC Pi22 population, which most closely matches the population estimates obtained from interviews with local hunters, was sensitive to a small increase in hunting rate. Under current hunting rates of two tapir per year, the mean time to extinction (TE) for populations was 17.7 years. Negative population growth (-0.01) and high PE (0.96) were calculated for a hunting scenario of only four adult tapir per year. A hunting rate of only one individual per year would see harvest levels equal 0.2 annual productivity. Recent hunting estimates suggest that more than four tapir were hunted in three of the last eight years. The PNC Pi49 population, which is likely to be an overestimate, achieved negative population growth (-0.03) and high PE (0.90) when seven tapir (four ♀ three ♂) were harvested each year. This level of hunting is likely to have been exceeded in two of the last eight years.

Hunting rates are not known from PNPB, but hunting is known to be ongoing in both sites surveyed for the present study. Considering the conservative population estimate (Pi79), a high (>0.05) PE was reached at a hunting rate of seven tapir (four ♀ three ♂) per year, and negative population growth at a hunting rate of 13 tapir (seven ♀ six ♂) per year. An annual hunting rate of only four tapir is sufficient to exceed 0.2 of productivity.

The PNSRT Pi=135 population maintained a PE <0.05 until a harvest rate of 27 tapir (14 ♀ 13 ♂) per year was modelled, and maintained positive mean population growth until a harvest rate of 37 tapir (19 ♀ 18 ♂) per year. Under the estimated hunting level for Sierra de Agalta National Park (10 tapir per year, 0.33 of annual productivity), the PNSRT Pi=135 population had a mean growth rate of 0.14, and mean PE of zero, suggesting that this level of hunting is sustainable. Since the year 2000, PNSRT has experienced almost five times as much deforestation as Sierra de Agalta National Park (10.43% compared to 2.19%), which is likely to mean that PNSRT has a concomitantly higher hunting rate resulting from increasing edge effects (Woodroffe & Ginsberg 1998; Broadbent *et al*. 2008).

The results of simulations for the BRP population suggested that this population may be considerably more robust, with positive mean growth maintained until a hunting rate of 380 or 510, and sustainable annual rate of hunting (PE <0.05) of 270 or 420 adult tapir (Pi2943 and Pi4415 respectively).

The three regions of tapir occupancy were proportionately more resistant to hunting pressure. The hunting rates reported in Sierra de Agalta National Park in 2007 are, however, almost at 0.2 of productivity for the whole Sierras region, under the conservative population estimate (0.2 x P = 14 tapir per year; hunting estimate in Sierra de Agalta National Park = 10 tapir per year). Hunting rates are not known from the Nombre de Dios region, but given the high levels of deforestation in Texiguat Wildlife Refuge (3.51%) and Nombre de Dios National Park (4.10%), which are similar to those in PNC (3.91%), it is likely that this region is experiencing hunting levels that approach or exceed 0.2 x productivity (4 or 11 tapir per year, depending upon the Pi). Although the hunting rates reported for the two protected areas in La Moskitia are likely to be underestimates, as the interviews were carried out in a small proportion of villages in those reserves, the La Moskitia tapir population is likely to be sufficiently large and remote to survive considerably elevated hunting rates.

Populations in PNC are predicted to lose approximately 20% of genetic diversity in the next 100 years, far exceeding conservation management thresholds that aim to retain a minimum of 90% of genetic diversity over 100 years (Frankham *et al*. 2014). The population in the Nombre de Dios region is predicted to lose approximately 5% of genetic diversity over the next 100 years, which although below the maximum ‘acceptable’ threshold, is still likely to reduce population fitness appreciably (Frankham *et al*. 2014).

Table 6.5: Demographic parameters for simulations of Baird's tapir populations.

Basic demographic and genetic parameters for Baird's tapir population simulations under a range of hunting rates in four study sites (Cusuco National Park, Pico Bonito National Park, Sierra Rio Tinto National Park, Rio Platano Biosphere Reserve) and three regions of tapir occupancy (Nombre de Dios Region, The Sierras Region, La Moskitia Region) in Honduras.

Means are from 1000 simulations using the programme Vortex 10 (Lacy 2014).

Pi: initial population size; r: stochastic population growth rate; PE: probability of extinction; TE: time to extinction.

The sustainable rate of hunting as per Naranjo and Bodmer (2007) is also given.

Study Site / Region	**Pi**	**Productivity (P) (Pi/2)*0.45**	**Naranjo & Bodmer (2007) sustainable rate of hunting (x/P = 0.2)**	**Hunting rate (tapir/year)**	**Mean r (SD)**	**PE**	**Mean TE (years)**	**Genetic Diversity (SD)**	**Mean final population (SD)**
Cusuco National Park	21	5	1	0	0.14 (0.11)	0	N/A	0.88 (0.03)	212 (6)
				1♂	0.13 (0.11)	<0.01	8.0	0.88 (0.03)	210 (16)
				1 ♀	0.11 (0.11)	0.17	18.0	0.87 (0.04)	174 (81)
				2*	0.10 (0.12)	0.21	17.7	0.85 (0.05)	167 (86)
				4	-0.01 (0.28)	0.96	8.8	0.84 (0.05)	9 (41)
	46	10	2	0	0.14 (0.10)	0	N/A	0.91 (0.02)	212 (6)
				2*	0.13 (0.10)	<0.01	21.0	0.90 (0.02)	211 (9)
				4	0.11 (0.11)	0.03	20.4	0.89 (0.03)	205 (36)
				5 (2♀/ 3♂)	0.11 (0.11)	0.06	17.5	0.89 (0.03)	197 (52)
				7 (4♀/ 3♂)	-0.03 (0.22)	0.90	12.1	0.88 (0.03)	21 (63)

Pico Bonito National Park	79	18	4	0	0.15 (0.09)	0	N/A	0.97 (<0.01)	1000 (13)
				7(3♀/4♂)	0.14 (0.10)	<0.01	14.3	0.96 (0.01)	995 (66)
				7 (4♀/3♂)	0.13 (0.10)	0.06	22.9	0.96 (0.01)	943 (228)
				13 (7♀/6♂)	-0.05 (0.28)	0.90	12.4	0.94 (0.02)	98 (297)
	236	53	11	0	0.16 (0.01)	0	N/A	0.98 (<0.01)	999 (17)
				26	0.12 (0.11)	0.05	23.2	0.97 (0.01)	949 (214)
				27(14♀/13♂)	0.11 (0.12)	0.10	22.3	0.97 (0.01)	897 (301)
				37(19♀/18♂)	<-0.01 (0.27)	0.77	14.9	0.97 (0.01)	227 (417)
Sierra Rio Tinto National Park	135	30	6	0	0.16 (0.10)	0	N/A	0.97 (<0.01)	767 (15)
				10^{+}	0.14 (0.10)	0	N/A	0.96 (0.01)	765 (15)
				15(7♀ / 8♂)	0.13 (0.10)	0.02	22.1	0.96 (0.01)	749 (108)
				15(8♀ / 7♂)	0.11 (0.11)	0.13	22.3	0.96 (0.01)	667 (255)
				21(11♀/10♂)	<-0.01 (0.25)	0.81	15.2	0.96 (0.01)	148 (301)
	298	67	13	0	0.16 (0.10)	0	N/A	0.97 (<0.01)	767 (14)
				10^{+}	0.14 (0.10)	0	N/A	0.97 (<0.01)	766 (16)
				33(16♀/17♂)	0.10 (0.10)	0.03	23.5	0.97 (<0.01)	742 (123)
				33(17♀/16♂)	0.10 (0.11)	0.06	23.6	0.97 (<0.01)	720 (176)
				43(22♀/21♂)	-0.01 (0.26)	0.76	15.8	0.97 (0.01)	181 (322)

Rio Platano Biosphere Reserve	2344	527	105	0	0.16 (0.09)	0	N/A	>0.99 (0)	6455 (88)
				270	0.11 (0.10)	0.03	24.3	>0.99 (0)	6181 (1297)
				280	0.11 (0.10)	0.05	22.3	>0.99 (<0.01)	6080 (1460)
				390	-0.04 (0.27)	0.85	14.6	>0.99 (<0.01)	969 (2292)
	3604	811	162	0	0.16 (0.09)	0	0	>0.99 (0)	6455 (82)
				420	0.09 (0.10)	0.04	22.7	>0.99 (0)	6117 (1311)
				430	0.08 (0.11)	0.06	20.7	>0.99 (0)	6008 (1522)
				560	< -0.01(0.21)	0.70	16.6	>0.99 (0)	1806 (2091)
Nombre de Dios Region	81	18	4	9 (4♀ / 5♂)	0.13 (0.10)	0.04	19.3	0.96 (0.01)	1486 (308)
				9 (5♀ / 4♂)	0.11 (0.12)	0.24	20.2	0.96 (0.01)	1174 (664)
				13 (7♀ / 6♂)	-0.05 (0.28)	0.90	12.7	0.95 (0.01)	142 (446)
	253	57	11	28	0.13 (0.10)	0.03	21.2	0.98 (<0.01)	1502 (262)
				29(14♀/15♂)	0.13 (0.11)	0.05	23.2	0.98 (<0.01)	1471 (339)
				41(21♀/20♂)	-0.02 (0.30)	0.83	14.7	0.98 (<0.01)	262 (581)
The Sierras Region	314	71	14	35(17♀/18♂)	0.13 (0.10)	0.03	23.0	0.98 (<0.01)	1706 (281)
				35(18♀/17♂)	0.12 (0.11)	0.05	24.4	0.98 (<0.01)	1661 (394)
				51(26♀/25♂)	-0.03 (0.30)	0.83	15.3	0.98 (<0.01)	293 (654)
	692	156	31	79(40♀/39♂)	0.10 (0.10	0.04	23.7	0.99 (<0.01)	1672 (349)
				80	0.10 (0.11)	0.05	22.2	0.99 (<0.01)	1659 (383)
				109(55♀/54♂)	<-0.01 (0.25)	0.75	16.1	0.99 (<0.01)	441 (757)

La Moskitia Region	2384	536	107	280	0.01 (0.07)	0.04	24.9	>0.99 (<0.01)	8455 (1787)
				290	0.01 (0.07)	0.07	21.7	>0.99 (<0.01)	8118 (2340)
				380	-0.06 (0.22)	0.77	16.1	>0.99 (<0.01)	1983 (3654)
	3894	888	178	460	0.01 (0.07)	0.05	21.1	>0.99 (0)	8184 (1876)
				479	0.01 (0.07)	0.07	22.7	>0.99 (0)	8041 (2153)
				600	-0.04 (0.17)	0.65	16.9	>0.99 (<0.01)	2994 (4050)

6.5. Discussion

6.5.1. Population size and available habitat estimates

Although population estimates for Baird's tapir in Honduras have been generated previously (Flesher 1999; Castellanos *et al*. 2008), this is the first study to attempt to calculate population size from encounter rates with tapir spoor collected in the field. The population density estimates generated for the four protected areas surveyed here (0.03 tapirs/km^2 - 0.86 tapirs/km^2) are in-line with estimates from other countries (Naranjo 2009). In a study in the Lacandon Forest in Mexico, Naranjo & Bodmer (2007) suggested that densities of 0.05-0.20 tapirs/km^2 are to be expected in heavily hunted/relatively un-hunted regions respectively, but tapir have been recorded to live in densities up to 3.03 tapirs/km^2 in parts of Costa Rica (Gonzalez-Maya 2008), the upper estimates from this study are, therefore, not biologically unreasonable.

Flesher (1999) estimated that the population of tapir in north eastern Honduras was between 520 and 2760 individuals in the year 1999, and a 2004 aerial survey of available habitat estimated the national population at 1859 individuals (Castellanos *et al*. 2008). Using population estimates from each of the 13 protected areas, this study calculated that Honduras may support 2800 - 4585 tapir, with all but approximately 400 living in the La Moskitia region. This revised figure is considerably higher than the current IUCN population estimate of >500 individuals (Castellanos *et al*. 2008). The IUCN figure probably represents an underestimate of the population remaining in La Moskitia, which is supported by the previous population estimate in the region (Flesher 1999). Research from Mexico that used similar techniques to those presented here suggests that the actual number of tapir remaining in Mexico may be nearly double the IUCN estimate of >1500 (Naranjo 2009).

To improve the accuracy of the population predictions, a higher proportion of the remaining protected areas should be surveyed, in addition to more complete sampling of the four reserves surveyed here (Funston *et al*. 2010). Given its large size, and the fact that it still retains a vast amount of potential habitat, a comprehensive survey of the protected areas that make up the La Moskitia region should be seen as a priority.

Previous estimates of tapir populations were generated by estimating the amount of suitable habitat remaining in Honduras (Flesher 1999; Castellanos *et al*. 2008). Flesher (1999) estimated that 10,400 km^2 – 11,500 km^2 of inhabitable forest remained in north eastern Honduras. His calculations did not include Rus Rus National Park, Warunta National Park or Mocoron National Park, which together make up 1911 km^2 of available remaining habitat. The present estimate of 14,682 km^2 remaining habitat in north eastern Honduras totals 12,771 km^2 with the removal of these three protected areas, which is in line with Flesher's calculation. The inclusion of these protected areas in the present population estimate is valid as tapir have been confirmed to inhabit Rus Rus National Park (N. Estrada, pers. comm.), and it is unreasonable to assume that they do not also inhabit the other two protected areas, which are adjacent, and currently experiencing among the lowest levels of deforestation of all the 13 protected areas included in this analysis (1.30% deforestation in Warunta National Park, and 1.44% in Mocoron National Park since the year 2000).

6.5.2. Stochastic modelling

Although PHVAs have been applied to Baird's tapir before, this is the first time that a modelling exercise has been conducted for specific Baird's tapir populations experiencing an immediate threat of extirpation. The results of the VORTEX analysis demonstrate the importance of rapid management of the hunting levels currently experienced in PNC, and the importance of controlling hunting levels in all of the remaining regions of tapir occupancy in Honduras.

Without prompt intervention to control hunting rates, the population of Baird's tapir in PNC is predicted to be extirpated under current harvest rates (TE = 17.7 years; Pi21, under current hunting rates), and, according to the sustainable hunting rates suggested by Naranjo and Bodmer (2007), stands a high probability of extirpation from overharvesting even if hunting is reduced to only a single tapir per year. It is unrealistic to assume, given anthropogenic pressures in and around PNC, that hunting will be eradicated completely within the next decade. Even if hunting levels are reduced, the viability of this population is not guaranteed due to the expected loss of genetic diversity and evolutionary fitness caused by the population bottleneck that it has already experienced (Frankham *et al*. 2014).

The introduction of novel genes, through the establishment of migration corridors between PNC and other populations of tapir, is important if the PNC population is to maintain population fitness in perpetuity (Hogg *et al*. 2006; Bruford *et al*. 2010). Larger populations that have a high probability of extinction are predicted to go extinct before inbreeding depression affects population fitness.

Populations of tapir in the remaining 12 protected areas should not be viewed in isolation, as, for the time being, inhabitable corridors facilitating migration still exist between the protected areas that constitute the remaining three regions of tapir occupancy. The viability of Baird's tapir populations in the Nombre de Dios Cordillera region and the Sierras region may depend on the maintenance of connectivity between the six protected areas that make up these two regions, which would enable metapopulation dynamic processes to buffer these populations from extinction (Sodhi & Ehrlich 2010). Tapir occupancy is lower in the buffer zones than the core zones of the Parks surveyed (Chapter 4), but as long as forest cover exists it is possible that the areas of buffer zone between the protected areas can still act as a corridor for Baird's tapir migration, as tapir have been recorded in degraded forest (Fragoso 1991; Naranjo & Bodmer 2007; Dunn *et al.* 2012). These populations can, therefore, still be considered contiguous rather than isolated. The forest between these protected areas is already highly degraded (Figure 6.1), and without intervention the tapir populations in these protected areas are likely to become completely isolated in the next few years.

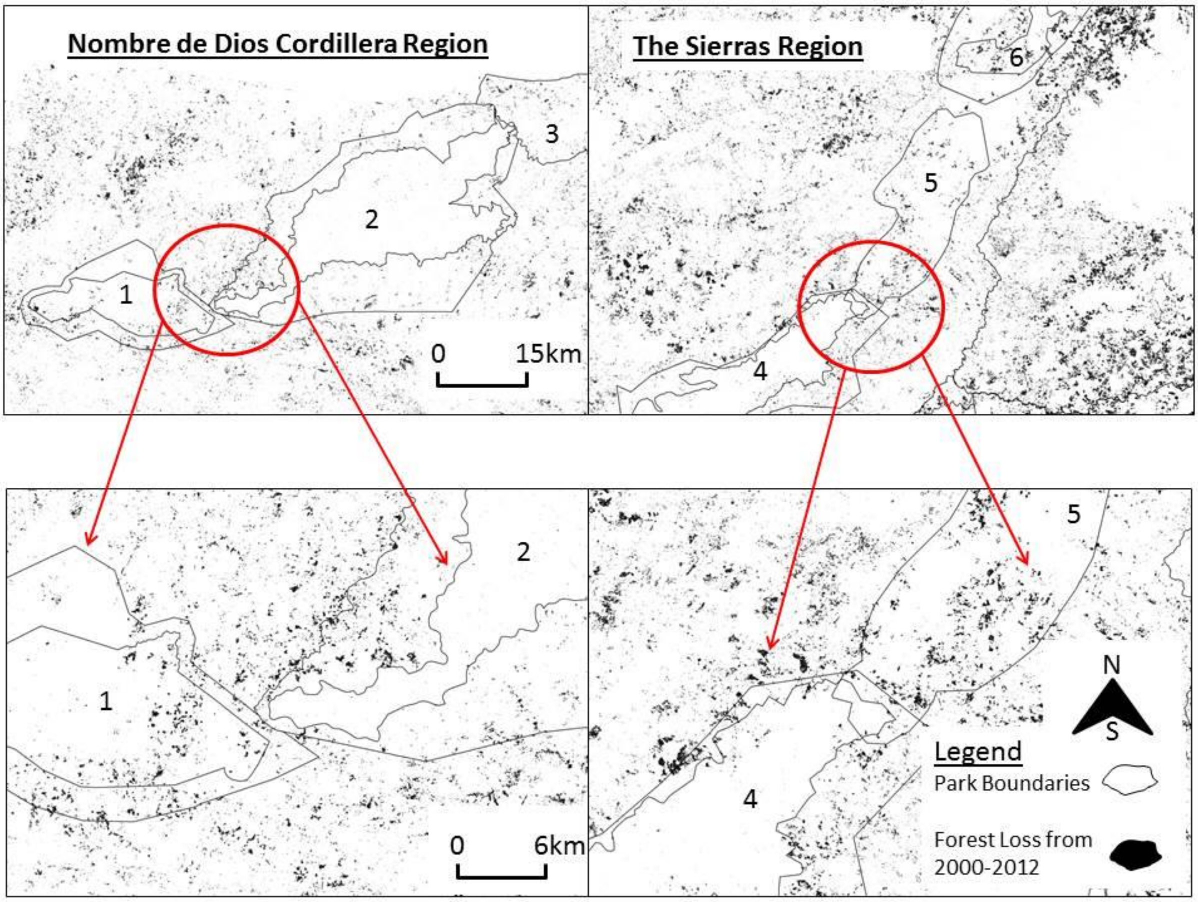


Figure 6.1: Deforestation between adjacent protected areas in the Nombre de Dios Cordillera Region and the Sierras Region in Honduras.
Deforestation between 2000 and 2012 (black) was high in the areas between the Parks (1: Texiguat Wildlife Refuge; 2: Pico Bonito National Park; 3: Nombre de Dios National Park; 4: Sierra de Agalta National Park; 5: El Carbon National Park; 6: Sierra Rio Tinto National Park). Deforestation data reproduced from Hansen *et al*. (2013) and analysed using ArcGIS 10.1.

Extinction probability is generally highest in island species, because island populations are both scarce and fragile due to the demographic constraints of isolation (Sodhi & Ehrlich 2010). Habitat fragmentation creates 'islands', and the negative effect of fragmentation on population viability may be compounded by hunting, which can lead to local extinction, as has been shown in lowland tapir (*Tapirus terrestris*) and white lipped peccary (*Tayassu pecari*) in Amazonia (Peres 2001).

Over-hunting is a recurring threat to endangered species, particularly large-bodied species with low rates of recruitment and low population sizes such as Baird's tapir (Bodmer & Robinson 2004; Wilkie *et al*. 2011). Similar studies have investigated the effect of over-hunting on PE in a range of taxa. A study by Hughes *et al.* (2011) identified an analogous situation in West Africa where common chimpanzee subspecies (*Pan troglodytes vallerosus*) populations are suffering a rate of harvest 2-13 times higher than sustainable levels and are predicted to go extinct within 20 years without prompt intervention. An interesting retrospective study by Turvey and Risley (2006) demonstrated how Steller's sea cow (*Hydrodamalis gigas*) was driven to extinction within 27 years of its discovery by a hunting rate that was seven times the sustainable level. This example demonstrates how rapidly a species or population can go extinct under similar pressures being experienced by Baird's tapir in PNC today.

In the absence of accurate estimates of hunting in all of the protected areas in Honduras, deforestation rate can be used as a proxy for probable levels of population disturbance, due to the common correlation between deforestation and hunting in developing countries with expanding agricultural frontiers (Sodhi & Ehrlich 2010). The BRP has lost 5.88% of forest since 2000, higher than PNC, and the adjacent protected areas of Patuca National Park and Tawahka Asangni Biosphere have experienced very high levels of deforestation (15.29% and 9.72% respectively). The interior of the BRP remains largely intact with minimal disturbance, and it is likely that the tapir population in La Moskitia exhibits a source-sink metapopulation model, whereby positive population growth in the source population (the interior of the BRP) replenishes peripheral sink populations that would display negative population growth in the absence of immigration (Hanski & Simberloff 1997). PNPB is experiencing the lowest levels of deforestation of all four study sites (1.36% total deforestation, only 0.15% inside the core zone), and it is likely that the core zone is acting as a source population for peripheral areas, particularly in the north of the Park, which are more disturbed, and where hunting was reported to be ongoing.

6.5.3. Conclusions

The results of the Honduras-wide PHVAs demonstrate the importance of performing an accurate census of Baird's tapir in Honduras. The Honduran metapopulation could be six times more resistant to hunting-related extinction risk than assumed under the IUCN population estimate of >500 individuals (Castellanos *et* al. 2008). Despite this, the Honduran metapopulation is fragmented into a minimum of four sub-populations, each of which is susceptible to further fragmentation due to ongoing deforestation, and the concomitant increase in extinction threat due to habitat loss and hunting (Sodhi & Ehrlich 2010).

If effective population size:census population size (*Ne:Nc*) ratios in Baird's tapir conform to the currently accepted figure of 0.1-0.14, only the La Moskitia population is likely to have an

Ne of over 50, which is the accepted minimum for avoiding inbreeding depression in the short-term (five generations; Soule 1980); and no populations, not even the entire Honduran metapopulation, retain a sufficiently large *Ne* (currently suggested as 500) to retain evolutionary potential in the long term (in perpetuity; Jamieson & Allendorf 2012). In light of recent analyses of inbreeding depression in both captive and wild populations, Frankham *et al*. (2014) recommend that MVP figures be revised upwards to 100/1000, which would place Baird's tapir in an even more precarious position. Although it is difficult to define exact numbers for MVPs *per se*, and that formulae such as those above can be seen as rules of thumb at best, it is generally accepted that populations become increasingly vulnerable to extinction the smaller they become (Lindenmayer *et al*. 1993).

The analyses presented here refer not only to habitat loss and hunting-related population pressures, but to the loss of genetic diversity and inbreeding as further confounding variables in driving population decline (Bruford *et al.* 2010). Both of these factors have been shown to influence extinction probability in wild populations (e.g. Saccheri *et al*. 1998), and maximizing the genetic viability of tapir populations has been set as a priority by the Tapir Specialist Group (Medici *et al.* 2005). Baird's tapir demonstrate very low rates of genetic diversity (Chapter 3), but until further work has been done it is not possible to know how robust the species is to population declines related to inbreeding and the loss of genetic variation.

Species extinction is typically driven by a series of anthropogenic (habitat loss, over-exploitation, introduced species, pollution, climate change) and environmental variables (demographic stochasticity, ecological stochasticity, genetic stochasticity, natural catastrophes; Frankham *et al*. 2014). Baird's tapir populations in Honduras are currently likely to be susceptible to both types of extinction threat. Controlling hunting is, however, likely to be the most appropriate method to avoid the extinction of the populations analysed here.

The results of this PHVA suggest that the hypothesis that Baird's tapir are declining as a result of anthropogenic pressures is unlikely to be false. Existing hunting levels in PNC and the Sierras Region will continue to cause negative growth in tapir populations unless they can be controlled. While human demographic conditions in Honduras remain in their current state, and law enforcement remains nominal in remote communities, poaching of all species will continue unabated, and it will be the most endangered species, such as Baird's tapir, that will be the first to suffer and will take the longest to recover.

Chapter 7. General Discussion

7.1. Thesis Overview

Outside of the protected areas of La Moskitia, Baird's tapir is at risk of local extinction across much of its range in Honduras as a result of habitat loss and fragmentation, and the concomitant rise in hunting, exacerbated by low levels of genetic diversity and poor connectivity between existing populations. The genetics component of this study (Chapter 3) is the first to be undertaken in Honduras, and although the results presented here are not sufficient to address hypotheses relating to isolation and gene flow, these data contribute to the growing evidence suggesting that Baird's tapir displays very low levels of genetic diversity (albeit at mitochondrial DNA), which potentially reduces their adaptive potential in the face of ongoing environmental and climatic change. Range-wide patch occupancy analyses support the hypothesis that Baird's tapir occupancy and population density correlate with factors relating to human activity, creating a distribution bias that is likely to be driven by unregulated hunting as well as a combination of other environmental factors (Chapter 4). The hypothesis that encounter rates with tapir spoor will reduce over time is supported by the observation that ongoing hunting is causing a population decline in PNC (McCann *et al*. 2012; Chapter 5). Although it was not possible to address hypotheses relating to isolation and gene flow using genetic data, deforestation has either already caused or is likely to lead to the isolation of Baird's tapir populations in seven of the 11 protected areas where the species has been confirmed to occur in Honduras (Chapter 6), which is likely to accelerate population declines in those protected areas. The PHVA conducted on Baird's tapir in PNC suggests that they could be effectively extirpated from this former stronghold in fewer than 20 years if recent hunting trends are not reversed (Chapter 6; McCann *et al*. 2012). Existing population estimates for Baird's tapir are likely to be overly conservative; only La Moskitia, however, is likely to retain a population large enough to remain viable for the foreseeable future (Frankham *et al*. 2014).

7.2. Baird's tapir distribution and abundance in Honduras

As no live tapir were observed during the present study, the conclusions presented here are based upon encounters with tapir spoor, which is highly conspicuous and is a generally reliable indicator of tapir presence. With the addition of the proposed Sierra Rio Tinto National Park (PNSRT), Baird's tapir are now confirmed to be present in 11 protected areas in Honduras, covering a total of 19,731 km^2. The amount of habitat available for tapirs is, however, shrinking rapidly. Even inside these protected areas deforestation remains high, and much of the habitat is degraded and in constant use by farmers and hunters. Hunting was reported in all of the protected areas surveyed, and deforestation is evident inside the 11 protected areas in which tapir have been recorded, averaging 6.03% in the 12 years from 2000 – 2012 (range 1.36% – 15.29%; Table 6.2; Hansen *et al*. 2013).

In addition to the 11 reserves in which Baird's tapir presence has been confirmed, it is highly likely that they also persist in Warunta National Park (653 km^2) and Mocoron National Park (682 km^2) in La Moskitia, which are contiguous with the BRP, and which are experiencing low levels of deforestation at this time (Chapter 6). Tapir may also occur in three other National Parks (Montana de Yoro National Park, 153.5 km^2; La Muralla National Park, 384 km^2; Botaderos National Park, 1006 km^2; N. Estrada pers. comm.). These three remaining Parks are isolated and an analysis of the deforestation levels here suggests that they are likely to be experiencing similar pressures as described for PNC (Table 7.1; Hansen *et al*. 2013). The small size of Montana de Yoro and La Muralla National Parks, and the high rates of deforestation in La Muralla and Botaderos National Parks makes the long-term survival of Baird's tapir in these protected areas unlikely, if populations persist there at all (Lawes *et al*. 2000).

Table 7.1: Forest loss in three National Parks in Honduras where Baird's tapir presence is suspected. The possibility that Baird's tapir occur in these three Parks was suggested by N. Estrada (pers. comm). Forest loss data were derived from Hansen *et al*. (2013) and calculated using the Spatial Analyst tool in ArcGIS 10.1.

Park name	Park area (km^2) Core / Buffer	% Remaining forest lost since year 2000 Total/Core/Buffer
Montana de Yoro National Park	153.5	1.59
La Muralla National Park	138 / 246	4.08 / 2.73 / 4.84
Botaderos National Park	389 / 617	6.94 / 5.36 / 7.93

Although more work is required to validate the population estimates generated here (Figure 6.2), current estimates for Baird's tapir by the IUCN (Castellanos *et al*. 2008) are likely to be conservatively low (Naranjo 2009). The long-term viability of Baird's tapir in Honduras is, however, uncertain. Under current theory of minimum viable population sizes, it is likely that La Moskitia is the only region in Honduras to retain a viable population of Baird's tapir, unless conservation efforts are greatly improved (Frankham *et al*. 2014; Chapter 6). To maximise the impact of conservation efforts on Baird's tapir, conservation resources should be concentrated in the BRP and other protected areas in La Moskitia, which is likely to retain one of the largest populations of Baird's tapir in Central America (Naranjo 2009; Castellanos *et al*. 2008; Chapter 6).

The results presented here support the hypothesis that tapir distribution is mediated by human activities on a local and national scale, though the precise determinants of occupancy and population density are situation specific (Chapter 4). Baird's tapir are likely to be restricted to the most inaccessible parts of the few protected areas in which they remain, especially in instances where regular hunting is ongoing (Garcia *et al*. 2012). Tapir do not appear to exhibit a natural aversion to human disturbance such as deforestation *per se* (Chapter 4), and spoor was frequently recorded near the forest edge and in remote plantations during this study, as has been previously reported (Naranjo & Bodmer 2007; Dunn *et al*. 2014). The critical factor in determining Baird's tapir occupancy in Honduras appears to be hunting, which is supported by previous studies (Tobler 2002; Lira-Torres *et al*. 2004; Medici *et al*. 2005; Naranjo & Bodmer 2007; Tejeda Cruz *et al.* 2009). Local anecdotes suggest that tapir are rarely targeted in Honduras, but are usually poached incidentally by hunters who primarily target species such as red brocket deer (*Mazama Americana*) and paca (*Cuniculus paca*). Many hunters use dogs to flush their quarry, which increases the chance of encountering a tapir, which would be highly elusive to hunters operating without dogs. Other hunters wait in ambush in tree platforms in areas of high large mammal activity. Tree platforms were frequently encountered in PNC, even deep inside the core zone of the Park.

Due to ongoing pressures from deforestation and hunting, it is likely that the species' range will continue to contract, and population numbers will decline as isolated populations succumb to poaching pressure.

7.3. Baird's tapir population viability

7.3.1. Cusuco National Park

The discovery that the transect network in PNC appears to have altered the distribution of tapir such that it is no longer possible to monitor population trends using transect surveys in isolation, and has potentially increased the extinction threat to the species, is the most significant result of this thesis. The reduction in population density inferred by the declines in occupancy and detectability (Figure 5.3) observed over the duration of this study supports the hypothesis that Baird’s tapir populations are declining as a result of anthropogenic pressures.

The observation that the creation of the transect network in PNC has caused a distribution bias in Baird’s tapir raises interesting questions for other researchers where the target species is susceptible to anthropogenic activities. This is significant, as transects have been the *modus operandi* of biodiversity monitoring for many decades (Anderson *et al*. 1979). The statistical power of these conclusions would have been improved had off-transect surveys been conducted with similar effort to on-transect surveys for the duration of the study. With only three years of off-transect data, a circumspect interpretation of the conclusions is necessary, and it is important that off-transect surveys in PNC continue so that this situation can continue to be monitored.

The suggestion that hunters are using the transect network to access the Park and that the creation of the transect network has therefore increased the extinction threat to the species, is part of a larger story of ongoing degradation of the Park. Deforestation has increased in a linear fashion in the core zone of PNC since the year 2000 (Figure 5.2), and although the creation of the transect network has certainly made PNC more accessible to hunters, and although it is undeniable both that hunters are using the trails and that the population of tapir has declined since the start of the study, it is impossible to rule out the possibility that this would have happened irrespective of the creation of the transect network. It is possible that the very low levels of deforestation in 2006 (1 hectare) could be attributable to a temporary reticence of local loggers to access the Park after the upsurge in conservation activity there in 2006, a phenomenon that has been identified in other protected areas (Campbell *et al*. 2011; Laurance 2013).

Ongoing conservation efforts in PNC (Chapter 7.5) may reduce hunting rates to sustainable levels, but it is possible that this population is no longer viable due to a loss in genetic diversity as a result of the recent population decline (Frankham *et al*. 2014; Figure 6.5). Baird’s tapir has been shown to demonstrate very low levels of genetic diversity (Norton & Ashley 2004 a, b; Ruiz-Garcia *et al*. 2012; Chapter 3), which would be further exacerbated in PNC by population isolation and demographic contraction, meaning that this population may be unviable in the long term even if hunting is controlled (Frankham *et al*. 2014).

7.3.2. The Honduran metapopulation

Habitat fragmentation and isolation have long been recognised as having a negative effect on population survival (Wilcox & Murphy 1985), due in part to the impact of edge effects (Woodroffe & Ginsberg 1998). Chapter 6 discussed the potentially damaging effect of deforestation in the buffer zones linking the six protected areas that make up the Nombre de Dios Cordillera region and the Sierras region, which could lead to the isolation of those six protected areas. Isolation increases access to remaining fragments (Bonaudo *et al.* 2005) by increasing the Park boundary edge:area ratio (Chapter 4), which is likely to reduce the amount of habitat available for tapir occupancy.

Local extirpation is likely to occur more rapidly if these six protected areas become isolated (Table 6.5). Perhaps most at threat is the population in PNSRT. Although this Park is relatively large (882 km^2), it has seen very high levels of forest loss in both the core zone and buffer zone since the year 2000, especially in the valley that separates it from the BRP. In the Ojo de Agua site in PNSRT, the forest was accessed via a newly constructed mining road, and, as has been shown before, the creation of roads into previously inaccessible areas is a precursor for habitat and biodiversity loss, particularly of vulnerable species such as Baird's tapir (McLellan & Shackleton 1988; Nelson & Hellerstein 1997). The Texiguat Wildlife Refuge, which at 332.7 km^2 is the third smallest of the 11 protected areas where Baird's tapir remain (after PNC and Nombre de Dios National Park), is also threatened. An analysis of the deforestation data downloaded from Hansen *et al*. (2013) shows that there has been a 5% (16.25km^2) loss of forest in the area joining PNPB and the Texiguat Wildlife Refuge since the year 2000. Unless this rate of deforestation can be contained, it is likely that the tapir of the Texiguat Wildlife Refuge will be isolated from the rest of the Nombre de Dios Cordillera region, which will greatly increase the extinction risk to this small population by reducing connectivity to the wider metapopulation of the species in this part of Honduras (Lawes *et al*. 2000; Sodhi & Ehrlich 2010).

Loss of genetic diversity in the Nombre de Dios region and Sierras region is predicted to be within acceptable levels (<5% in the next 100 years; Frankham *et al*. 2014; Figure 6.5). If the six protected areas in these two regions do become isolated, loss of genetic diversity may begin to impact population viability and compound the conservation situation of this population (Lacy 1997; Frankham *et al*. 2014).

The deforestation data presented in Chapter 6 show that even the La Moskitia region is rapidly losing forest cover, which has been attributed in part to the rise in land conversion relating to the laundering of money from the transportation of illegal narcotics through the area (McSweeney *et al.* 2014). It is likely that Baird's tapir occupancy and population density will have been affected by these developments, particularly in Patuca National Park, which has seen the largest amount of deforestation of all the protected areas since 2000 (15.29%; Table 6.2). The BRP largely remains intact, which adds further weight to the suggestion that this protected area is among the most important refuges for Baird's tapir across its entire range.

The suite of factors that contribute to the extinction threat to Baird's tapir are complex and situation-specific. Certainly the effective management of the protected areas where tapir remain must be seen as the critical factor in conserving this iconic species in Honduras over the coming years (Bruner *et al*. 2001), but ultimately only a drastic improvement in the enforcement of anti-poaching and deforestation laws in Honduras is likely to guarantee the survival of the species in all but the most remote locations.

7.4. Future work

Like many other large forest-dwelling ungulates, Baird's tapir are difficult to observe in the wild as they live at low population densities in the most inaccessible parts of their habitat (Garcia *et al*. 2012). No live tapir were observed during this study, but indirect observations such as spoor counts and non-invasive genetic sampling can provide robust data for ecological studies on elusive species (Hines *et al*. 2010; Stanton *et al*. 2014). The techniques developed here, including the use of non-invasive DNA sampling and patch occupancy modelling are applicable for a wide variety of species, and have helped answer questions relating to the distribution, abundance and conservation status of Baird's tapir in Honduras. Many questions relating to the Baird's tapir genetics and ecology remain unanswered, however, and should be prioritised for future research.

Genetics is likely to form an important part of future conservation efforts on Baird's tapir (Medici *et al*. 2005). Understanding mating systems, social structure, dispersal and effective population sizes will inform conservation management decisions (Frankham *et al*. 2014), and is already identified as a priority for future research by the Tapir Specialist Group (TSG;

Medici *et al*. 2005). This study was unable to address hypotheses relating to isolation and gene flow due to low genotyping success rate, and the results relating to hypotheses about genetic diversity and population structure require further development. In order to address these hypotheses, it will be important to develop a series of polymorphic microsatellite markers that can successfully amplify faecal DNA specifically for Baird's tapir. Importantly, this study confirmed that the existing markers are insufficiently reliable for use in such projects (Chapter 3).

For any non-invasive genetics study on elusive forest mammals, one of the major difficulties to overcome is collecting a sufficient number of samples for a statistically robust study. In PNC, the probability of encountering a fresh (<72 hours old) faecal pile was considerably higher during 'off-transect' surveys than during 'on-transect' surveys (Table 7.2). It is recommended that all sampling efforts in Parks with an existing transect network include an element of 'off-transect' work, to rule out the possibility of transects creating a sampling bias (Chapter 5). Studies that specifically aim to collect faecal samples should focus on locating fresh tracks, and then follow those tracks until they are lost, or until a fresh faecal sample is discovered.

Table 7.2: Faecal sample encounter rates per km from field surveys in Cusuco National Park (PNC), Honduras.
Faecal sampling surveys were carried out between June 2009 and August 2012. Samples were collected if the faecal pile was judged to be <72 hours old. Two extra faecal samples, bringing the total number of samples collected in PNC to 85, were collected by non-research personnel and included in genetic analysis (Chapter 3).

Years	Distance walked	Number of samples	Mean distance walked/sample (km)
On Transect (2009-2012)	194.3	4	36.13
Off Transect (2011 & 2012)	101.85	79	4.24

Multiple faecal samples were occasionally collected from the same location (Figure 7.1). Previous studies have identified that tapirs use latrines consisting of multiple dung piles in the same location, either deposited by the same or by different individuals (Naranjo & Cruz-Aldan 1998; Fragoso *et al*. 2003; Garcia *et al*. 2012). In the present study, of the 150 encounters with faeces (of any age) for which latrine data were collected, 104 (69.33%) were discovered in association with at least one other dung pile. Using genetic techniques to identify the number of individuals using these latrines would be an interesting area of further study, and would shed further light on the social structure of the species.

Figure 7.1: Baird's tapir dung in a communal latrine.
Samples were photographed in the Rio Platano Biosphere Reserve (BRP), Honduras. The figure shows three samples in discrete piles, of three different ages. Sample age was estimated by a minimum of two trained observers based upon moisture content and other environmental clues. Sample 3 is partially submerged in water.

Estimates of hunting levels were obtained through informal interviews with local guides, but could have been greatly improved given a formal study across all of the communities bordering PNC. Hunting is illegal in Honduras, and there is considerable reticence to report its occurrence, which could lead to the underestimation of hunting levels in some areas (Knapp *et al*. 2010). A study investigating hunting rates in PNC through interviews with multiple individuals in villages surrounding the Park would provide a more accurate assessment on hunting levels, and would enable the comparison of hunting trends with deforestation and other quantifiable variables.

The data-collection for the present study could also have been augmented through the use of a camera trapping survey. Camera traps were used and successfully captured images of tapir in PNC, PNPB and PNSRT during the present study, but financial and logistical constraints prohibited the development of a formal population survey in any of the sites. Camera traps may be used for presence/absence sampling, or as a method of capture-mark-recapture sampling, and have also been used in standard monitoring surveys on many species and on the spatio-temporal dynamics of animal activity in a study area (e.g. Tobler *et al*. 2008; Kays *et al.* 2009). Baird's tapir leave conspicuous spoor, so using camera traps for presence/absence studies is unnecessary, but this methodology could provide excellent information on population numbers and social structure (Karanth & Nichols 1998). To effectively census the tapir populations in Honduras, a large number of camera traps would be required. Using 35 camera trap stations, totalling 14,577 camera trap hours (mean 17.35 days per camera) in the core zone of PNC, Reid *et al*. (2014, unpublished data) recorded only four tapir with no recaptures. Increasing the number of camera trap hours would increase the probability of capturing more individuals and obtaining recaptures, from which population estimates could be calculated for the Park (Tobler *et al*. 2008).

7.5. Conservation application and recommendations

Prioritising resources for conservation is an important and challenging task for conservation practitioners (Wilson *et al.* 2006). Protected areas may be ranked in terms of criteria such as levels of species richness, endemism or irreplaceability (the potential of a site to contribute to conservation goals), or in terms of existing threats and current levels of protection (Kerr 1997; Wilson *et al.* 2006; Le Saout *et al*. 2013). Two Honduran protected areas (PNC and PNPB) were included in a recent list of the top 100 most irreplaceable sites in the world (Le Saout *et al*. 2013), with both sites scoring particularly highly in terms of their contribution to the conservation of threatened amphibians.

Although the BRP was not included in the top 100 list, it is a UNESCO world heritage site, and due to its vast size should be seen as one of the most important refuges for tapir and many other threatened and emblematic species such as harpy eagles, jaguar and white-lipped peccary. The BRP is currently threatened by accelerating levels of illegal deforestation (Hansen *et al*. 2013; McSweeney *et al*. 2014) and by poaching near communities and along rivers (Dunn *et al*. 2012). Despite these threats, the BRP still retains approximately 94% of its original forest cover (Table 6.2) and is likely to support one of the largest populations of Baird's tapir in Central America (Flesher 1999; Chapter 6). Investment in conservation here would be more likely to preserve a greater biodiversity in the long term than in PNC and PNPB, which are smaller and more isolated.

One method of investment that has been shown to have a positive impact on the biodiversity of protected areas is the establishment of conservation research centres. Such centres may help protect biodiversity either actively - by discouraging poachers and other offenders, or passively - by providing economic benefit for local communities (Laurance 2013). Active research sites have been shown to have higher abundance of wildlife and lower levels of poaching than surrounding areas (e.g. Campbell *et al.* 2011; Hoppe-Dominik *et al*. 2011; N'Goran *et al*. 2012).

The results presented in Chapter 5 highlight some of the potential problems with opening up a National Park for biodiversity research. To mitigate these issues, researchers and conservation practitioners must consider how best to ensure the integrity and security of their study sites in the long term, including after research activity has been concluded. A thorough Biodiversity Impact Assessment should be considered for all projects that intend to modify the study site in a way that might impact the biodiversity contained therein (Geneletti 2003).

The second main form of investment in conservation is in effective patrolling and law enforcement (Plumptre *et al*. 2014). Carrillo *et al*. (2000) monitored mammal abundance in two National Parks in Costa Rica with similar environmental characteristics but different hunting restrictions. They found that mammal abundance in the more heavily hunted Park was 6-28% that of the protected Park. The greatest disparity in abundance occurred in those species that are hunted for food, which is the case for Baird's tapir. Interestingly they also identified a decline in mammal abundance in the protected Park during a three year period when hunting vigilance was reduced. Poaching has previously been shown to be negatively correlated with patrol effort (Leader-Williams *et al*. 1990) and positively correlated with distance from ranger stations (Plumptre *et al*. 2014).

Many conservationists have been reluctant to advise the use of law enforcement to protect endangered species and argue for a solely people-oriented approach to conservation (Wilshusen *et al.* 2002; Baral & Heinen 2005). In many cases, however, law enforcement may be the only remaining course of action to prevent the extinction of certain species, particularly those species implicated as 'conflict commodities' or those involved in the trade in animal products (Poudyal *et al*. 2009). Increasing the size of ranger forces and the spend in anti-poaching is now often seen as the only remaining option in the conservation of certain species in the short term (Plumptre *et al*. 2014), and it is likely that military and military-trained personnel will be deployed to protect biodiversity with ever increasing frequency in the years to come (Draulans & van Krunkelsven 2002; Dobson & Lynes 2008; Ferreira & Okita-Ouma 2012).

In the case of PNC, with the identification of high levels of illegal deforestation and the threat of local extirpation of the Baird's tapir population in as little as 17 years, regular military patrols were established in the Park in 2012. It is hoped that the militarisation of PNC that commenced in August 2012 will precipitate a decline in hunting and deforestation. Reports from the Institute of Conservation and Forestry (ICF), who manage PNC, indicated that deforestation accelerated during the 6 week hiatus in the patrols when local elections were held in December 2012, in which time two tapir were also poached. Evidence from the field suggests that without continued military presence the conservation situation in PNC will decline very rapidly. Only two tapir were reported to have been poached in 2013 (R. Alvarenga, pers. comm.). Although this is less than levels in 2011 and 2012, the reduction could be due to hunters experiencing a lower encounter rate with tapir because of the lower population density and the distribution bias reported in Chapter 5; or due to changes in anthropogenic pressures linked to trends in illegal activity in PNC; the increase in policing in the Park; or it could simply be a random effect.

Bruner *et al.* (2001) demonstrated that protected areas are generally effective at preserving biodiversity, and that even a minimal amount of 'management' of protected areas is sufficient to have a positive impact on biodiversity when compared to non-protected areas. This raises the important point that heterogeneous effort in the management of protected areas is likely to impact the efficacy of protected areas in maintaining biodiversity (Leader-Williams *et al*. 1990; Plumptre *et al*. 2014). The decline of Baird's tapir in PNC was partly attributable to the lack of management of the Park, which was one of the first conservation problems that needed to be addressed. Data presented in Chapters 5 and 6, and in McCann *et al*. (2012), were used to support the application to place ICF in full charge of the management of PNC, and to authorise the militarisation of PNC in August 2012, in an attempt to curb the rate of poaching and deforestation.

7.6. Conclusions

From the evidence presented in this thesis and the experiences gained over the duration of this study, it seems probable that without improved conservation management efforts, Baird's tapir could be extirpated from large parts of its range in Honduras. Ongoing monitoring projects are vital to enable the identification of conservation crises and to focus resources for intervention on those areas that most need them (Wilson *et al*. 2006). Survey methodologies should be continually reviewed to ensure accurate reportage of the conservation situation, as once-valid methodologies may lose their efficacy due to unforeseen changes in population structure (Chapter 5).

The development of conservation strategies that prioritise reducing levels of hunting and deforestation in the short-term and maximising the evolutionary potential of Baird's tapir in the long-term is critical for the survival of the species, but implementing such strategies against the backdrop of high levels of civil unrest is another matter altogether. The socio-political situation in Honduras is incredibly complex and in many ways is detrimental to the survival potential of Baird's tapir and other endangered species (McSweeney *et al*. 2014). The Honduran government have, however, demonstrated a willingness to listen to advice and a desire to conserve iconic species such as Baird's tapir using conservation strategies that are informed by science. It is vital that this enthusiasm be nurtured and that current efforts to conserve biodiversity in Honduras be maintained and expanded to include the protection of existing habitat corridors between protected areas (Chapter 6).

Guaranteeing the viability of Baird's tapir populations in Honduras will require further investment in law enforcement in the existing protected area network (Wilson *et al*. 2006) and in infrastructure for ecotourism to ensure the long-term financial viability of the protected areas. It is of paramount importance that the maintenance of biodiversity and the integrity of wild places be included in our political and economic planning, and I believe that those conservation practitioners who are able should see it as a moral responsibility to lobby policy makers to increase investment in the maintenance of biodiversity and in habitat preservation, clearly among the most pressing global issues we face today.

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Useful URLs:

www.earthtrends.wri.org

www.iucnredlist.org

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Appendices

Appendix 1:

McCann, N. P., Wheeler, P. M., Coles, T., & Bruford, M. W., 2012. Rapid ongoing decline of Baird’s tapir in Cusuco National Park, Honduras. Integrative Zoology 7: pp. 420-428.

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ORIGINAL ARTICLE

Rapid ongoing decline of Baird's tapir in Cusuco National Park, Honduras

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Abstract

During the International Tapir Symposium 16–21 Oct 2011, the conservation of Baird's tapir (*Tapirus bairdii*) in Honduras received a boost with the signing of a memorandum of understanding between the Minister Director of the Honduran Institute of Conservation and Forestry (ICF) and the Tapir Specialist Group (TSG). Despite this agreement, accelerating levels of hunting and habitat loss continue to pose a threat to Baird's tapir in Honduras. An ongoing study in Cusuco National Park in northwestern Honduras has been monitoring changes in population dynamics of Baird's tapir since 2006 through the collection of occupancy data. The study has identified an increase in hunting pressure, coinciding with a drastic decline in the encounter rate with Baird's tapir spoor. Here, we examine the significance of a range of demographic variables on Baird's tapir occupancy in Cusuco National Park using the software PRESENCE, and simulate the effects of different management strategies on the future dynamics of the population using the stochastic simulation software VORTEX. The predictions of the theoretical population models are compared to observed changes in occupancy levels. We found that non-intervention resulted in the local extinction of Baird's tapir within a very short time frame, but that various intervention models enabled the population to recover to near carrying capacity. Occupancy and extinction probability were shown to respond markedly to the increase in hunting pressure; and occupancy models supported the future population predictions generated by VORTEX. Our study suggests that immediate intervention is required to reduce hunting pressure to near historical levels to prevent the imminent local extinction of the species.

Key words: Baird's tapir, Honduras, population viability analysis, PRESENCE, VORTEX

INTRODUCTION

Despite national laws that prohibit hunting and international regulations that prohibit trade in body parts, Baird's tapir [*Tapirus bairdii* (Gill, 1865)] populations continue to decline due to poaching and habitat loss across their range (IUCN 2008). In this context, understanding the possible future population dynamics of endangered species is crucial to their conservation (e.g. Bruford *et al.* 2010). The ability to predict future population outcomes given a range of possible environmental variables has become a key factor in the creation of many conservation programs (Alcakaya & Sjogren-Gulve 2000). Central to this concept is the use of popu-

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lation viability analysis (PVA) (Lacy 1993). PVA incorporates stochastic population modeling tools to simulate and potentially predict the future dynamics of threatened populations based upon a range of environmental parameters, often with the principle aim of identifying and estimating parameter values associated with the increased probability of extinction (PE) (Lacy 1993). PVA models can incorporate environmental variables, anthropogenic threats to survival and other biotic and abiotic parameters that influence the population dynamics of a species to estimate the PE. The program VORTEX (Lacy 1993) has been used extensively in PVA to estimate PE in threatened (predominately K-selected) animal populations, and has previously been used in PVAs for all 4 tapir species, *Tapirus terrestris* (Linnaeus 1758) (Medici *et al*. 2007; Medici 2010; Gatti *et al.* 2011), *Tapirus indicus* Desmarest, 1819 (Medici *et al*. 2003), *Tapirus pinchaque* (Roulin, 1829) (Lizcano *et al*. 2005) and *T. bairdii* (Barongi *et al.* 1996; Medici *et al*. 2005). A comprehensive review of 21 PVA studies conducted by Brook *et al.* (2000) concludes that population predictions made using a range of PVA software programs did not differ significantly from observed population trends in those populations. However, these findings have been criticized (e.g. Coulson *et al.* 2001) and caution must be applied to any prediction due to the stochastic nature of individual and population dynamics, the highly heterogeneous response of different species to identical pressures and, in particular, to uncertainties and potential inaccuracies in parameterization due to lack of information (Ludwig 1999; Coulson *et al*. 2001).

Baird's tapir is the largest terrestrial mammal in the Neotropics, with a range extending from southern Mexico to northern Ecuador (Medici *et al.* 2005). Baird's tapir is classified as 'endangered' by the International Union for Conservation of Nature (IUCN), with an estimated population of ±5000 mature individuals across its range. Honduras represents a stronghold in the Baird's tapir range, with a conservative estimate of 500 individuals, representing approximately 10% of the global population (IUCN 2008). Cusuco National Park (PNC) is a 224 km^2 national park in the Merendon mountains near the major conurbation of San Pedro Sula, in northwestern Honduras. Since 2006, systematic studies focusing on the ecological determinants of Baird's tapir occupancy have been carried out as part of a series of biodiversity studies by teams coordinated by UK-based research-tourism operator Operation Wallacea (Long 2009). The identification of spoor along a network of transects has been used as an indicator of tapir presence.

In the 5 years prior to 2009, only 2 tapirs were confirmed as having been hunted within the park boundaries. Between January 2010 and June 2011, a minimum of 6 tapirs was hunted in PNC (R. Alvarenga, pers. comm.). This increased rate of harvest has coincided with a rapid increase in deforestation largely attributed to the activity of illegal coffee growers (Operation Wallacea, unpubl. data). Due to increasing anthropogenic disturbance in the park, and a drastic reduction in the spoor encounter rate in 2011, a park-specific PVA is required to assist the park authorities to initiate an effective and well-informed conservation program to avoid the local extinction of Baird's tapir, one of the most charismatic species in the park.

Ecological occupancy surveys might incorrectly identify a patch of habitat as being empty of the target species and it is important to account for false absences when presenting conclusions about habitat occupancy. The software program PRESENCE (MacKenzie *et al.* 2006) is designed to mitigate this bias when detection rates are below 1. Using spatially referenced binary encounter data, models can be created that describe competing hypotheses about the study system. The model that best describes the data is chosen using 'goodness of fit' concepts, such as Akaike's information criterion (as per MacKenzie *et al*. 2003) or other maximum likelihood tests, which provide an information-theoretic method for model selection (MacKenzie *et al.* 2003). Patch occupancy analysis using PRESENCE is being increasingly incorporated into individual population studies and species management plans (e.g. Karanth *et al*. 2011; Sarmento *et al*. 2011), as it specifically addresses the central problem of imperfect detection, which has been a key confounding problem in distribution studies on rare and illusive species.

Conservation programs aim to ensure the sustainability of a species or population on the basis of minimum population size estimates (Thomas 1990). Sustainability is difficult to accurately define and published definitions of minimum viable population (e.g. the 50/500 rule, as per Franklin 1980; Soule 1980) can, at best, be described as 'rules of thumb' and are widely criticized (e.g. Traill *et al*. 2007; Flather *et al*. 2011). In this paper, we define sustainability as positive population growth over a period of 10 generations (100 years), irrespective of population size.

The objective of this study is to carry out predictive modeling on the population of Baird's tapir in PNC to compare potential management strategies and conservation priorities for the species (Possingham *et al.* 1993) and, specifically, to parameterize and quantify the effect of uncontrolled hunting. Modeling future population trajectories for Baird's tapir in PNC under different assumptions could inform the development of an effective conservation strategy for the species across its range.

MATERIALS AND METHODS

Study site and occupancy analysis

A network of 7 camps have been established in PNC, with 49 800 m of transects, organized approximately at the 4 points of the compass around each camp (Fig. 1). The camps are opened and managed by Operation Wallacea during a 2 month sampling season between June and August each year. Each transect was marked at 50 m intervals to give a simple and robust method for recording the location of tapir records on a transect. Data on Baird's tapir occupancy were gathered by recording encounters with tapir spoor (direct sightings, feces, tracks and evidence of foraging) taken on transect surveys walked by researchers working for Operation Wallacea. The location of all novel encounters were recorded on a Garmin Vista HCX GPS unit and the distance along the transect was also recorded.

Data were recorded in each of the years 2006, 2008, 2009, 2010 and 2011, with identical sampling effort between years. Sites located in the highly impacted buffer zone of the park were not included in the analysis. The program PRESENCE 3.1 (Hines 2010) was used to select the model of tapir occupancy best supported by the data from a candidate set of 57 models that variously incorporated the effects of location and year on initial occupancy rate, colonization, extinction rate and probability of detection. Model selection was based on a combination of model fit assessed by Akaike's information criterion, likelihood ratio tests and inspection of model output to ensure model convergence had been achieved.

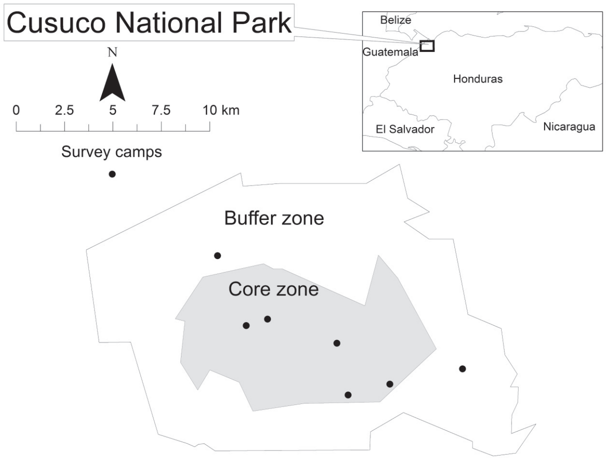

Figure 1 Map of Cusuco National Park (PNC) showing location of the park within Honduras and the arrangement of camps within the park.

Stochastic modeling

The program VORTEX 9.99b (Lacy 2010) was used to simulate population projections with life history parameters based on a combination of published and unpublished data on Baird's tapir and the closely-related lowland tapir (*T. terrestris*) (Medici *et al.* 2007; Medici 2010; Gatti *et al*. 2011) and on 2 previous PVA studies conducted on the species (Barongi *et al.* 1996; Medici *et al*. 2005). The breeding system was changed from monogamous (as per the 2005 PVA) to polygynous for the purposes of the present paper. Tapirs are not known to mate exclusively and the use of a monogamous breeding system in VORTEX would introduce inaccuracy into the model. Additional unpublished data were derived from hunters and guides living close to PNC, from observations in the field and from conversations with tapir researchers with detailed knowledge of tapir population dynamics.

Scenarios using initial population sizes of Baird's tapir from 10 (lowest estimate of the PNC population) to 70 individuals (carrying capacity estimate of the PNC population) at intervals of 5 were simulated. A total of 1000 iterations were run for each scenario over a timeframe of 100 years, which is the equivalent of approximately 10 generations (IUCN 2008).

Three main models were explored for the population viability analysis: no intervention, whereby hunting could continue unchecked; partial intervention, whereby hunting was reduced to a range of levels below the current level; and immediate intervention, whereby all harvesting of individuals was modeled to stop from $T = 0$, allowing the population to change through stochastic processes only. The effect of the current rate of harvest of 3 adults per annum (H3) was modeled in the no intervention model. Incrementally increasing rates of harvest were compared for the partial intervention model, from the original rate of harvest (Ho) of 1 adult per gender every 5 years, through 1 adult per gender every 4 years (H1,4), 1 adult per gender every 3 years (H1,3), 1 adult per gender every 2 years (H1,2), to 1 adult per gender per year (H1,1). The aim of this modeling exercise was to create a series of population projections under different harvesting pressures, and to calculate the level at which harvesting becomes unsustainable, leading to the extinction of the PNC population; and the level of harvesting at which the population is able to maintain growth under normal stochastic processes.

A recurring criticism of PVA modeling is that it lacks validation in real systems (Beissinger & Westphal 1998; Brook *et al.* 2000). Population density estimates for Baird's tapir are based on very few studies and show little consistency (Naranjo 2009). For the present study, population and carrying capacity estimates were derived from field observations and unpublished data from local guides and hunters. We used the change in occupancy estimates over time to provide a check of the population trend predictions generated in VORTEX.

RESULTS

The encounter rate with Baird's tapir spoor remained broadly consistent between 2006 and 2010 but exhibited a major decrease between 2010 and 2011 (Table 1).

Stochastic modeling

Biologically relevant initial population size (Pi) figures of Pi = 20 (conservative population estimate for PNC), Pi = 30 (probable population estimate) and Pi = 40 (optimistic population estimate) were chosen for analysis, based upon these estimates. The current estimate of 30 individuals falls within acceptable boundaries, as suggested by previous studies (e.g. Naranjo 2009; Naranjo & Bodmer 2007), giving a density of 0.39 tapirs·km^{-1} within the 77 km^2 core zone of PNC.

Under the no intervention model, VORTEX results predicted a negative growth rate under all relevant initial population (Pi) size parameters. All models gave a 100% PE over 100 years.

Under a partial management strategy, negative growth was predicted in all but 2 models (H1 Pi = 20 and H1,2 Pi = 20), where hunting rate was modeled as high and Pi conservatively low. The PE was >5% on all models, with the exception of Ho Pi = 40, which is probably they most realistic model for the real state of the park before the increase in hunting rate.

A comparison of the current rate of harvest *versus* the full range of partial intervention models demonstrates the impact of implementing an intervention strategy and enables the assessment of sustainable rates of harvest given different starting populations. Population simulations using the current rate of harvest suggest that the local population will be extinct within the next few years independent of the Pi. The exact time to extinction is dependent upon the Pi, but in 1000 iterations of the model, only 1 population survived, even with an optimistically large Pi of 40 individuals. The qualitative predictions of the effects of hunting on population growth did not vary substantially with initial population size: a harvest rate of 1 adult per gender per 3 years (strategy H1,3) enabled a positive net growth rate. Higher rates of harvest caused zero growth or a negative growth rate in all but 1 scenario (H1,2 Pi = 40), which showed a growth rate of <1%. Under a Pi of 20, population growth under the Ho, H1,4 and H1,3 strategies resulted in the population reaching a stable level considerably beneath the carrying capacity of the park (given as 70 individuals, SD 10). Under a harvest rate of H3, a PE of 1 was achieved after only 15 years and mean population extinction (TE) occurred after 6.5 years.

Under a Pi of 30 (Fig. 2), the same pattern was observed, although the only hunting strategy that experienced 100% population extinction was the current rate of harvest (H3), where PE = 1 was achieved after 33 years. H1,1 resulted in a negative population growth and a PE of 0.97. H1,2 Showed zero growth and a 51% chance of extinction. Scenarios run for strategies Ho, H1,4 and H1,3 showed populations stabilizing at between 40 and 50 individuals. TE figures doubled between hunting strategies H3 and H1,1 and H1,1 and

Table 1 The number and frequency of encounters with tapir spoor along transects in Cusuco National Park between 2006 and 2011

Year	2006	2007	2008	2009	2010	2011
Number of encounters	152	DD	144	163	151	22
Frequency of encounter·km^{-1}	4.66	DD	4.42	5.0	4.63	0.67

H1,2, after which they stabilized at around 50 years.

Under a Pi of 40, Ho, H1,4 and H1,3 saw positive population growth and population stability, with over 50 individuals, close to the park carrying capacity of 70 (SD 10). H1,2 achieved a non-negative but negligible growth rate of 0.7%. The H1,1 scenario gave an 89% chance of extinction; and H3 achieved a PE of 0.999, effectively a 100% chance of extinction. TE estimates described a similar pattern, approximately doubling between hunting strategies H3, H1,1 and again between H1,1 and H1,2, before the rate of increase reduced considerably for more effective intervention strategies.

Under the immediate intervention model, all simulated populations experienced a positive growth rate. The PE was very low (2%) even under a conservative scenario of Pi = 20. The PE became negligible (<1%) given a Pi of 30 or more.

Occupancy analysis

Among the 4 best fitting models, 3 included 'year' as a covariate of local PE (eps), indicating strong support in the data for annual changes in this parameter (Table 2). The top-ranked model included 'year' as a covariate of both colonization and PE but did not significantly improve the model fit over the simpler second-ranked model (likelihood ratio test, $P > 0.100$). Therefore, the latter was chosen as that which most parsimoniously explained the data.

The estimates of interest are the derived estimate of occupancy [*psi(t)*]: the estimate of occupancy for each year (Table 3). *Psi(t),* estimates suggest that the population was growing slowly before the catastrophic decline in the 2010–2011 inter-year period, as was suggested by the raw occupancy data The 95% confidence intervals for *psi(t)* lie within acceptable boundaries, although cau-

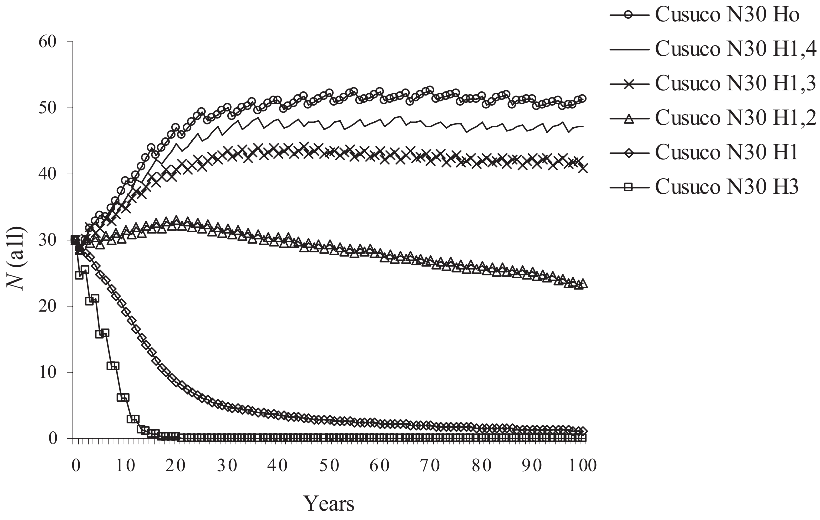

Figure 2 Population trend under various hunting pressures: original hunting pressure (Ho) of 2 adults every 5 years, 1 adult per gender every 4 years (H1,4), 1 adult per gender every 3 years (H1,3), 1 adult per gender every 2 years (H1,2), 1 adult per gender every 2 years (H1,2), 1 adult per gender per year (H1,1), and current hunting pressure of 3 individuals per year (H3) given a Pi of 30.

Table 2 Selection of the most parsimonious model. Models show the importance of covariates in explaining the data, as given by the program PRESENCE (Hines 2010). Covariates considered were: initial population size (psi), colonization (gamma), probability of extinction (eps) and probability of detection (p)

Model	AIC	Delta AIC	AIC weight	Model likelihood	Number of par	2×log likelihood
psi,gamma(year), eps(year),p(.)	539.39	0.00	0.6154	1.0000	10	519.39
psi,gamma(.), eps(year),p(.)	540.52	1.13	0.3498	0.5684	7	526.52
psi(.)eps(year), p(.)	545.61	6.22	0.0274	0.0446	6	533.61
Psi(year),gamma (.),p(.)	548.43	9.04	0.0067	0.0109	7	534.43

AIC, Akaike's information criterion.

Table 3 Statistical output for the psi,gamma(.),eps(year),p(.) model showing inter-year values for *psi(t)* (derived estimate of occupancy) as given by the program PRESENCE (Hines 2010)

Model	Year	*psi(t)*	95% confidence interval
psi,gamma(.),eps(year),p(.)	2006–2008	0.3775	0.2003–0.5547
psi,gamma(.),eps(year),p(.)	2008–2009	0.4839	0.3295–0.6383
psi,gamma(.),eps(year),p(.)	2009–2010	0.5255	0.3168–0.7342
psi,gamma(.),eps(year),p(.)	2010–2011	0.1449	0.0457–0.2440

tion must be applied to any precise conclusions drawn from these results.

DISCUSSION

Although PVAs have been applied to Baird's tapir before, this is the first time that a modeling exercise has been conducted for a specific Baird's tapir population experiencing an immediate threat of extinction. The results of the VORTEX analysis demonstrate the importance of rapid management of the hunting levels currently experienced in PNC. Under current harvest levels, 100% of the simulations reveal that under a no-intervention model, local extinction will occur, even with an optimistically high initial population value of 40 individuals. Partial intervention models described a much lower PE, which was reduced to 0% under an immediate intervention model. Results from the immediate intervention model suggest that even if the population has fallen to as low as 20 individuals, there is a 98% chance that the population will survive over a period of 100 years. Under the original rate of harvest, the probability of local extinction would remain at approximately 5% provided the population had not been reduced to fewer than 30 individuals and the population would stabilize just below the expected carrying capacity of the park. The partial intervention models suggest a harvest rate of 1 adult per gender, every 3 years, approaches sustainability and will result in positive population growth even if the population has been reduced to 20 individuals. Under this model, the PE is reduced to 21% given a Pi of 30, and 9% under a Pi of 40, which is at the lower-limit of population sustainability. Scenarios run at historical levels of hunting describe a more positive outlook. If the population has dropped to as low as 20 individuals, it still has a 75% probability of survival under original rates of harvest, rising to a 94% probability of survival given an initial population of 30, which is a more realistic starting value. Assuming population stability lies between H1,3 and Ho harvest rates, current rates of harvest are between 4.5 and 7.5 times higher than sustainable rates, depending on the Pi. TE figures clearly demonstrate the impact of intervention and suggest that an initial reduction in the hunting rate could buy considerable time for the population, during which management strategies could be implemented to assist in the further reduction of harvesting and the establishment of recruitment mechanisms.

It is clear from these results that non-intervention is not a viable management strategy. Without prompt intervention, the population of Baird's tapir in PNC is predicted to rapidly become extinct under current harvesting rates and faces a high PE even under greatly reduced rates of harvest. It is unrealistic to assume, given the human demographic situation in and around PNC, that hunting will be eradicated completely within the next decade or more, so the partial intervention models provide more realistic population predictions. Observations at the beginning of the study suggested that a population of approximately 40 individuals might have been present in the park. The sharp increase in the harvest rate since 2009 has caused a catastrophic crash in the population, with the VORTEX simulations predicting rapid extinction without prompt intervention. It is important that hunting be reduced so that a maximum of 2 adult tapirs are harvested every 3 years; a failure to contain hunting to within these rates is predicted to create negative growth and result in the eventual extinction of the population.

Over-hunting is a recurring threat to endangered species, particularly large-bodied species with low rates of recruitment and low population sizes (Bodmer & Robinson 2004). Similar studies have investigated the effect of over-hunting on PE in a range of taxa. A study by Hughes *et al.* (2011) identifies an analogous situation in West Africa where common chimpanzee subspecies *Pan troglodytes vellerosus* (Gray, 1862) populations are suffering a rate of harvest 2–13 times higher than sustainable levels and are predicted to be extinct within 20 years without prompt intervention. An interesting retrospective study by Turvey & Risley (2006) demonstrates how Steller's sea cow was driven to extinction within 27 years of its discovery by a hunting rate that was 7 times the sustainable level. This example demonstrates how rapidly a species or population can become extinct under similar pressures being experienced by Baird's tapir in PNC today.

The theoretical population models predicted by the VORTEX analysis are validated by variations in occupancy and PE derived from real data collected in the field. The occupancy estimates generated by PRESENCE broadly match the mean population trends predicted by VORTEX. Under the traditional hunting model, experienced before 2010, stochastic models predict a steady increase in population over time until carrying capacity is reached. This matches the derived occupancy estimates that suggest an increase in the occupancy rate from 2006 to 2010, rising from 0.38 to 0.52, before a sudden decline to 0.15 during the 2010–2011 interyear period, when harvest rates increased dramatically. Stochastic models also predict an immediate population decline as a result of an increase in the harvest rate, which is equally supported by the derived occupancy estimates.

Although the data collected in 2011 describe an encounter rate one-seventh of that in previous years, it is highly improbable that the population itself has been reduced to one-seventh of its size in only 2 years. It is more likely that the population being sampled during the surveys represents a fraction of the overall population of the park, and that hunting has removed the majority of the sample population, whose territories overlapped the transect network used by loggers and hunters to access the park. In addition to surveying all transects in 2011, 25 000 m of trails were walked off transect, in areas predicted to have a comparatively high density of tapirs because of their remoteness and historically high abundance of tapir spoor. The relative abundance of tapirs was found to be higher in these areas than along the transects (3.08 encounters·km^{-1} compared to 0.67 encounters·km^{-1}), but still considerably less than the on-transect encounter rate from 2006–2010 (mean 4.68 encounters·km^{-1}). It should be noted that these occupancy results require extension. A complete survey of PNC designed to incorporate all areas in the park will be carried out in 2012. Although accurate locations for the harvesting sites do not exist, local information suggests that 2 tapirs were killed near each of the 3 core-zone camps, where occupancy rates have traditionally been high. As such, previously occupied patches might now be recorded as unoccupied because insufficient time has passed to allow for re-colonization from other parts of the park. It is a reasonable assumption that the transect data accurately reflect a decline in the abundance of tapirs in the park, but also that the original sample population was small and represented a fraction of the overall population in the park.

The analyses presented here do not incorporate the loss of genetic diversity and inbreeding as confounding variables. Both of these factors have been shown to influence PE in wild populations (e.g. Saccheri *et al.* 1998) and maximizing the genetic viability of a population must be seen as a priority in creating any future management plan for Baird's tapir (Bruford *et al.* 2010). It is highly likely that the population will have already suffered a serious decline in genetic diversity that might make the population unviable in the long term (Lacy 1997; Traill *et al.* 2007). Isolation and small population size are thought to reduce individual and population fitness as a result of inbreeding and the loss of genetic diversity (Hogg *et al.* 2006). Facilitating immigration through the establishment of habitat corridors should be seen as a priority for the future management of the species. Demographic (and genetic) rescue by the translocation of tapir from other parts of Honduras should be considered as another possible management strategy if the genetic diversity of the population is proven to be dangerously low (Bouzat *et al.* 2009). Demo-genetic rescue has been shown to be an effective method of restoring population viability in a range of taxa (e.g. Westemeier *et al.* 1998; Hedrick & Fredrickson 2010). Hogg *et al.* (2006) describe the genetic rescue of a population of bighorn sheep founded by 12 individuals and isolated at a population of approximately 40 individuals. The translocation of individuals into this population restored fitness-related trait values in descendants of the migrant animals. Individual tapirs with a high genetic value to the PNC population should be sought as candidates for translocation (Goncalves da Silva *et al.* 2010). An increase in genetic variability, brought about by the input of novel genotypes, would minimize the deleterious effect of inbreeding and help protect against stochastic environmental events such as an outbreak of disease. Analysis of genetic samples collected in Cusuco between 2009 and 2012 will be conducted in late 2012 and the results will be suggested for incorporation into future management strategies for Baird's tapir in PNC.

Local survival of Baird's tapir in PNC depends very much on 2 factors: the remaining population size and the future rate of harvest. These results highlight the importance of conducting an accurate population census in PNC, which will increase the precision of the predictive models. Generating an accurate census of the population of Baird's tapir in PNC is the first critical step in estimating how robust the population is to hunting and to potentially confounding environmental variables related to the loss of genetic diversity.

Controlling hunting is the only way to avoid the extinction of this population. While human demographic conditions remain in their current state and law enforcement remains nominal in remote communities, poaching of all species will continue unabated and it will be the most endangered species, such as Baird's tapir, that will be the first to suffer and will take the longest to recover.

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