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Glucocorticoid Metabolites and GPS Radio Collar Telemetry in Wildlife Conservation: The Jane Goodall Institute Mandrill Release Project in the Republic of Congo

Miles Woodruff

Abstract

Wildlife populations are being depleted globally by pressures associated with the growing human population and non-human primate populations are in sharp decline. The Tchimpounga Chimpanzee Rehabilitation Sanctuary in the Republic of Congo cares for orphaned primates with the goal of reintroducing them to the wild when appropriate. The primary aim of this study was to reintroduce the mandrills held at Tchimpounga into Conkouati-Douli National Park following the International Union for Conservation of Nature (IUCN) Guidelines as closely as possible. In preparation for the release we built an enclosure at the selected release site and tested the global positioning system (GPS) collars the animals would be wearing. At the end of the study we retrieved the GPS collars and found fewer successful fixes than expected and analysed the collar fix success rates in relationship to each individual's use of three-dimensional space and mass in an effort to understand the lack of successful fixes. We found that vegetation density and collar height within the vegetation significantly affected fix success rates. Our post-release data indicated larger animals spent more time on the ground than smaller animals, and that smaller animals had more successful

fixes. We found variation in GPS collar function and that how the animals interact with their three-dimensional (3D) environment affects collar function. If animals in a study group spend different amounts of time at different heights in the forest it could bias the data. Researchers should thus test the collars they will be using for height bias in circumstances where the release subjects have a 3D relationship with the environment around them. We recommend accounting for an animals' use of three-dimensional space in GPS collar studies where the species is not fully terrestrial and vegetation, topography, or human-made structures are likely to interfere with their collars' access to satellites.

We also used non-invasive faecal sampling to measure the mandrills' glucocorticoid metabolite levels as a biological proxy for their stress response to each stage of the release. The findings suggest that faecal glucocorticoid metabolites can be used to capture the biological response to the stages of reintroduction. All mandrills had an increase in glucocorticoid metabolite values post transfer. It took 4 weeks for the glucocorticoid metabolite values to decrease although there was variation amongst individuals. We recommend using faecal glucocorticoid metabolite analysis in release projects to inform decisions about how long the study species should be held in a pre-release enclosure to overcome the stress of transfer and habituate to their surroundings prior to being released. The findings of this study also highlighted that different animals reacted differently to the stages of the release process, thus researchers should assess animals as individuals rather than a group to assure maximum animal welfare through the release process.

Ultimately, through scientifically testing aspects of this release project we gained insight to inform future mandrill releases as well as wildlife release projects generally. We recommend GPS collars are tested in the release area and the results are reviewed prior to fitting the collars to the animals. GPS collar studies should account for an animal's 3D relationship with topographical obstruction and vegetation within their environment because systematic differences in forest usage

can bias collar data. Finally, we recommend sanctuary release projects use soft release methods unless hard release had been thoroughly validated for the species under representative circumstances.

GPS Radio Collar Telemetry and Glucocorticoid Metabolites in Wildlife Conservation: The Jane Goodall Institute Mandrill Release Project in the Republic of Congo

Miles Woodruff

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Contents

Abstract ..... **i**

Contents ..... **v**

List of Figures ..... **xii**

List of Tables ..... **xix**

List of Abbreviations ..... **xxi**

Declaration of Contributions ..... **xxii**

Statement of copyright ..... **xxiv**

Acknowledgements ..... **xxv**

Chapter 1: Introduction **1**

1.1 Primate conservation **1**

1.2 Translocation **3**

1.3 Primate release **4**

1.3.1 Release strategy 5

1.3.2 Failure points in release projects 5

1.3.3 Release site assessment 7

1.3.4 Post-release monitoring 8

1.3.5 Selecting release candidates 9

1.3.6 Mandrill behaviour and ecology 9

1.3.7 Mandrill conservation status, threats, and conservation strategies 11

1.3.8 Mandrill release 12

1.3.9 Narrative background on the study design 15

1.3.10 Thesis structure 16

Chapter 2: General methods 17

2.1 Principles of re-introduction 17

2.2 Aims 17

2.3 Study locations 18

2.4 Republic of Congo 19

2.5 Tchimpounga Reserve 19

2.6 Conkouati-Douli National Park 20

2.6.1 Study animals 22

2.7 Assessing the suitability of the release stock 23

2.8 Tchimpounga enclosure 25

2.8.1 Habitat requirements and release site selection 27

2.9 Survey findings 30

2.10 Site selection 32

2.11 Pre-release enclosure 33

2.12 Release group structure 37

2.12.1 Transfer to the release site 37

2.13 Practice collars 38

2.14 GPS Collars 39

2.15 Behavioural observations 40

2.16 Post-release monitoring 42

2.17 Ethical approvals and research authorisations 42

Chapter 3: Testing GPS collars in preparation for a primate release 44

3.1 List of authors and affiliations:.....44

3.2 Abstract 45

3.3 Introduction 46

3.4 Methods 51

3.4.1 Test 1: Presence of a simulated animal 52

3.4.2 Test 2: Collar orientation 53

3.4.3 Test 3: Habitat type.....54

3.4.4 Test 4: Height in the canopy 55

3.4.5 Test 5: Height in a ravine 56

3.5 Data analysis 57

3.6 Results: 58

3.6.1 Test 1: Presence of a simulated animal 58

3.6.2 Test 2: Collar orientation 59

3.6.3 Test 3: Habitat type.....62

3.6.4 Test 4: Height in the canopy 65

3.6.5 Test 5: Height in a ravine 66

3.7 Discussion 68

3.7.1 Test 1: Presence of a simulated animal 68

3.7.2 Test 2: Collar orientation 69

3.7.3 Test 3: Habitat type.....69

3.7.4 Test 4: Height in the canopy 70

3.7.5 Test 5: Height in a ravine 70

3.8 Conclusion 71

3.9 Acknowledgements 73

Chapter 4: Height bias in GPS Collar Studies: a post-release study of mandrills 74

4.1 List of authors and affiliation: 74

4.2 Abstract 75

4.3 Introduction 76

4.3.1 Habitat introduces systematic bias in GPS collar data 76

4.3.2 Models for correcting bias 78

4.3.3 Hypothesis 1: body mass and behaviour 79

4.3.4 Hypothesis 2: collar accuracy and height above the ground 79

4.3.5 Hypothesis 3: fix success and body mass 79

4.3.6 Hypothesis 4: fix success and time of day 79

4.3.7 Hypothesis 5: fix success and habitat 80

4.3.8 Hypothesis 6: collars and handheld GPS data 80

4.4 Methods 80

4.5 Analysis 82

4.6 Results 83

4.6.1 Hypothesis 1: body mass and behaviour 83

4.6.2 Hypothesis 2: collar accuracy and height 83

4.6.3 Hypothesis 3: fix success and body mass 86

4.6.4 Hypothesis 4: fix success and time of day 87

4.6.5 Hypothesis 5: Fix success and habitat 89

4.6.6 Hypothesis 6: Collars and handheld GPS data 92

4.7 Discussion 92

4.7.1 Implications for wildlife collar studies 95

4.8 Potential areas of research to understand and reduce height bias. 96

Chapter 5: Faecal glucocorticoids as a biological measure of welfare during captive transfers and primate release 98

5.1 List of authors and affiliations:.....98

5.2 Abstract 99

5.3 Introduction.....99

5.4 Methods 105

5.4.1 Study site.....105

5.4.2 Study animals.....106

5.4.3 Tchimpounga enclosure.....107

5.4.4 Pre-release enclosure 107

5.4.5 Release 108

5.4.6 Release methods.....109

5.4.7 Faecal sampling collection and processing.....110

5.4.8 Validation of the enzyme immunoassay:.....112

5.4.9 Statistical analysis 114

5.4.10 Accounting for the effect of drying time 116

5.5 Ethical Note.....116

5.6 Results 117

5.6.1 Prediction 1: Transfer to the pre-release enclosure will lead to an increase in FGCM values. 117

Prediction 2: The FGCM response associated with the transfer to pre-release enclosure will decrease over a period of weeks 117

5.6.2 Prediction 3: Release will lead to an increase in FGCM values. 119

5.6.3 Prediction 4: The FGCM response associated with the release will decrease over a period of weeks. 121

5.6.4 Prediction 5: The magnitude of the glucocorticoid response at release will be less than at transfer to the pre-release enclosure. 123

5.6.5 Prediction 6: FGCM values will be lower post-release than in the sanctuary. 124

5.7 Discussion 125

5.7.1 Prediction 1: Transfer to the pre-enclosure will lead to an increase in FGCMs 126

5.7.2 Prediction 2: The FGCM response associated with the transfer will decrease over a period of weeks. 127

5.7.3 Prediction 3: Release will lead to an increase in FGCM values. 128

5.7.4 Prediction 4: The FGCM response associated with the release will decrease over a period of weeks 129

5.7.5 Prediction 5: The magnitude of the glucocorticoid response at release will be less than at transfer to the pre-release enclosure. 130

5.7.6 Prediction 6: FGCM values will be lower post-release than in the sanctuary. 130

5.8 Conclusions and recommendations 131

5.8.1 Planning construction and populating the enclosure 131

5.8.2 Sample Processing 132

5.8.3 Group dynamics 133

5.9 Acknowledgments 133

Chapter 6: Discussion 134

6.1.1 Release strategy 138

6.1.2 Other key lessons 140

6.1.3 Considerations for the selection of release candidates 142

6.1.4 Conclusion 144

References 145

Appendix 168

List of Figures

FIGURE 1.1 DISTRIBUTION OF MANDRILLS IN CENTRAL AFRICA. MANDRILL DISTRIBUTION SHOWN IN RED. COUNTRY BORDERS SHOWN IN WHITE. BLACK LINE THROUGH THE CENTRE OF THE RED AREA FOLLOWS THE OGOOUÉ RIVER AND MARKS THE BOUNDARY BETWEEN THE TWO SUBSPECIES OF MANDRILL. IUCN RED LIST OF THREATENED SPECIES. VERSION 2012 12

FIGURE 2.1 MAP OF SOUTH WESTERN GABON AND REPUBLIC OF CONGO SHOWING TCHIMPOUNGA RESERVE IN GREEN ON THE RIGHT AND CONKOUATI-DOULI NATIONAL PARK IN BLUE ON THE LEFT. RED SHOWS THE MANDRILL DISTRIBUTION FROM IUCN. COUNTRY BORDERS ARE SHOWN IN WHITE. 18

FIGURE 2.2 MAP OF THE RELEASE SITE AND BASE CAMP IN RELATIONSHIP TO TCHIMPOUNGA. CONKOUATI IS IN BLUE ON THE LEFT AND TCHIMPOUNGA IN GREEN ON THE BOTTOM RIGHT. RED DENOTES THE MANDRILL DISTRIBUTION ACCORDING TO IUCN 20

FIGURE 2.3 NUMBER OF SNARES FOUND IN CONKOUATI-DOULI NATIONAL PARK PER YEAR BY FOREST RANGERS BETWEEN 2007 AND 2011. UNPUBLISHED DATA COMPILED BY WCS. GUARDS PATROL THE PARK AND TURN IN THE SNARES THEY FIND AT THE END OF THEIR MISSION. 21

FIGURE 2.4 NUMBERS OF MANDRILL CARCASSES CONFISCATED BY FOREST RANGERS IN CONKOUATI-DOULI NATIONAL PARK BY YEAR BETWEEN 2007 AND 2011. DATA COMPILED BY WCS CONGO. ECO-GUARDS CONDUCTED VEHICLE SEARCHES AT THE ENTRANCES TO THE PARK AND DOCUMENTED THE BUSHMEAT THEY CONFISCATED. ECO-GARDS ALSO PATROLLED THE PARK AND KEPT RECORDS OF THE CONFISCATED MEAT AND SNARES (VANLEEUWE, 2012) 22

FIGURE 2.5 FAR LEFT ENCLOSURE FRONT VIEW 26

FIGURE 2.6 FRONT VIEW FAR RIGHT ENCLOSURE 26

FIGURE 2.7 CORNER PLATFORMS, SWINGS, HAMMOCKS AND BAMBOO ENRICHMENT STRUCTURES 26

FIGURE 2.8 THE ORIGINAL CHAIN-LINK CORRIDOR LINKING ENCLOSURES 1 AND 3 26

FIGURE 2.9 PERMANENT CORRIDOR CONNECTING ENCLOSURES 2 AND 3 26

FIGURE 2.10 EXAMPLE OF DAILY PROVISIONS WITH A MIX OF AVAILABLE PRODUCE AND WILD FRUIT. 27

FIGURE 2.11 MAP OF PRE-RELEASE SURVEY TRANSECTS IN CONKOUATI-DOULI NATIONAL PARK. EACH RED AND BLUE VERTICAL LINE REPRESENTS A 1 KM TRANSECT. THE GRID IS IN THE UTM QUADRATE SYSTEM AND EACH SQUARE IS 1 KM^2. THE LIGHT BLUE IS THE FULLY PROTECTED AREA IN THE PARK. THE WHITE AREA IS IN THE INHABITED BUFFER ZONE. THE DARKER GREEN AREA WITH THE RED TRANSECTS IS ZONE A AND THE LIGHTER GREEN AREA WITH THE BLUE TRANSECTS IS ZONE B. THE DARK BLUE LINE RUNNING DOWN THE MAP IS A RIVER LARGE ENOUGH TO POSE A NATURAL BARRIER FOR MANDRILLS. THE RED DOT NEAR THE RIVER TOWARDS THE TOP OF THE MAP IS CAMP POUNBOU AND THE RED DOT NEAREST THE LOWER HALF OF THE MAP IS CAMP FALCON. 29

FIGURE 2.12 TRANSECT RESULTS FROM THE PRE-RELEASE SURVEY CONDUCTED IN CONKOUATI-DOULI NATIONAL PARK. EACH DOT INDICATES A TRACK OR SIGN ENCOUNTERED ON A 1 KM LINE TRANSECT. A) HUMAN SIGNS, B) ELEPHANT SIGNS, C) CHIMPANZEE SIGNS..... 31

FIGURE 2.13 MAP OF TRANSECTS AT THE RELEASE SITE 33

FIGURE 2.14 LOCATION OF MANDRILL BASE CAMP IN RELATION TO THE MANDRILL RELEASE SITE 33

FIGURE 2.15 DESIGN OF THE PRE-RELEASE ENCLOSURE COMPOUND..... 35

FIGURE 2.16 PRE-RELEASE ENCLOSURE 36

FIGURE 2.17 EXTENDED CORRIDOR FROM THE PRE-RELEASE ENCLOSURE TO THE RIVER. 36

FIGURE 2.18 OUTDOOR CORRIDOR ON THE PRE-RELEASE ENCLOSURE. 36

FIGURE 2.19 UNASSEMBLED PRACTICE COLLAR WITH INTERNAL WEIGHTS IN HAND AND FINISHED COLLAR WRAPPED IN DUCT TAPE RESTING ON RETAINING WALL..... 39

FIGURE 2.20 ADOLESCENT MALE MANDRILL WITH COLLAR EXPANDER 40

FIGURE 3.1 EMPTY COLLARS AND COLLARS FITTED TO SIMULATED ANIMALS PLACED UPRIGHT ON A PLATFORM IN OPEN SAVANNA..... 53

FIGURE 3.2 COLLARS FITTED TO SIMULATED ANIMALS, PLACED ON PLATFORM IN OPEN SAVANNA. THE COLLARS WERE ORIENTED AT 0°, 45°, 90°, 135° AND 180° TO TEST FOR AN EFFECT CAUSED BY COLLAR ORIENTATION. 53

FIGURE 3.3 LOCATION OF THE FOUR PLATFORMS LOCATED IN REPRESENTATIVE FOREST TYPES (RIPARIAN, SECONDARY FOREST, FOREST FRAGMENT, SAVANNA) FOUND IN THE RELEASE AREA. THE PRE-RELEASE ENCLOSURE WAS NEXT TO THE RIPARIAN PLATFORM LOCATION. 54
FIGURE 3.4 COLLARS FITTED TO SIMULATED ANIMALS AND PLACED UPRIGHT ON PLATFORMS AT A) 18.8 M AND B) 0.5 M TO TEST FOR THE EFFECT OF HEIGHT ON COLLAR PERFORMANCE. THE PLATFORM AT 18.8 M WAS LOCATED DIRECTLY ABOVE THE PLATFORM AT 0.5 M. FOREST STRUCTURE IN MUCH OF THE RELEASE AREA HAD C) SPARSE SECONDARY GROWTH WITH D) DENSE MARANTACEAE UNDERGROWTH. IN C) I AM AT ~5 M DIRECTLY BELOW THE COLLAR PLATFORM; D) WAS TAKEN AT ~1.5 M. 56
FIGURE 3.5 THE FORESTED RAVINE USED FOR TEST 5 (HEIGHT IN A RAVINE). THE TREE USED IN THE TEST IS HIGHLIGHTED WITH A WHITE RECTANGULAR BOX 57
FIGURE 3.6 COLLARS PLACED AT THREE HEIGHTS IN A TREE LOCATED IN A RAVINE 57
FIGURE 3.7 3D FIX PERCENTAGE FOR EMPTY COLLARS AND COLLARS FITTED TO SIMULATED ANIMALS (SA). COLLARS WERE ON A 0.5 M PLATFORM IN OPEN SAVANNA. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS, OTHERS ARE GPS-ONLY COLLARS. 58
FIGURE 3.8 MEAN TIME TO FIX FOR EMPTY COLLARS AND COLLARS FITTED TO SIMULATED ANIMALS (SA). BOXPLOTS SHOW THE MEDIAN (BLACK BAR) AND THE FIRST AND THIRD QUARTILES (BOXES). WHISKERS SHOW THE MAXIMUM AND MINIMUM VALUES EXCLUDING OUTLIERS (POINTS). COLLARS WERE ON A 0.5 M PLATFORM IN OPEN SAVANNA. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS, OTHERS ARE GPS-ONLY COLLARS. 59
FIGURE 3.9 MEAN 3D FIX PERCENTAGE FOR COLLARS FITTED TO SIMULATED ANIMALS AND POSITIONED AT 0°, 45°, 90°, 135°, 180° AWAY FROM UPRIGHT. THE COLLARS WERE ON A 0.5 M PLATFORM IN OPEN SAVANNA. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS; OTHERS ARE GPS-ONLY COLLARS. 60
FIGURE 3.10 MEAN TIME TO FIX FOR COLLARS FITTED TO SIMULATED ANIMALS AND POSITIONED AT 0°, 45°, 90°, 135°, 180° AWAY FROM UPRIGHT. BOXPLOTS SHOW THE MEDIAN (BLACK BAR) AND FIRST AND THIRD QUARTILES (BOX). WHISKERS SHOW THE MAXIMUM AND MINIMUM VALUES EXCLUDING OUTLIERS

(POINTS). THE COLLARS WERE ON A 0.5 M PLATFORM IN OPEN SAVANNA. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS; OTHERS ARE GPS-ONLY COLLARS..... 61
FIGURE 3.11 MEAN 3D FIX PERCENTAGE FOR COLLARS FITTED TO SIMULATED ANIMALS AND PLACED IN FOUR FOREST TYPES (RIPARIAN, SECONDARY FOREST, SAVANNA, FOREST FRAGMENT). COLLARS WERE ON A 0.5 M PLATFORM IN OPEN SAVANNA. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS; OTHERS ARE GPS-ONLY COLLARS. 63
FIGURE 3.12 MEAN TIME TO FIX FOR COLLARS FITTED TO SIMULATED ANIMALS AND PLACED IN FOUR FOREST TYPES. BOXPLOTS SHOW THE MEDIAN (BLACK BAR) AND FIRST AND THIRD QUARTILES (BOX). WHISKERS SHOW THE MAXIMUM AND MINIMUM VALUES EXCLUDING OUTLIERS (POINTS). COLLARS WERE ON A 0.5 M PLATFORM IN OPEN SAVANNA. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS; OTHERS ARE GPS-ONLY COLLARS. 64
FIGURE 3.13 MEAN 3D FIX PERCENTAGE OF COLLARS FITTED TO SIMULATED ANIMALS AND PLACED AT TWO HEIGHTS IN A SECONDARY FOREST WITH DENSE UNDERGROWTH. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS; OTHERS ARE GPS-ONLY COLLARS..... 65
FIGURE 3.14 MEAN TIME TO FIX FOR COLLARS FITTED TO SIMULATED ANIMALS AND PLACED AT TWO HEIGHTS IN A SECONDARY FOREST WITH DENSE UNDERGROWTH. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS; OTHERS ARE GPS-ONLY COLLARS. 66
FIGURE 3.15 MEAN 3D FIX PERCENTAGE FOR COLLARS FITTED TO SIMULATED ANIMALS AND PLACED AT THREE HEIGHTS IN A SECONDARY FOREST LOCATED IN A RAVINE. COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS; OTHERS ARE GPS-ONLY COLLARS. 67
FIGURE 3.16 MEAN TIME TO FIX FOR COLLARS FITTED TO SIMULATED ANIMALS AND PLACED AT THREE HEIGHTS IN A SECONDARY FOREST LOCATED IN A RAVINE. BOXPLOTS SHOW THE MEDIAN (BLACK BAR) AND FIRST AND THIRD QUARTILES (BOX). WHISKERS SHOW THE MAXIMUM AND MINIMUM VALUES EXCLUDING OUTLIERS (POINTS). COLLARS 659801 AND 659802 ON THE LEFT OF EACH COLUMN PANEL ARE ARGOS COLLARS, OTHERS ARE GPS-ONLY COLLARS. 68

FIGURE 4.1 THE PERCENTAGE OF TIME EACH MANDRILL SPENT IN EACH OF FOUR HEIGHT CATEGORIES POST-RELEASE. NAME CODES ARE ORDERED BY THE ANIMAL'S BODY MASS WITH THE LARGEST MANDRILL ON THE LEFT AND THE SMALLEST ON THE RIGHT. 83
FIGURE 4.2 LOCAL CONVEX HULL (95%, NEIGHBOURS: 30) OF GPS COLLARS IN THE STATIONARY COLLAR TEST. THE BLUE POLYGON WAS CREATED USING THE 3D POINTS COLLECTED AT 0.5 M, THE WHITE POLYGON WAS CREATED USING 3D POINTS COLLECTED AT 21 M AND THE RED POLYGON WAS CREATED USING 3D POINTS COLLECTED AT 29 M. 84
FIGURE 4.3 KERNEL UTILISATION DISTRIBUTION DENSITY OF GPS COLLARS IN THE STATIONARY COLLAR TEST. THE BLUE POLYGON WAS CREATED USING 3D POINTS COLLECTED AT 0.5 M. THE WHITE POLYGON WAS CREATED USING THE 3D POINTS COLLECTED AT 21 M AND THE RED POLYGON WAS CREATED USING 3D POINTS COLLECTED AT 29 M. 85
FIGURE 4.4 KERNEL UTILISATION DISTRIBUTION DENSITY OF THE HEIGHT IN THE CANOPY TEST. THE BLUE POLYGON WAS CREATED USING 3D POINTS COLLECTED AT 0.5 M. THE RED POLYGON WAS CREATED USING 3D POINTS COLLECTED AT 18.8M..... 85
FIGURE 4.5 LOCAL CONVEX HULL (95%, NEIGHBOURS: 30) DENSITY OF GPS COLLARS IN HEIGHT IN THE CANOPY TEST. THE BLUE POLYGON WAS CREATED USING 3D POINTS COLLECTED AT 0.5 M. THE RED POLYGON WAS CREATED USING 3D POINTS COLLECTED AT 18.8 M. 86
FIGURE 4.6 PERCENTAGE OF 3D AND RESOLVED FIXES BY BODY MASS. THE COLLAR ON THE LEFT WAS FAULTY. ... 87
FIGURE 4.7 PERCENTAGE OF 3D FIXES AND NON 3D FIXES SPLIT BY NIGHT AND DAY ATTEMPTS. 88
FIGURE 4.8 LOCAL CONVEX HULL (95% 40 M) FOR 3D AND RESOLVED DAYTIME FIXES. THE IMAGE ON THE TOP LEFT IS A COMPILATION OF ALL OF THE RANGES OVERLAID, FOLLOWED BY MANDRILL KM IN YELLOW, OB IN GREEN, VDL IN BLUE, MAD IN PURPLE AND DOM IN RED 90
FIGURE 4.9 SUCCESSFUL DAYTIME 3D FIXES. THE IMAGE ON THE TOP LEFT IS ALL 3D DAYTIME FIXES FROM ALL COLLARS, FOLLOWED BY MANDRILL KM IN YELLOW, OB IN GREEN, VDL IN BLUE, MAD IN PURPLE AND DOM IN RED. 91
FIGURE 4.10 POINT DISTRIBUTION OF GPS COLLAR DATA (A, B) AND HAND-HELD GPS DATA (C)..... 92

FIGURE 5.1 RESULTS OF ASSAYS TESTING THE REACTIVITY OF ANTIBODIES TO CORTISOL, CORTICOSTERONE AND CORTISOL METABOLITES 69A AND 72T USING FAECAL SAMPLES FROM A FEMALE MANDRILL BEFORE AND AFTER A ROUTINE HEALTH CHECK. SAMPLES WERE COLLECTED IMMEDIATELY FROM KNOWN INDIVIDUALS IN THE MORNING BEFORE THE HEALTH CHECK AND APPROXIMATELY 24 HOURS AFTER THE HEALTH CHECK. 114

FIGURE 5.2 COMPARISON OF FGCMS IN MANDRILLS DURING THEIR FINAL MONTH AT THE SANCTUARY AND THEIR FIRST WEEK IN THE PRE-RELEASE ENCLOSURE. EACH LINE REPRESENTS ONE INDIVIDUAL. 117

FIGURE 5.3 WEEKLY FGCM VALUES FOR MANDRILLS DURING THE FIRST FOUR WEEKS POST TRANSFER TO THE PRE-RELEASE ENCLOSURE. EACH LINE REPRESENTS ONE INDIVIDUAL 118

FIGURE 5.4 WEEKLY FGCM VALUES FOR MANDRILLS IN GROUPS 1-3 DURING THE FIRST FOUR WEEKS AFTER TRANSFER TO THE PRE-RELEASE ENCLOSURE. EACH LINE REPRESENTS AN INDIVIDUAL. GREEN INDICATES GROUP 1, DARK BLUE GROUP 2 AND RED GROUP 3. 119

FIGURE 5.5 COMPARISON OF FGCMS IN MANDRILLS DURING THEIR FINAL MONTH OF SAMPLING WHILE HOUSED IN THE PRE-RELEASE ENCLOSURE AND DURING THEIR FIRST WEEK POST-RELEASE. EACH LINE REPRESENTS ONE INDIVIDUAL 120

FIGURE 5.6 COMPARISON OF FGCMS IN GROUPS 1-3 DURING THE FINAL MONTH IN THE PRE-RELEASE ENCLOSURE AND THE FIRST WEEK POST-RELEASE. EACH LINE REPRESENTS AN INDIVIDUAL. GREEN INDICATES GROUP 1, BLUE GROUP 2, CRIMSON GROUP 3. 120

FIGURE 5.7 WEEKLY FGCM VALUES OF MANDRILLS DURING THE FIRST FOUR WEEKS POST-RELEASE. EACH LINE REPRESENTS ONE INDIVIDUAL. 121

FIGURE 5.8 WEEKLY FGCM VALUES FOR MANDRILLS IN GROUPS 1-3 DURING THE FIRST FOUR WEEKS POST-RELEASE. EACH LINE REPRESENTS AN INDIVIDUAL. GREEN INDICATES GROUP 1, BLUE GROUP 2, RED GROUP 3. 122

FIGURE 5.9 FGCM LEVELS FOR MANDRILLS IN THE PRE-RELEASE ENCLOSURE AND DURING THE FIRST WEEK POST-RELEASE. EACH LINE REPRESENTS ONE INDIVIDUAL. 123

FIGURE 5.10 FGCM LEVELS FOR MANDRILLS IN THE PRE-RELEASE ENCLOSURE AND DURING THE FIRST WEEK POST-RELEASE. EACH LINE REPRESENTS ONE INDIVIDUAL. GREEN INDICATES GROUP 1, BLUE GROUP 2, RED GROUP 3. 124
FIGURE 5.11 FGCM LEVELS FOR MANDRILLS IN THE SANCTUARY AND AFTER RELEASE. EACH LINE REPRESENTS ONE INDIVIDUAL. A: UNADJUSTED VALUES. B: VALUES ADJUSTED FOR DRYING TIME 125

List of Tables

TABLE 2.1 MANDRILLS INVOLVED IN THE RELEASE PROGRAMME. MANDRILL NAME, SEX, ID CODE, APPROXIMATE AGE AT RELEASE, RELEASE GROUP, DATE TRANSFERRED TO THE PRE-RELEASE ENCLOSURE, RELEASE DATE, DAYS IN PRE-RELEASE ENCLOSURE, RELEASED WITH OR WITHOUT COLLAR. 24

TABLE 2.2 DETAILS OF THE RELEASED MANDRILLS AND THEIR TRACKING COLLARS 38

TABLE 3.1 3D FIX RATES FOR EMPTY COLLARS (EMPTY) AND COLLARS FITTED TO SIMULATED ANIMALS (SA). COLLARS PLACED ON A PLATFORM IN AN OPEN SPACE WITH NO OBSTRUCTION OF SKY 58

TABLE 3.2 TIME TO FIX FOR EMPTY COLLARS (EMPTY) AND COLLARS FITTED TO SIMULATED ANIMALS (SA). COLLARS PLACED ON A PLATFORM IN AN OPEN SPACE WITH NO OBSTRUCTION OF SKY 59

TABLE 3.3 3D FIX RATES FOR COLLARS FITTED TO SIMULATED ANIMALS (SA) AND PLACED AT 0° (UPRIGHT), 45°, 90°, 135°, 180°. COLLARS PLACED ON A PLATFORM IN AN OPEN SPACE WITH NO OBSTRUCTION OF THE SKY. 60

TABLE 3.4 TIME TO FIX FOR COLLARS FITTED TO SIMULATED ANIMALS AND PLACED AT 0° (UPRIGHT), 45°, 90°, 135°, 180°. COLLARS PLACED ON A PLATFORM IN AN OPEN SPACE WITH NO OBSTRUCTION OF THE SKY. 61

TABLE 3.5 3D FIX RATES FOR GPS RADIO COLLARS FITTED TO SIMULATED ANIMALS AND PLACED ON PLATFORMS AT TWO HEIGHTS 65

TABLE 3.6 TIME TO FIX FOR COLLARS FITTED TO SIMULATED ANIMALS AND PLACED ON PLATFORMS AT TWO HEIGHTS IN A SECONDARY FOREST WITH DENSE UNDERGROWTH 65

TABLE 4.1 POINT SPREAD PERIMETER AND AREA FOR THE HEIGHT IN A RAVINE AND HEIGHT IN THE CANOPY TESTS CALCULATED USING LOCAL CONVEX HULL AND KERNEL DISTRIBUTION TOOLS IN ZOATRACK 84

TABLE 4.2 MANDRILL MASS AND PERCENTAGE OF COLLAR FIXES AT EACH QUALITY LEVEL 87

TABLE 5.1 INDIVIDUAL TRANSFER AND RELEASE DATES WITH NUMBERS OF SAMPLING DAYS AND FAECAL SAMPLES COLLECTED 110

List of Abbreviations

WCS	Wildlife Conservation Society
Conkouati	Conkouati-Douli National Park
PALF	Projet d'Appui à l'Application de la Loi sur la Faune
NGO	Non-governmental organisation
MEF	Ministère de l'Economie Forestière
Tchimpounga	Tchimpounga Chimpanzee Rehabilitation Sanctuary
IUCN	International Union for the Conservation of Nature
RSG	Reintroduction Specialist Group
Guidelines	Guidelines for Nonhuman Primate Reintroductions
FGCM	Faecal glucocorticoid metabolites
CIRMF	Centre International de Recherches Medicales de Franceville

Declaration of Contributions

Chapter 2

WCS Eco-guard data provided by Hilde Van Leeuwe Project Director, WCS Congo. Debby Cox Technical Advisor at the Jane Goodall Institute produced the PASA education report covering the work done in the area surrounding the release site (Appendix A). We built out a team of interdisciplinary advisors to support the project (Appendix B). The Jane Goodall Institute then conducted the surveys and provided the list of fruiting trees in the release area (Appendix C) Rebeca Atencia Country Director, Jane Goodall Institute Congo produced the Health Management and Sedation protocol (Appendix F) WCS produced the Survey methods used by the project (Appendix C; Appendix D)

Chapter 3

I conceived the aim, designed the study, collected, and analysed data then wrote the chapter, under the supervision of Professor R.A. Hill and Professor J.M. Setchell.

Chapter 4

I conceived the aim, designed the study, collected and analysed data then wrote the chapter, under the supervision of Professor R.A. Hill and Professor J.M. Setchell. Lilian Pintea entered the GPS coordinates into ARC GIS and produced an excel sheet with the tree heights and tree densities associated with the points and returned the raw data to me for processing.

Chapter 5

I conceived the aim, designed the study, collected samples, analysed data and wrote the chapter, under the supervision of Professor J.M. Setchell. I also oversaw and participated in the collection and processing of the faecal samples, along with G.T. Woodruff who oversaw the veterinary staff and project while I was out of the country for several weeks. The Disney Animal Kingdom partners conducted the assay validation and the hormone analysis. R. Atencia specified the release protocol. D. Cox instigated the project and oversaw much of the fundraising and logistics. F.N. Lambert processed the samples at the Disney labs under the oversight of C.J. Wheaton and S.R. Lavin. The Disney partners trained me in assay methodology.

Statement of copyright

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When I was first applying to Durham my primary advisor Jo asked me, "Are you sure you understand the implications of this commitment," I told her I did. I can assure you now Jo I had absolutely no idea what I was getting myself into. Jo on the other hand knew exactly what she was getting into because she had my application and writing sample. Without her prompt and ruthless feedback coupled with support above and beyond what anyone could hope for from an advisor I

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Chapter 1: Introduction

I committed to working on this mandrill release project long before I became affiliated with Durham or had a desire to conduct a PhD. I came into primate conservation work because I was frustrated that humans, just one of 505 recognized primate species (Myers and Rowe, 2017), had destroyed around half of the forest that once existed and 30% of the remaining forests were fragmented, secondary forest or generally degraded (Collomb et al., 2000). Over-exploitation of natural resources, habitat fragmentation, intentional and unintentional introduction of non-native species, chains of extinction, and the pet and bush meat trades are causing population level and total species extinctions in wildlife worldwide (Diamond, 1984, Ceballos and Ehrlich, 2002). In just over 200 years the human population has increased from 1 billion to more than 7.4 billion (Population Reference Bureau, 2016) and as populations increase the human disturbance of wildlife habitat increases with it. Humans are increasingly dependent on fragmented plots of land and resources vulnerable to the effects of climate change (Ehrlich and Harte, 2015). Non-human primates are among the animals most affected by human activity (Jerozolimski and Peres, 2003). Despite the efforts of conservation organisations, most species of primate are in rapid decline and will likely soon be extinct (Estrada et al., 2017). I came into primatology an enthusiast who wanted to help fix this issue by putting a cage full of monkeys back in the wild, so they could be free. I came into academic research when I realised how dangerously naïve I had been and that it would take a PhD worth of research and support to do the project properly.

1.1 Primate conservation

How primate conservation is carried out over the next 50 years is critical. Many species of primate are facing extinction and the strategies conservation groups form and

the methods they use to implement them have the potential to reverse, stay, or cause the extinction of the species they are working with. The circumstances for wildlife are expected to worsen as a majority of the recent and projected future growth in the human population is happening in stressed ecosystems held by unstable governments which are already supporting stressed human and wildlife populations (Melorose et al., 2015). Congo, Equatorial Guinea and Cameroon are among the countries most affected and are expected to see 100-200% increases in the human population, while Gabon's population is expected to experience 50-100% increase between 2015 and 2050 (United Nations, 2015). These countries are host to several threatened or endangered species of primate. Conservation strategies in countries like these therefore need to be effective in holding off the impending extinctions under increasing human pressure.

It is illegal to hunt or own protected wildlife species without the appropriate authorisation (Nash, 2005). The Convention on International Trade in Endangered Species of Wildlife Fauna and Flora (CITES) regulates trade in wildlife specimens to ensure trade does not threaten the survival of those species (CITES, 2010). Government authorities confiscate animals from people who have them in their possession illegally (Klemm, 1993). Confiscated animals can then be euthanised, maintained in captivity for life, or released into the wild (IUCN, 2002).

Euthanising threatened, endangered and critically endangered species is morally complicated. Wildlife sanctuaries have arisen to rehabilitate and provide long term care for confiscated animals (Rosen et al., 2002). Long-term captive care is expensive (CITES, 2010). These sanctuaries are rapidly reaching, or have exceeded, their carrying capacity (Faust et al., 2011). Limited primate sanctuary capacity has made the release of primates into the wild a goal for many primate sanctuaries (Trayford and Farmer, 2013).

1.2 Translocation

The International Union for Conservation of Nature (IUCN) is a union of government agencies, states, and non-governmental organisations from around the world that address conservation issues at local, regional, and global levels (IUCN, 2002). IUCN currently defines "translocation" as the human-mediated movement of living organisms from one area for free release into another (IUCN/SSC, 2013). Translocation can be intentional or unintentional and is subdivided into multiple categories including introduction, re-introduction, and re-stocking (IUCN, 1987). Reintroduction is the reintroduction of an organism into an area where they no longer exist (Kleiman et al., 1994). Here I discuss conservation releases, which is the movement of an animal from one location for release in another location to benefit the population species, the ecosystem and not only the animals being released (IUCN/SSC, 2013). A primary aim of conservation translocations is to reduce the possibility that a single major event could threaten a species' survival by increasing the species' geographic distribution within its natural historical range (Swaisgood, 2010). Other aims of release projects are to re-establish a keystone species, increase or maintain biodiversity and or provide long-term economic benefit to local people (Kleiman et al., 1994). "Rescue/welfare" releases are a subset of conservation translocations involving the movement of primates from one area to another to save them from hazardous situations, mitigate conflicts with humans, releasing captive primates to ease pressure on sanctuaries or improve the individual's welfare (Soorae and Baker, 2002). In this thesis, I refer to rescue/welfare releases when using the term release, or release project.

In accordance with the IUCN Guidelines, translocations must be justified with clear objectives, identification and assessment of risks and have performance measures in place(IUCN/SSC, 2013). IUCN's recommended process for planning a release project is outlined in their Guidelines for Reintroduction and Other Conservation Translocations

(IUCN/SSC, 2013). To briefly summarise, IUCN recommends the process begins with assessing if a translocation is or is not a good option for the circumstance. Where translocation is appropriate a plan for how the release will be carried out should be generated, including the assessment and selection of the release area and the specific release strategy. The key considerations when setting up a release strategy are acclimatising the animals to the release area, the group composition and number of animals being released, pre-release behavioural training, seasonality, and specific methods including methods for keeping animals in the release area post-release (Soorae and Baker 2002). Biological and social feasibility, regulatory compliance, and resource availability for completing the project should then be assessed, followed by a risk assessment of the project. The project should have defined goals, objectives, benchmarks and monitoring protocols followed by an exit strategy. Post-release the viability of the group should be monitored, and the outcomes of the project should be shared.

1.3 Primate release

The Reintroduction Specialist Group (RSG) is a network within the IUCN that is using reintroduction as a tool for addressing the loss and restoration of biodiversity (IUCN, 2010). Release into the wild is costly and can endanger wild populations (CITES, 2010). Primate releases have often had low success rates (Mathews et al., 2004), taken unscientific approaches, not monitored release individuals and had low reporting and sharing of results (Fischer and Lindenmayer, 2000). As a result, IUCN produced the Guidelines for Nonhuman Primate Reintroductions (2002) as a practical set of methods for release project managers to follow. The IUCN guidelines for re-introduction suggest a set of principles to guide the set-up a release project (IUCN, 1998). IUCN does not condone releasing animals unless it is conducted in accordance with their Guidelines and is well planned and carefully executed (Soorae and Baker, 2002).

Since the conception of the IUCN Guidelines for re-introduction, which I refer to hereafter as “the Guidelines” (IUCN, 1998), they have been updated (IUCN/SSC, 2013) and guidelines have also been generated for great apes (Beck et al., 2007) and gibbons (Campbell et al., 2015). The IUCN Specialist Group has also published Global Re-Introduction Perspectives featuring reintroduction case studies in an effort to share the learnings and outcomes of re-introductions (Soorae, 2008, 2010, 2011, 2013, 2016).

1.3.1 Release strategy

The Guidelines separate release strategies into two categories: 1) soft release, where animals receive pre-release training, are housed temporarily in a pre-release enclosure at the release site, and receive post-release supplementary food and training (Herrero et al., 1986) and 2) hard release, where animals do not receive training or support before or after the release (Kleiman, 1989). Soft release is thought to be preferable to hard release, as it helps the animals adjust to the new environment (Soorae and Baker, 2002). The Guidelines include holding the animals in the transfer cage as compliant with a soft release (Soorae and Baker, 2002). Short no stays in enclosures may be adequate for some species, but species-specific recommendations derived from biological measures for the minimum optimum duration an animal or group of animals should spend in the pre-release enclosure would be useful to reduce potential negative consequences associated with the cumulative effects of stress to the animals concerned.

1.3.2 Failure points in release projects

Many factors may result in the failure of a release project. Some potential causes of primate release failure include availability of shelter, lack of provisioning, fragmentation of the group post-release, presence of hunters at the release site, aggressive indigenous populations and rearing in captivity (Konstant and Mittermeier, 1982). Additional considerations may include naivety to predators, failure to appropriately plan

for and fund the project, shifts in government policy, lack of support from local communities and the stress associated with release into the wild (Soorae and Baker, 2002).

The stress associated with being released is a major causal factor in the failure of release projects, as chronic stress negatively influences animal health, cognitive processes, and behavioural competence (Teixeira et al., 2007). Release comes with many stressors including sedation, transfer, finding food and coping with environmental and social stressors. The physiological effects of stressors can be cumulative (Aguilar-Cucurachi et al., 2010), but it is not well understood how long it takes animals to recover from the physiological stress of various stages of the release process. It is important to gain a better understanding of these relationships to improve release outcomes and decrease chronic stress-related mortality.

The IUCN recommendations to reduce the cumulative effect of stress on release subjects include holding animals in a pre-release enclosure and supplementing their diets post-release. Species and individuals within species have different responses to translocation due to individual biological, ecological and social needs (IUCN, 1998). These differences may mean that species and individuals differ in the optimum time spent in a pre-release enclosure. Having a standardised method for measuring and aggregating the biological responses of individuals within a species to measured durations in a pre-release enclosure will remove some of the guess-work from this process. How time in a pre-release enclosure affects an individual's biological stress response is unknown. Cortisol is the primary glucocorticoid in non-human primates and a key component of the physiological stress response (Davenport et al., 2006). Faecal samples can be used to measure glucorticoid cortisol metabolites (FGCMs) non-invasively as a biological marker for the stress response (Touma and Palme, 2005). Stress can also be measured behaviourally through self-directed behaviours (SDB) including auto-grooming, yawning,

body shake and scratching, which are displacement activities associated with stress in primates (Schino et al., 1988). SDBs can be used as non-invasive indicators to of emotional states in primate social interactions (Maestripieri et al., 1992) and are commonly used to measure anxiety (Manson and Perry, 1999). However, cortisol levels and SDB were not found to corelate in wild baboons (Higham et al., 2009) and in captive baboons cortisol levels increased with crowding but SDB did not (Pearson et al., 2015). Thus, non-invasive measures of corticoid metabolites may be a more appropriate method for measuring the incremental effects of environmental changes during a release project than SDB.

1.3.3 Release site assessment

According to the Guidelines a release site must be within a protected area in the primate's natural home range, support the animal's nutritional needs, and not expose the primates to human predation. Understanding the human dimensions of the potential release area is critical to site selection and the success of the release program (IUCN/SSC, 2013). The relationship with the local human population is critical for a project's success. If the animals pose a physical threat to the local community or interfere with their crops they will likely be seen as pests. Therefore, the assessment should provide an understanding of any hunting activities in the release area and ensure the proposed site is far enough from villages and farming operations to limit the probability of crop-foraging (Soorae and Baker, 2002). The site must also be approved by local authorities and be accessible. The most efficient way to determine whether a release site is suitable is to survey a proposed area to measure these aspects. Wildlife surveys give an understanding of size and distribution of wildlife populations in an area (Kuhl et al., 2008). They are also used to assess the intensity and distribution of regional threats in protected areas to provide measurable data for assessing current management strategies (Kuhl et al., 2008).

Distance sampling is commonly used for estimating population size and density in biological surveys (Buckland et al., 2010, Thomas et al., 2010). In distance sampling observers travel along a compass heading recording the perpendicular distance to any signs of wildlife detected from the centre of the transect line (Buckland et al., 2010, Thomas et al., 2010). Transects should be randomly located or systematically spaced with random start points (Thomas et al., 2010), rather than clustered around points of access or trails (Buckland et al., 2010). Indirect survey methods then use statistical calculations to infer animal density from dung and nests counts (Plumptre, 2000, Buckland et al., 2010).

1.3.4 Post-release monitoring

Post-release monitoring is essential to gauge the success of a release project (Soorae and Baker, 2002). Without monitoring there is no way to know if an animal survived long enough post-release to establish itself in the release environment, reproduce and lead to the successful colonisation of an area. Monitoring also provides insight into how released animals behave in the wild, informing future releases and providing insight into species-specific behaviour in a free environment (Soorae and Baker, 2002).

Very High Frequency (VHF) or Ultra High Frequency (UHF) radio-collars can be fitted to an animal allowing it to be tracked remotely via with a receiver and earphones (Honess and Macdonald, 2011). Global positioning system (GPS) collars track an animal's movements using satellites and either store the information on the radio-collar or transmit the animals location to an external device (Honess and Macdonald, 2011). In densely forested environments where visibility is limited and with species who have long day journey lengths post-release monitoring requires the aid of a radio or GPS collar.

GPS collars are widely used in wildlife studies (Tomkiewicz et al., 2010) and make it possible to collect ranging data on release candidates remotely. Remote tracking reduces potential risks for the animals and observers inherent in placing a human close to

a wild animal (Williamson and Feistner, 2003). However, animal behaviours (foraging, sitting, climbing, resting), topographic features, and forest density may affect the collar's ability to connect with satellites and obtain a "fix" of the animal's location (Rempel et al., 1995). Missed fixes introduce a bias in ranging data that must be accounted for if it is systematic. Tests measuring the effects of collar position on fix rate have been performed with empty collars (Bêlant, 2009). Collars may, however, interact with the animal wearing them, improving the fix rate. Thus, previous tests may have overstated the effect of collar position on fix success, and further tests of the effects of collar position and fix rates in various habitat types are needed. It is important to understand and correct for biases introduced by the GPS collars because they can lead to skewed sampling across a geographic area and inaccurate range estimates (Frair et al., 2004).

1.3.5 Selecting release candidates

The Guidelines recommend several considerations for the selection of release candidates including: is the population from a stock that is demographically and genetically appropriate for release; has captivity introduced behavioural abnormalities; have the animals been given sufficient training opportunities; is the release group structure appropriate; do the animals have physical mobility issues or a transmittable disease; and are the animals human oriented and a danger to locals. Other considerations may include the social cohesion of the release subjects (Wimberger et al., 2010) and the presence of pregnant females or females with dependent young (Peignot et al., 2008).

1.3.6 Mandrill behaviour and ecology

The genus *Mandrillus* includes two species of African monkey, the mandrill (*Mandrillus sphinx*) and the drill (*Mandrillus leucophaeus*). Although their superficial morphology is similar to that of *Papio*, examination of skeletal anatomy shows mandrills are more closely related to *Cercocebus* (Fleagle and McGraw, 1999). The distribution of

the two species is split by the Sanaga River in Cameroon with the mandrill found to the south of the river in Cameroon, Equatorial Guinea, Gabon and Republic of Congo (Figure 1.1) and the drill found north of the river in Cameroon and Nigeria (Grubb, 1973). Mandrills are highly sexually dimorphic and males have 3-3.4 times the body mass of females (Setchell et al. 2001; Hill, 1970). Males of both species have colourful rumps, short tails and long canines; mandrills have bright blue and red faces and drills have entirely black faces (Grubb, 1973). There are two distinct subspecies of mandrills isolated from one another by the Ogooué river in Gabon (Figure 1.1).

Both male and female mandrills can develop bright red and blue colour on their faces but subordinate males have reduced development of secondary sexual traits (Setchell and Dixson 2001a). This reduced development may relate to avoiding inter-male conflict (Setchell and Dixson 2001b; Setchell and Wickings 2005). Male mandrills experience a growth spurt when aged 7 years, peripheralise from their natal group at 6-8 years and are fully grown at 9-10 years (Setchell and Dixson, 2002, Setchell, 2003). Adult male mandrills have large canines averaging 45 mm in length, which can inflict serious injury (Leigh et al., 2008). Mandrills produce a secretion from a gland in their chest which they rub on objects in their territory, presumably to signal their presence and aid in mate selection (Setchell et al. 2010; Setchell et al. 2011).

Dominant male mandrills are more likely to have offspring than subordinates (Charpentier et al., 2005) but young males of 3.8 years or more sneak copulations (Setchell, Charpentier, and Wickings 2005). Males mate-guard high-ranking females who are more likely to be fertile and females base their selection of males on genetic dissimilarity and bright colour (Setchell and Huchard 2010; Setchell and Wickings 2002; Setchell 2005; Setchell et al. 2010). Females also form coalitions to counteract the size difference between them and males and exert control in the group (Setchell et al., 2006). Under semi-free ranging conditions, females show their first sexual swelling at

approximately 3.6 years, begin reproducing around 4.7 years and have a seasonal peak in mating between July-September (Setchell and Wickings 2004; Setchell et al. 2002).

Little is known about the behaviour of wild mandrills because they are difficult to both habituate and follow (Harrison, 1988). Mandrills live in multi-male multi-female groups, with most adult males living on the periphery or having only a seasonal presence (Abernethy et al., 2002). Ecological studies have established that mandrills live in both undisturbed and disturbed forests and are primarily terrestrial (Hoshino, 1985, Garcia and Jesus, 1997) with a preference for Marantaceae and rocky forests (Rempel et al., 1995). They eat a frugivorous diet, supplemented with insects, seeds, pith, flowers, root, and fungi (Harrison, 1988, Rempel et al., 1995). Estimations of day journey length based on observer follows range from 150 m to 15 km (Harrison, 1988, Rempel et al., 1995). Radio-collared captive-born mandrills had a day journey length of between 1.3 ± 0.9 km when released (Peignot et al., 2008). Collared wild mandrills had a day journey length of between 4.9 ± 1.9km (White et al., 2010).

1.3.7 Mandrill conservation status, threats, and conservation strategies

IUCN lists mandrills as Vulnerable and the United States Fish and Wildlife Services lists them as Endangered (IUCN 2012, USFWS 2013). Mandrills are hunted heavily and need protection (Abernethy et al., unpublished IUCN status update, 2019). Mandrill populations are difficult to survey because they travel quickly, live in isolated and difficult terrain, have large ranges, do not leave nests and their faeces deteriorate rapidly (Imong and Okeke, 2009). Transect surveys did not work well for counting drill groups (Astaras, 2009). Vocalisations are the most common indication of mandrill presence (Imong and Okeke, 2009). At the time of this study no vocalisation-based survey has been conducted and population size across the mandrill range is unknown.

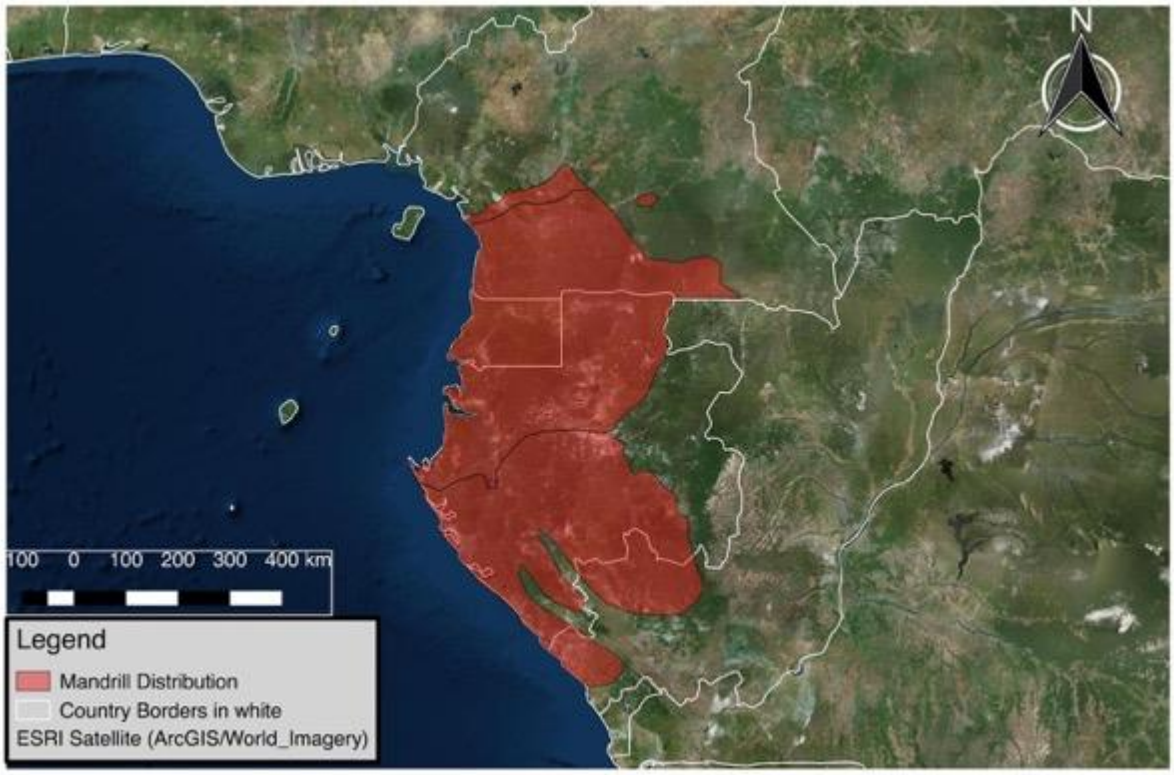

Figure 1.1 Distribution of mandrills in Central Africa. Mandrill distribution shown in red. Country borders shown in white. Black line through the centre of the red area follows the Ogooué river and marks the boundary between the two subspecies of mandrill. IUCN Red List of Threatened Species. Version 2012

1.3.8 Mandrill release

The Centre International de Recherches Medicales de Franceville (CIRMF), a medical research facility, conducted the only mandrill release published at the time of this study (Peignot et al., 2008). CIRMF conducted the release to avoid overcrowding and degradation of their colony's captive environment. CIRMF acknowledged that IUCN does not recommend release projects to dispose of surplus animals but felt release was appropriate in their circumstance for the following reasons: 1) Mandrills are vulnerable to extinction; 2) Mandrills can survive in a broad range of habitats and the captive colony had many behavioural and environmental training opportunities in their enclosures; 3) Mandrills are a socially adaptive species and the release group was selected for cohesiveness; 4) Food plant species were abundant enough at the release site to support

the historical wild primate population. That population has declined leaving excess food resources available.

CIRMF released 36 captive-bred mandrills into Lekedi Park, Gabon, with 33% mortality in the first year. The release was a partial soft release that included the holding the animals for 12 days in a pre-release enclosure located in savanna adjacent to a forested area and the use of VHF tracking collars on six of the animals. The animals were provisioned twice a day in the enclosure but were not provisioned during the 8 weeks following the release. After 8 weeks the animals were provisioned regularly as needed. Additional concessions were made to support pregnant females and account for seasonal fruit availability. The release group was joined by a wild adult male mandrill in the third year (Peignot et al., 2008) but the only documentation of how this influenced the group's behaviour was a brief note on a shift in ranging (Peignot et al., 2008). It is likely the captive-born mandrills who affiliated with the wild mandrill benefited from his knowledge of the forest and predator awareness, as previously demonstrated in golden lion tamarins (Stoinski et al., 2003).

In 2009, Tchimpounga partnered with H.E.L.P. to conduct several small hard releases of mandrills in Conkouati-Douli National Park, Congo. Tchimpounga was given the confiscated mandrills with the expectation they would be released where possible. Tchimpounga did not have the funding or space to permanently house the mandrills. The justification for the release was as follows: 1) Mandrills are vulnerable to extinction; 2) They believed mandrills were not territorial and that males would be accepted into wild populations; 3) The release site was approved by wildlife authorities; 4) Chimpanzees had been released into the area successfully; 5) The site was within the mandrill's natural distribution; 6) Eco-guards patrolled the area regularly; 7) The presence of JGI staff and funding would provide additional support to the park; 8) The confiscated animals were left in their care for the purpose of being released.

JGI transferred the mandrills to a release site in the morning and released them in the afternoon at a site where supplemental food was readily available. JGI intended for the releases to be soft and to provide supplemental food then track the animals for 6 months but the mandrills left the site separately within hours or days after release and were not seen again. It was not possible to provide support for or gather further data on the animals because they were not collared. No further data are available for the initial JGI releases.

Both projects followed some but not all of the Guidelines. The CIRMF project knew the health status of the animals, held them in a pre-release enclosure, collared key individuals, included experts in the study design, followed up post-release using VHF tracking, provided post-release nutrition and published the results of the release. It is difficult to say where JGI complied with the guidelines because the results were not formally published.

The key lessons from the projects are that animals held in an enclosure on a savanna for 12 days and animals released from pet carriers both left the area in hours or days even when food was present. The use of radio-collars is essential for monitoring the progress of mandrills post-release. The authors of the CIRMF release recommend that future releases conduct a soft release, avoid releasing pregnant females or those with dependent infants, and do not overestimate the benefits of social and ecological pre-release experience. (Peignot et al., 2008).

The release rationale provided by the Jane Goodall Institute for this release project are as follows: 1) It will lead to the increased protection of the species living within the research area. 2) It will return wild-born mandrills to the wild. 3) It will raise awareness of the plight of mandrills in the Congo. 4) The release methods and results will be published to inform future release projects and provide insight into mandrill behaviour

in the wild. 5) Release of confiscated animals back into the wild is expected by the Congolese government.

1.3.9 Narrative background on the study design

Prior to my arrival in the Congo JGI had conducted a survey and selected an area to conduct the release. While planning this PhD we were unsure whether the release would occur, due to instability in the region and the general challenges of primate release. My supervisors suggested I form my initial study questions around steps that were likely to occur during the preparation for the release rather than focus on the mandrill release itself, so I would have sufficient data to write my PhD if the release did not happen. I formed questions around the function of the radio-collars we would be using for the release and a behavioural study intended to aid in the selection of candidates for release. This was a prudent strategy. When I arrived in the Congo our permission to conduct the release in the selected area was revoked and the financial crisis made funding the project more difficult than expected. We could not purchase the collars in the timeframe we had hoped. Significant challenges of this magnitude and greater happened frequently throughout the project.

I did not include some components of my research formally in this thesis because of time constraints. They were part of my learning process and our due diligence and informed our decisions along the way. These components included: a pre-release behavioural study intended to aid the assessment of release candidates by monitoring their social and self-directed behaviour before during and after transfer to a new enclosure; collection of fingernail clippings and hair samples to include in the hormone study and morphological data from the mandrills during the health checks; post-release behavioural data on feeding and affiliation. There are no resources available to complete these studies.

I was not actively involved in some aspects of the project that were important and fulfilled requirements of the guidelines. These activities included multiple wildlife and botanical surveys in parks and reserves in the Republic of Congo prior to my involvement in the project, leading JGI to decide on Conkouati-Douli National Park (Conkouati) as a release site; a survey to assess attitudes and beliefs in the villages near the study area; and a community awareness programme in the villages near the release area (Appendix A).

1.3.10 Thesis structure

This thesis consists of seven chapters. In this chapter I have introduced the state of wildlife conservation, release into the wild and specifically nonhuman primate release and introduced the study species. In Chapter 2 I present the general methods of the project. I first broadly describe the study area and the details of the pre-release survey we conducted to select the release site. Next, I introduce the study animals. Finally, I briefly describe the soft release methods we used during the project including the pre-release enclosure, radio collars, and the post-release feeding and observation programmes. Chapter 3 details the series of tests we conducted with stationary GPS collars to understand their function in the release area and formulate predictions about how the collars would function post-release. Chapter 4 examines the effect of an animal's use of three-dimensional space on radio-collar function. Chapter 5 investigates changes in faecal glucocorticoid metabolites (FGCMs) levels during the release process. We monitored FGCMs because they are a non-invasive biological proxy for stress widely used as an indicator of an animal's well-being in the field of wildlife endocrinology. In Chapter 6 I provide a summary of the project and a synthesis of the results and key findings.

Chapter 2: General methods

> *"Poorly planned or executed releases or (re-) introduction programmes are no better than dumping animals in the wild and should be vigorously opposed on both conservation and humane grounds"* (IUCN, 2002, p. 14)

2.1 Principles of re-introduction

In this release we sought to follow the International Union for Conservation and Nature (IUCN) Guidelines for Non-Human Primate re-introduction (Soorae and Baker, 2002) as closely as possible. We did this by consulting the Guidelines and members of our multi-disciplinary team (Appendix B) throughout the release process.

2.2 Aims

The primary aim of the project was to successfully release the mandrills housed at the Tchimpounga with no mortality. We hoped to establish a group that bred successfully and reared their young to populate the release area and fortify existing groups in the park. The secondary aim was to conduct studies throughout the release that would inform wildlife conservation practices and improve welfare in release programs and translocations. To do this we established a multidisciplinary team with the relevant skillsets and affiliations required to successfully reach our aims and incorporated additional members when necessary during the process. We refined the aims, objectives and timeframe of the project and made a contract between the project partners. We outlined the expected contributions and outcomes of the project and the project partners

in a memorandum of understanding between the Jane Goodall Institute and Durham University. We later added the hormone study and created an agreement between the Jane Goodall Institute, Durham University and Disney outlining three resulting publications and the associated authorship. The project faced many challenges that caused us to update our timeline multiple times. The initial goal during my time with the project was to conduct the release in June 2012-May 2013. We released the first group of animals in March of 2014.

2.3 Study locations

We conducted fieldwork in two parks in Republic of Congo (Congo) (Figure 2.1). The animals were rehabilitated at the Tchimpounga Reserve in southern Congo (UTM 32 M 814303 9500175) and released into the Conkouati-Douli National Park (UTM 32 M 774300 9567971).

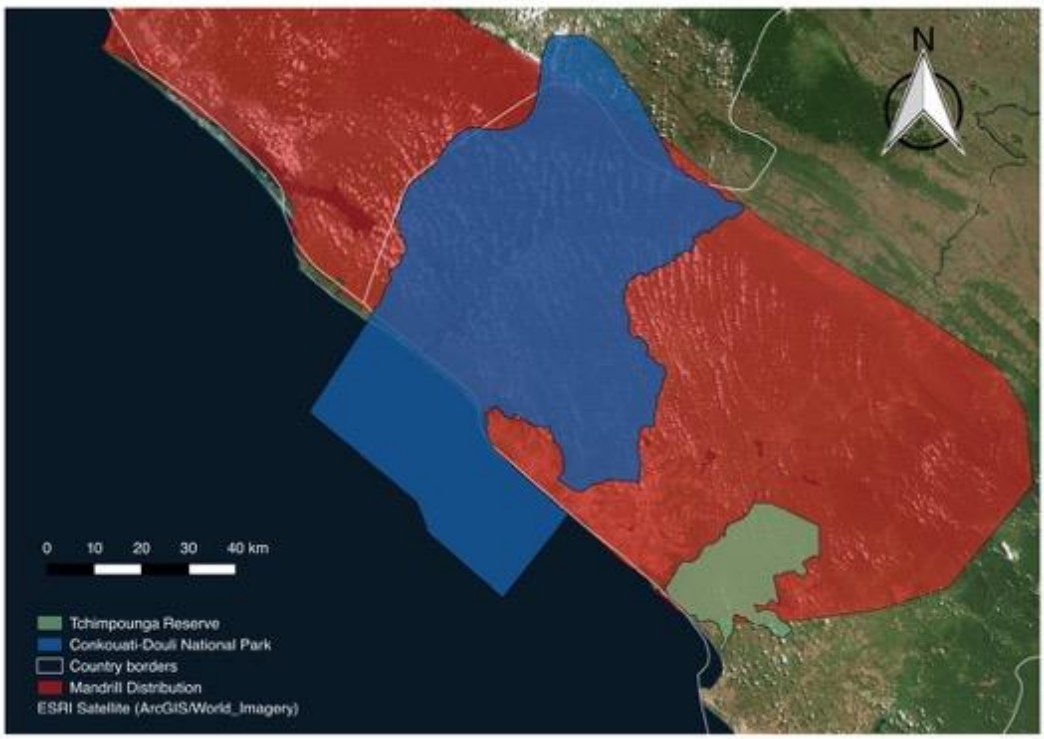

Figure 2.1 Map of south western Gabon and Republic of Congo showing Tchimpounga Reserve in green on the right and Conkouati-Douli National Park in blue on the left. Red shows the mandrill distribution from IUCN. Country borders are shown in white.

2.4 Republic of Congo

The Republic of Congo is located in central Africa. The economy is driven by extractive industry and production of agricultural goods (CNSEE, 2009). China, Europe, Lebanon, Malaysia and Singapore are all heavily invested in extractive concessions within the country (Tessa et al., 2012). The human population is growing with trends towards urbanisation (UNFPA, 2016). Bushmeat is widely consumed, especially by urban populations, and current trends indicated the practice will lead to the disappearance of a majority of the species of wildlife in the country (Mbete et al., 2011). The country has 10 protected areas ranging in size from 94-1,354,600 ha with a total of 3,752,00 ha of protected area (UICN/PACO, 2012).

2.5 Tchimpounga Reserve

Tchimpounga Reserve (Figure 2.2) was founded in 1995 as a chimpanzee sanctuary. It is an area of 55,526 ha approximately 33 km from Pointe-Noire. The primary source of funding of the reserve is the Jane Goodall Institute. The reserve recently acquired three islands and are in the process of transferring most of the ~160 chimpanzees housed at the sanctuary to the islands. In addition to chimpanzees Tchimpounga also regularly receives other species of wildlife in need of rehabilitation and care when they are confiscated by Congolese authorities with the expectation that they will rehabilitate them, care for them, and release them into the wild where possible. During this study Tchimpounga housed chimpanzees (*Pan troglodytes*), spot-nosed guenons (*Cercopithecus nictitans*), moustached guenons (*Cercopithecus cephus*), mandrills (*Mandrillus sphinx*) and African grey parrots (*Psittacus erithacus*) confiscated by Congolese authorities.

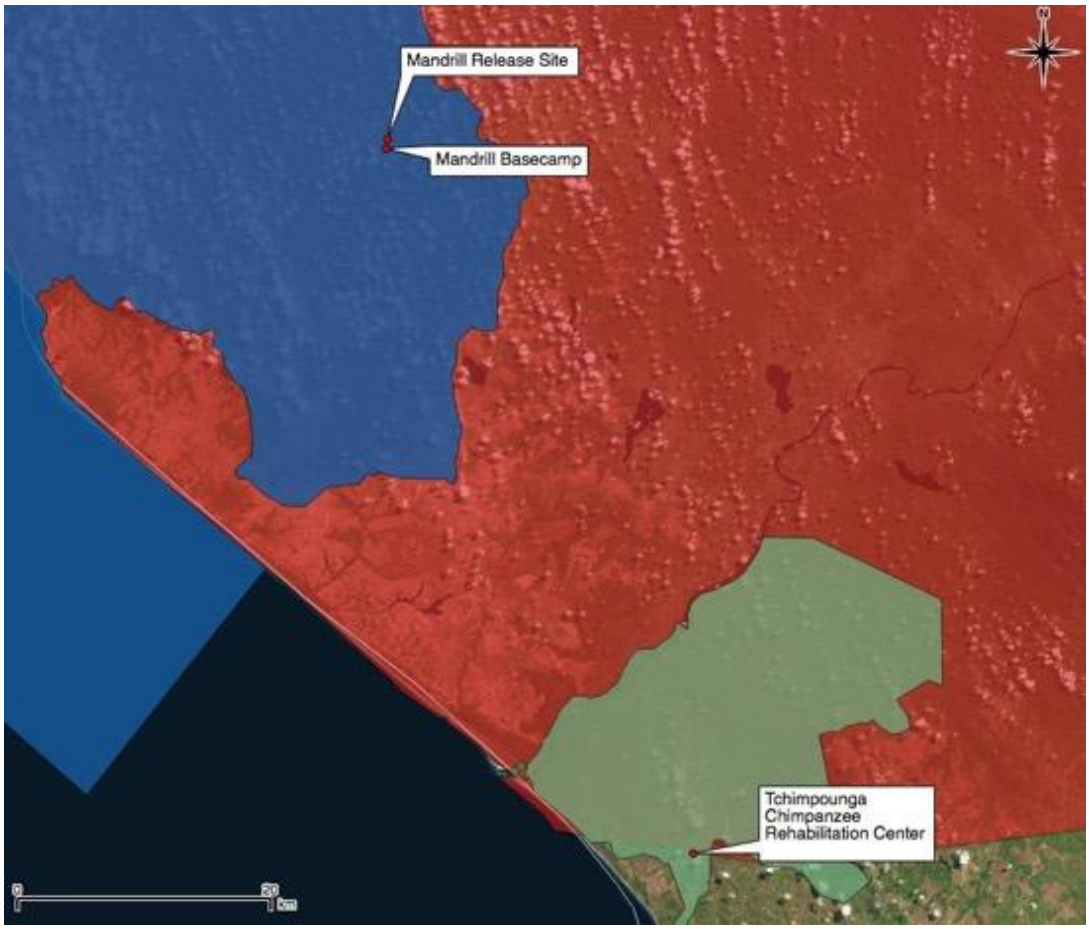

Figure 2.2 Map of the release site and base camp in relationship to Tchimpounga. Conkouati is in blue on the left and Tchimpounga in green on the bottom right. Red denotes the mandrill distribution according to IUCN

2.6 Conkouati-Douli National Park

Conkouati-Douli National Park (Conkouati) (Figure 2.2) was founded by the Congolese government in conjunction with the Wildlife Conservation Society (WCS) in 1999. The park is near the Gabonese border at latitude S3 59-44 8 and longitude E11 19-16 01 (UNESCO, 2012). It is the third largest of 10 parks in Congo and home to a wide variety of important flora and fauna facing pressures from people domestically and internationally (UICN/PACO, 2012). Approximately 7,000 people live in mixed-use buffer zones surrounding the park (UICN/PACO, 2012). A major challenge in the park is that 80% of the human population aged 16-25 years are unemployed and bushmeat hunting is an ongoing problem (UICN/PACO, 2012). The WCS attempted to work with

local authorities to regulate hunting in the park with ecoguards but withdrew their presence from the park in 2018 after the conclusion of this study. The park has an existing mandrill population, but no reliable mandrill population estimates because transect surveys are not useful for assessing mandrill populations (Section 2.11). There is however a record of carcasses confiscated in Conkouati between November and May of 1995 and 2006. The data report the quantity of small primate carcasses confiscated decreased by >95% over that period and counts of integrally protected species decreased by >89% (Vanleeuwe, 2012). The declining numbers of bushmeat confiscations in conjunction with a threefold increase in the quantity of snares removed by the guards (Figure 2.3) suggest a decreasing wild population of primates coinciding with increased human pressure, although mandrill carcass counts in the park show no particular patterns over time (Figure 2.4). The report does not control for ranger effort during the study, so it is not possible to know if the variation is affected by changes in ranger behaviour. To assess the level of the human threat in the release and the suitability of the habitat for the mandrills we conducted a line transect survey of the proposed release zone.

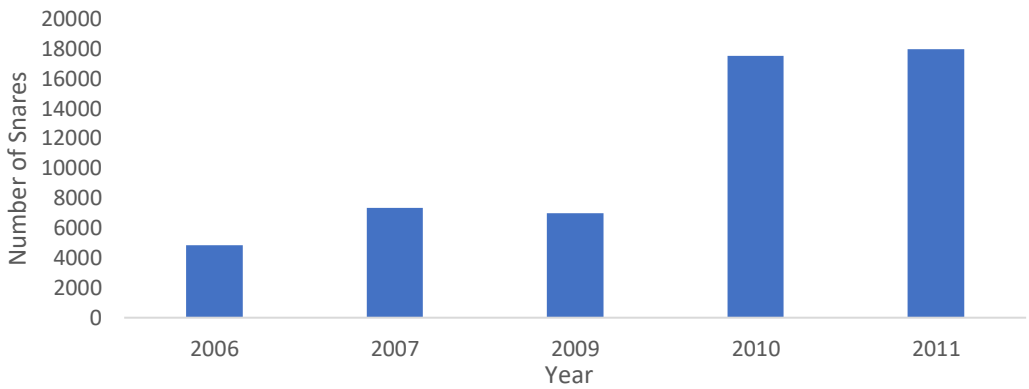

Figure 2.3 Number of snares found in Conkouati-Douli National Park per year by forest rangers between 2007 and 2011. Unpublished data compiled by WCS. Guards patrol the park and turn in the snares they find at the end of their mission.

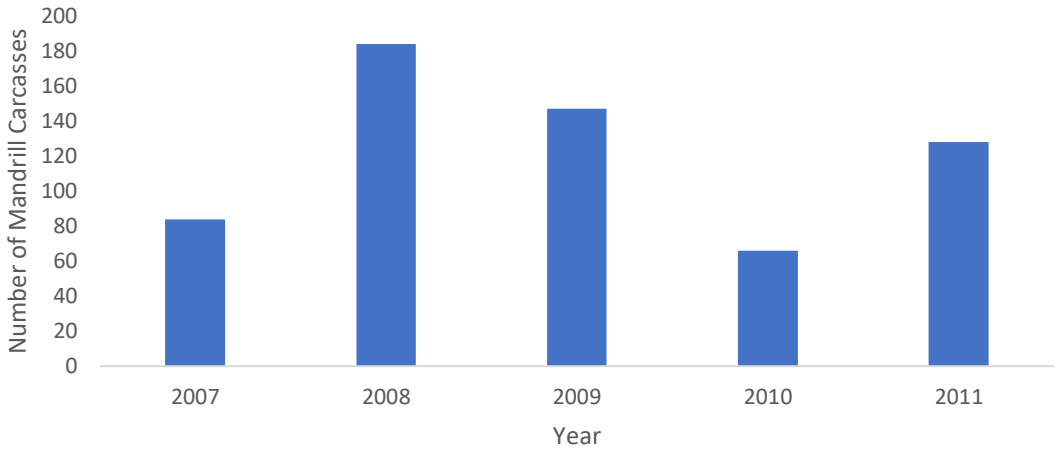

Figure 2.4 Numbers of mandrill carcasses confiscated by forest rangers in Conkouati-Douli National Park by year between 2007 and 2011. Data compiled by WCS Congo. Eco-guards conducted vehicle searches at the entrances to the park and documented the bushmeat they confiscated. Eco-gards also patrolled the park and kept records of the confiscated meat and snares (Vanleeuwe, 2012)

2.6.1 Study animals

The mandrills who participated in the study were bushmeat or pet trade orphans confiscated by, or with the approval of, the Congolese environmental law enforcement agency, the Ministère de l'Economie Forestière. After confiscation, they were transferred to Tchimpounga for long-term care. We quarantined all animals at the ranger station in a separate area of the park for >30 days and screened them for communicable diseases prior to integrating them into a group. Tchimpounga was responsible for 18 confiscated mandrills and three animals born during the study (total: 21 individuals; 14M/7F). Only the animals born during the study were known to be related to other members of the group. The initial study group (Table 2.1) was composed of five mandrills (2 females, 3 males) housed together in a stable group for over a year, aged approximately 4-11 years old. Mandrills that arrived later were added into the program where appropriate.

2.7 Assessing the suitability of the release stock

We did not do a genetic analysis of the mandrills in the release group as recommended by the Guidelines on the grounds that all the mandrills involved in the programme were confiscated in Congo, and therefore it is highly unlikely any of the animals are from a different subspecies. The two proposed subspecies of mandrill are separated geographically by the Ogooue River which runs through the middle of Gabon (Telfer et al., 2003). The portion of mandrill range in Congo is located south of the river.

The Guidelines provide several suggestions to determine if the release candidates will fulfil the needs of the taxon while maximising the survival prospects of the release individuals (Soorae and Baker, 2002). Wild mandrills live in multi-male multi-female groups that range in size between 15 to 845 individuals (Harrison, 1988, Abernethy et al., 2002). In captivity, lower ranking individuals are forced to be close to dominant individuals. In an attempt to meet the needs of the species and release a multi-male multi-female group that was likely to remain together post-release, we conducted a pre-release behavioural study which did not fall within the scope of this thesis. Based on the results of the study we did not include an adult male from the original group because he was aggressive towards staff. Aggressive individuals pose a threat to staff post-release where there is no barrier between the animals and those following the animals. This animal was particularly dangerous because his original owner hid treats in various pockets and encouraged the mandrill to climb on him to search for them. The staff also had a particular fear of this animal because he was aggressive towards them during feedings. The combination of the trained pocket searching behaviour and the staff fear caused the management to decide not to include the animal in the release.

During the study we received two additional animals we deemed inappropriate for release because one was less than a year old and the other had trouble walking and was aggressive towards observers.

Table 2.1 Mandrills involved in the release programme. Mandrill name, sex, ID code, approximate age at release, release group, date transferred to the pre-release enclosure, release date, days in pre-release enclosure, released with or without collar.

Mandrill Name	Sex	ID	Approximate age at release in years	Release Group	Transfer date	Release date	Days in Pre-release enclosure	Released with collar
George	Female	GEO	6	1	20.8.13	5.3.14	197	Collar
Dominque	Female	DOM	5	1	20.8.13	5.3.14	197	Collar
Kiki Mpaka	Male	KM	12	1	20.8.13	5.3.14	197	Collar
Obia	Male	OB	7	1	20.8.13	5.3.14	197	Collar
Madol	Male	MAD	5	1	20.8.13	5.3.14	197	Collar
Kiki Tchiali	Male	TCH	9	2	19.2.14	-	17	No collar
Gagaga	Male	GAG	9	2	19.2.14	12.3.14	21	Collar
Gayard	Male	GAY	4	2	19.2.14	12.3.14	21	No collar
Mobote	Female	MOB	4	2	21.2.14	12.3.14	19	No collar
Veiu de Loin	Male	VDL	6	2	21.2.14	12.3.14	19	Collar
Suzo	Male	SUZ	3	3	13.11.14	28.1.15	76	No collar
Nzelly	Female	NZ	3	3	13.11.14	28.1.15	76	No collar
Kento	Female	KEN	3	3	2.12.14	28.1.15	57	No collar
Brek	Male	BRK	4	3	2.12.14	28.1.15	57	No collar
Egeuo	Male	EGU	4	3	2.12.14	28.1.15	57	No collar
Disney	Female	DIS/BD	-	-	-	-	-	No collar
Mark	Male	MAR	-	-	-	-	-	No collar

2.8 Tchimpounga enclosure

At Tchimpounga the mandrills were housed in three enclosures with corrugated tin roofs, walls 3 m high, chain-link sides, dirt floors and a 1 m concrete brick foundation around the perimeter (Figure 2.5; Figure 2.6). The enclosures were divided into two separate areas by a chain link fence above a 1 m brick foundation and a sliding door. Each enclosure had diagonal structural elements passing through the centre and fire hose or hammocks as enrichment (Figure 2.7). There were also platforms made with planks and perches constructed from 4x4 beams in the corners to allow the mandrills to leave the ground. Only two of the enclosures were constructed when we sampled faeces sampling at the sanctuary and these were connected by a chain-link corridor (Figure 2.8) passing through the space where Enclosure 3 was later built. The total area of Enclosure 1 was approximately 30 m^2 and the total area of Enclosures 2 and 3 approximately 43 m^2. The enclosures were connected by a small raceway (Figure 2.9).

The enclosures were in alignment with the Pan African Sanctuary Alliance requirements of a three-dimensional holding facility (Farmer et al. 2009). The enclosures provided shelter from wind, rain and sun and were sturdy and well maintained. Each space was equipped with water bubblers to provide constant fresh water and was comprised of two adjoining rooms. The animals had sufficient space vertically and horizontally to avoid aggression. The mandrill enclosure at the sanctuary did not meet the space requirements set by the Global Federation of Animal Sanctuaries GAFA for Old World primate space requirements (GFAS, 2013); but without funds to build a larger enclosure the alternative would be to leave the animals in much worse conditions or euthanise them. To compensate for the lack of space the sanctuary supplied varied substrates (hay and dirt), hammocks and fire hoses as non-nutritional enrichment (Figure 2.7).

Figure 2.5 Far left enclosure front view

Figure 2.6 Front view far right enclosure

Figure 2.7 Corner platforms, swings, hammocks and bamboo enrichment structures

Figure 2.8 The original chain-link corridor linking Enclosures 1 and 3

Figure 2.9 Permanent corridor connecting Enclosures 2 and 3

JGI staff fed the captive mandrills approximately 2 kg each per day (Figure 2.10). The mandrills received a combination of seasonal fruit, leafy greens, rice, and sweet potatoes every morning and afternoon. JGI staff shut the sliding gate between the two sides of each enclosure during feeding to allow low-ranking animals access to food. Food was placed around the enclosure and on two feeding platforms approximately 40 cm x 70 cm each. Placement of the food encouraged behaviours such as climbing and jumping and made it difficult for more dominant individuals to monopolise access to the food.

Figure 2.10 Example of daily provisions with a mix of available produce and wild fruit.

During pre-release behavioural observations the mandrills often reacted aggressively towards observers who were standing close to one another or who passed objects to one another. The dominant female was particularly aggressive when two observers were near one another and appeared to focus her aggression on the person she perceived to be less dominant or less threatening. In an informal video-recorded test we observed that she was not aggressive when two staff were standing near the cage separately; however, when I approached, she became aggressive, head-bobbing and grunting at the other two observers. She only showed this type of aggression towards me when the veterinarian was present with a dartgun. Based on these findings we set the protocol that observers should not stand near one another or pass objects to each other in the presence of the mandrills.

2.8.1 Habitat requirements and release site selection

The Congolese government gave the project approval to conduct the release on the eastern boarder of the park. The Guidelines state that in preparation for a release it is necessary to assess the appropriateness of the site and its habitat for the release subjects (IUCN, 2002). Conkouati is in the mandrill's natural distribution and the area we were approved to assess was in a protected area. We conducted a pre-release survey in accordance with these guidelines.

My field team and I conducted the survey in a part of Conkouati approved by MEF for the mandrill release. We created the survey design with Distance survey design engine (Thomas et al., 2010). We selected a transect length of 1 km to make the information collected in this survey directly comparable with data collected in earlier prospecting surveys conducted by JGI. The study area consisted of 30 km^2 and 58 transects. We measured the presence of great apes, large to medium mammals, and humans and human activity in the study area using line transects and Distance (Thomas et al., 2010). The observation sheets (Appendix C) were adapted from sheets created by Fiona Maisels and Mike Fay at WCS (Appendix D).

We conducted the survey with armed ecoguards because there were hunters in the area. The total study area was 15 km wide and 20 km long. We divided the area into two survey zones, A and B (Figure 2.11). We conducted a higher resolution survey of Zone A, as it was further from villages and roads.

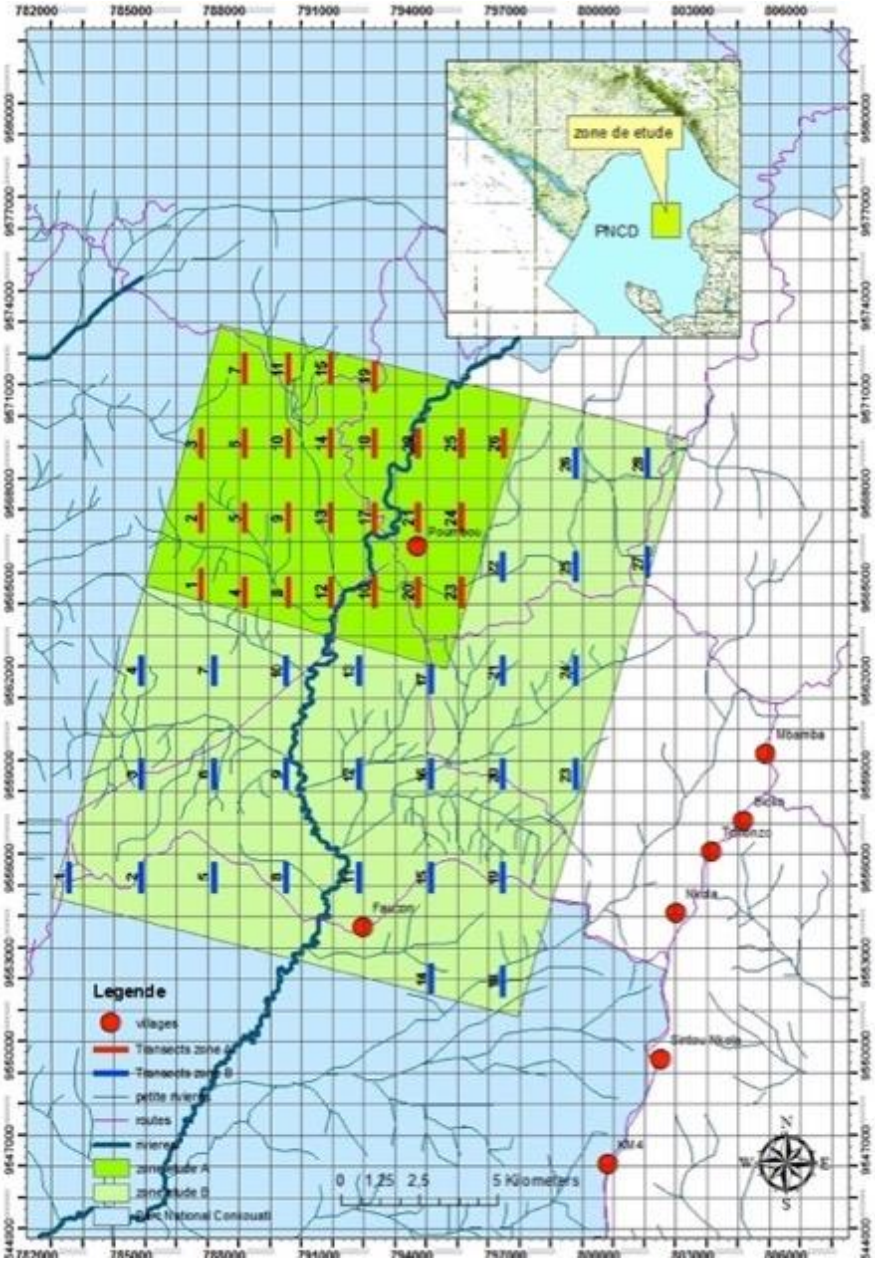

Figure 2.11 Map of pre-release survey transects in Conkouati-Douli National Park. Each red and blue vertical line represents a 1 km transect. The grid is in the UTM quadrate system and each square is 1 km2. The light blue is the fully protected area in the park. The white area is in the inhabited buffer zone. The darker green area with the red transects is zone A and the lighter green area with the blue transects is zone B. The dark blue line running down the map is a river large enough to pose a natural barrier for mandrills. The red dot near the river towards the top of the map is camp Pounbou and the red dot nearest the lower half of the map is camp Falcon.

2.9 Survey findings

At the time of the study the survey results had not been analysed for density so we used them as an indicator of presence/absence of animals and human signs. Large areas of the southern part of Zone B were composed of swamps, secondary forest and savanna. Zone A was composed almost entirely of continuous old growth forest. We found evidence that the habitat in both Zones A and B supported large mammals including elephants and chimpanzees (Figure 2.12). During the survey, we had two auditory contacts with mandrills and encountered multiple groups of poachers. The auditory contacts happened in Zone A showing that wild mandrills were currently using that area. The Southern part of Zone B had less human presence and no evidence of an existing population of wild mandrills. In addition to the human sign data we collected during the transects the guards also collected data from the poachers they stopped and interviewed as a part of their duties for the park. These interviews provided the additional information that people living outside the park hunted in both Zone A and Zone B.

a)

b)

c)

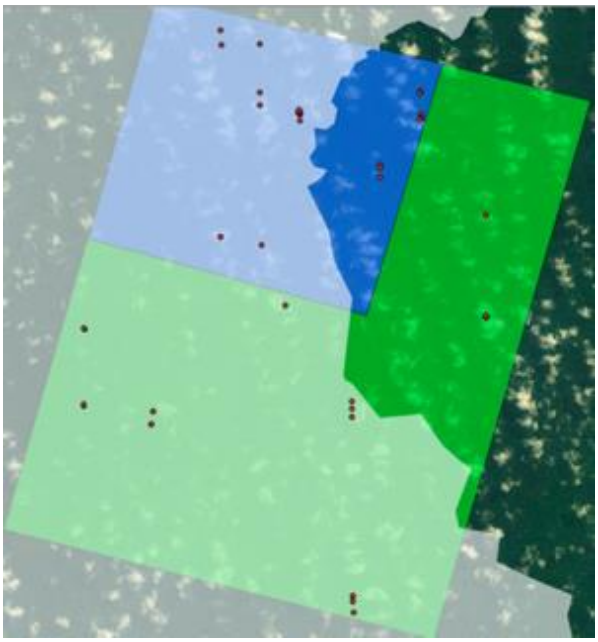

Figure 2.12 Transect results from the pre-release survey conducted in Conkouati-Douli National Park. Each dot indicates a track or sign encountered on a 1 km line transect. a) human signs, b) elephant signs, c) chimpanzee signs

Neither of the auditory contacts we had with mandrills during the survey occurred on a transect. Our findings that line transects are not an effective tool to survey *Mandrillus* are consistent with earlier studies (e.g. Astaras, 2009). Nevertheless, the contacts confirmed that mandrills were present in Conkouati. I also saw groups of mandrills on multiple occasions while working with a chimpanzee release project in the park in 2009.

The survey results indicated that the southern portion of Zone B was more appropriate for the mandrill release because of the lesser human presence and the lack of a wild mandrill population.

2.10 Site selection

We selected Camp Falcon as the release site because it was the furthest (~12 km) accessible point from local human settlements in our approved release area. The release area had abundant water, no signs of an existing mandrill population and fewer human signs than the rest of the surveyed area. JGI conducted a separate survey and found the surrounding area had an abundance of fruiting trees (Appendix E). The site was at the centre of a long-term logging concession. The concession was no longer active and the secondary forest had regenerated to the extent that it supports other species of primates. The base camp was placed at the junction of the old logging road and the Numbi river. The release site was located approximately 900 m north east of the base camp at an abandoned logging camp. Hunting is still an issue in the park and JGI funded additional WCS ecoguard patrols in the release zone to reduce illegal human activities during the project. The pre-release surveys and our presence at the release site led to additional patrols in the release area. As a result of working with the local government and the increased ecoguards hunting in the release area was discouraged and did not affect our release animals. We found no active snares on our transects (Figure 2.13) during the project, however at night we heard occasional gunshots and poachers crossed through the release area frequently when returning from hunting expeditions deeper in the park. We set up our primary camp to the south of the release site (Figure 2.14).

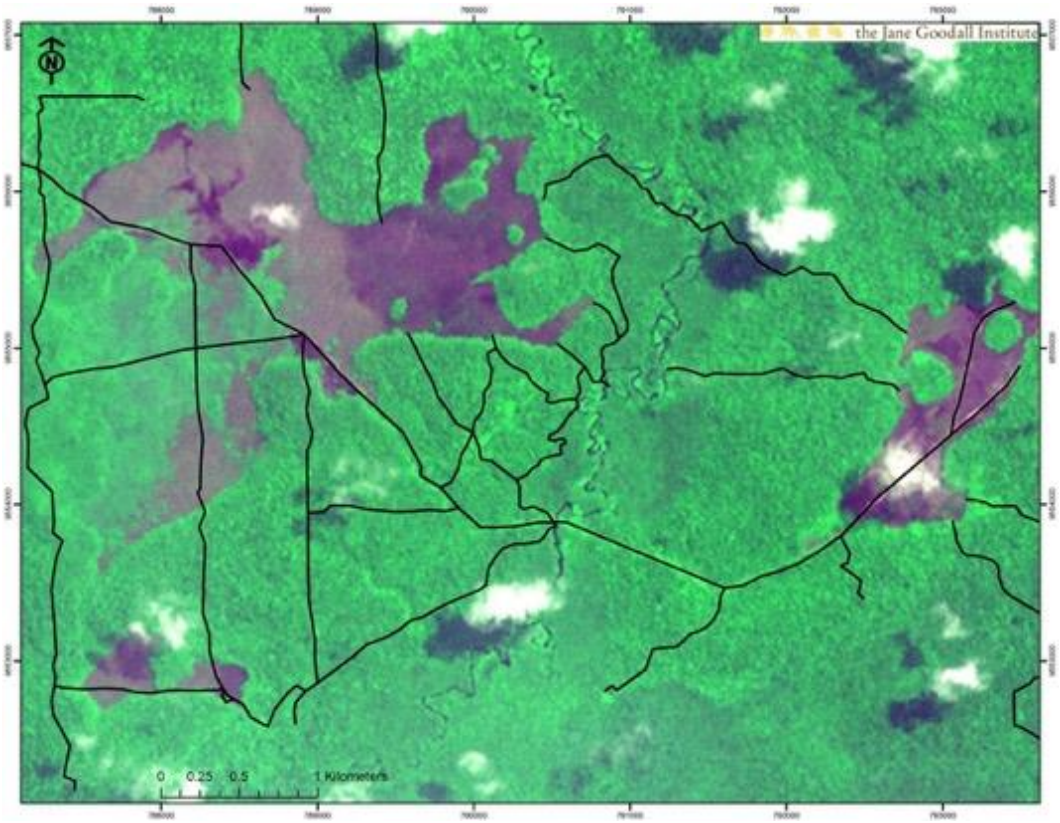

Figure 2.13 Map of transects at the release site

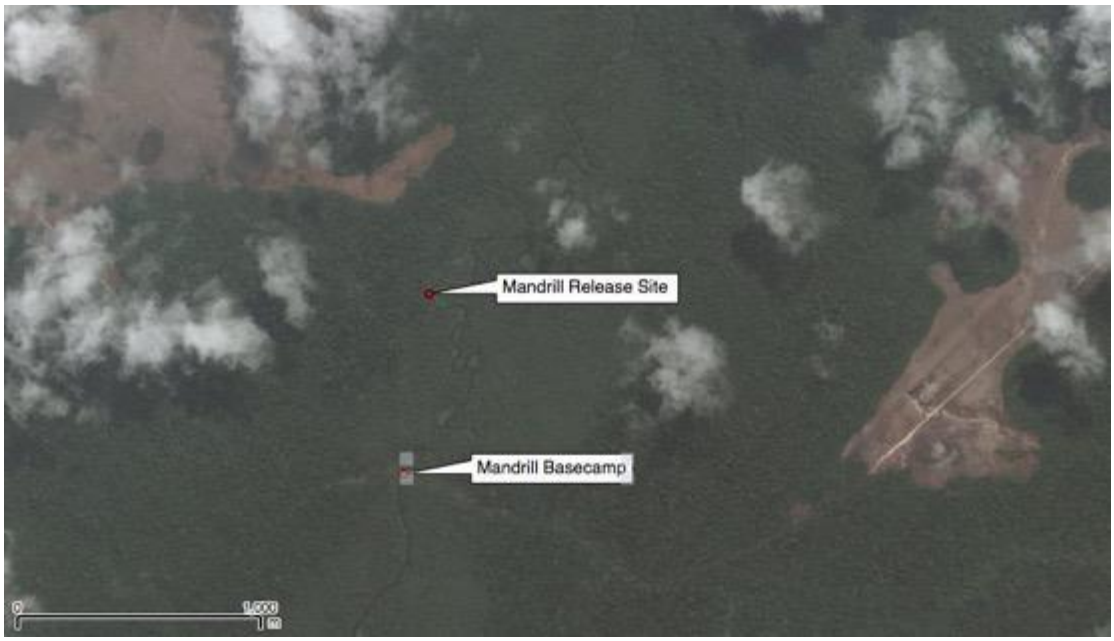

Figure 2.14 Location of mandrill base camp in relation to the mandrill release site

2.11 Pre-release enclosure

The pre-release enclosure was constructed at the release site from the same materials and in a similar fashion to the Tchimpounga enclosures (Figures 2.15, 2.16): a 1 m foundation

and a slider door, diagonal structural elements, firehoses or hammocks for enrichment, platforms, and perches. The three compartments had a total area of ~58 m^2 of covered space. A corridor to the river was later added for the safe transport of food the enclosures (Figure 2.17). Construction of the third compartment was completed after Group 1 was transferred to the release site. We included two chain link outdoor runs without roofs that allowed the mandrills to forage in the open air (Figure 2.18).

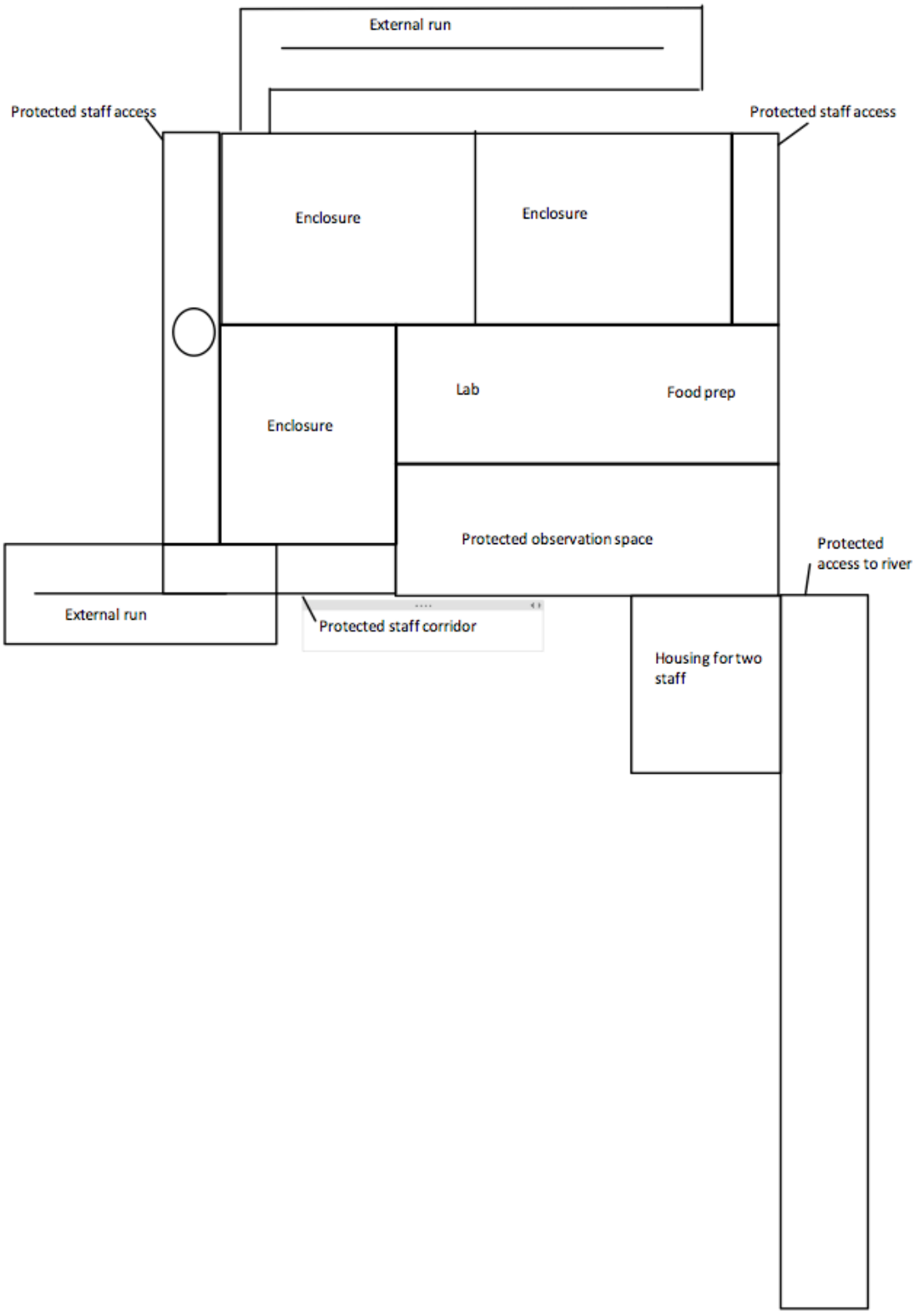

Figure 2.15 Design of the pre-release enclosure compound

Figure 2.16 Pre-release enclosure

Figure 2.17 Extended corridor from the pre-release enclosure to the river.

Figure 2.18 Outdoor corridor on the pre-release enclosure.

2.12 Release group structure

The mandrills were released in 3 groups each consisting of five individuals (Figure 2.1). JGI released the first ten mandrills in two groups to assure we had enough resources to track a smaller number of animals before dealing with a larger number. This method was selected based on the sanctuary manager's earlier experience releasing chimpanzees at H.E.L.P. Congo. We did not know whether the animals would disperse post-release and the project did not have the staff to find and retrieve 5-10 individuals in multiple locations. Separating the stable group into two smaller groups disrupted the group's dominance hierarchy and gave the animals in Group 2 much less time to habituate to the pre-release enclosure. The timing of the release was based on when it was logistically possible and did not take season into consideration. Any potential changes in available food resources were counter-balanced with the supplemental feeding.

The first five mandrills (Group 1) remained near the cage so we released the second group (Group 2) seven days later and removed two animals, one of which was removed from the release programme because it was aggressive towards staff and the other because it was aggressive towards the other mandrills (March 2014). We released a further eight new arrivals (Group 3) in January 2015.

2.12.1 Transfer to the release site

We sedated and performed a final health check on Group 1 using the methods detailed in Appendix F and transferred them to the pre-release enclosure in August 2013. We transferred Group 2 to the pre-release enclosure in two sub-groups in February 2014. We transferred Group 3 to the pre-release enclosure in two sub-groups in November-December 2014. In preparation for each release we fitted mandrills large enough to wear a collar (n = 7) with artificial collars to habituate them to wearing collars, then later replaced the artificial collars with radio-collars. In accordance with the accepted standard

set by the American Society of Mammologists committee (American Society of Mammalogists, 1998) collars were less than 5 % of the animal's body mass. We originally intended to house the animals at the release site for three months. However, a local village experienced some social unrest after we transferred Group 1 to the release site forcing us to delay their release for several months. The disruption also caused us to reduce the amount of time Group 2 spent in the pre-release enclosure. We held the animals in Group 1 in the pre-release enclosure for 197 days and the animals in Group 2 for 19-21 days prior to releasing them (Table 2.2).

Table 2.2 Details of the released mandrills and their tracking collars

Mandrill Name	ID	Sex	Approximate age at release (years)	Last recorded mass (kg)	Release Group	Release date	Collar type	Telonics Product model	Collar mass (g)	Collar mass as a percentage of body mass
Kiki Mpaka	KM	Male	12	34	1	5.3.14	Argos	TGW-4483H-3	582	1.71%
Gagaga	GAG	Male	9	20	2	12.3.14	Argos	TGW-4483H-3	582	2.90%
George	GEO	Female	6	12	1	5.3.14	GPS	TGW-4200-2	436	3.60%
Dominque	DOM	Female	5	9.3	1	5.3.14	GPS	TGW-4200-2	436	4.69%
Obia	OB	Male	7	16.5	1	5.3.14	GPS	TGW-4200-2	436	2.64%
Madol	MAD	Male	5	9.3	1	5.3.14	GPS	TGW-4200-2	436	4.69%
Veiu de Loin	VDL	Male	6	12	2	12.3.14	GPS	TGW-4200-2	436	3.63%

2.13 Practice collars

We fitted five mandrills with practice collars of approximately the size of the actual release collars. The real collars weighed 0.45 kg for the GPS units and 0.5 kg for the Argos collars. The practice collars were made of rectangular metal tubing with additional sections of metal welded to the tubing. The weighted rectangular tubing was then fixed to plastic straps with wire and wrapped in duct tape to eliminate any sharp edges. Three of the collars had weights on them to approximate the mass of actual collars whilst two of them had the weights removed because we were concerned that the mass exceeded 5% of the animal's body mass. We selected George, Dominique, Madol, Mpacka and Obia to wear the practice collars because they were in the first release group and large enough to wear them (Figure 2.19). The collars were fitted to the animals at the release site on January 22, 2014. The animals pulled at the collars intermittently and

scratched around them occasionally but showed no substantial reaction to them. We removed the practice collars during the final health check prior to release on 9 February 2014.

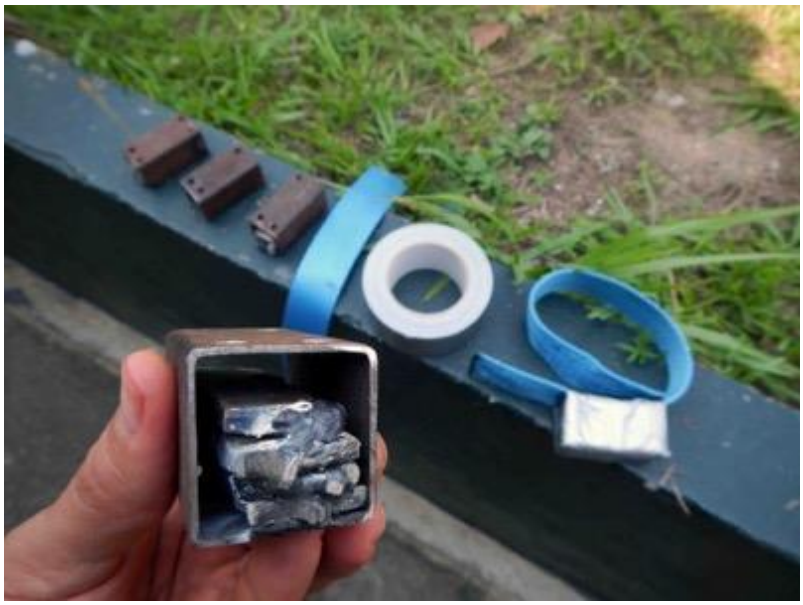

Figure 2.19 Unassembled practice collar with internal weights in hand and finished collar wrapped in duct tape resting on retaining wall

2.14 GPS Collars

Because of project delays we had to adjust collars past the pre-drilled holes because the animals grew during the study (Figure 2.20). We made extensions from straps that we prepared carefully to avoid edges that would irritate the animal's skin.

Figure 2.20 Adolescent male mandrill with collar expander

2.15 Behavioural observations

The 15-month duration of the behavioural study was based on the GPS collar battery life. We assumed it would be impossible to follow the animals after the collars dropped off. Nevertheless, the release site was staffed as of April 2019 as a buffer against human pressures in the area.

The mandrill ethogram (Appendix G) used in this study was based on existing ethograms. Mandrill behaviours were primarily based on those described in Mellen et al., (1981) and Setchell (1999), self-directed behaviours not specifically addressed in those ethograms were extracted from Castles et al., (1999). Aggression was divided into three levels ranging from non-physical to physical outlined in Otivac (2007) and the vocalisation components of the ethogram were extracted from Kudo (1987).

Observations were conducted at the sanctuary, in the pre-release enclosure, and post-release. We recorded behaviours using both instantaneous and continuous sampling methods (Martin and Bateson, 1986). We aimed to conduct daily behavioural

observations from 0630 h to 18:30 h seven days per week. The work was broken up into two shifts: 06:00-12:00 h and 12:00-18:30 h.

We collected 20-minute focal samples, each followed by a scan sample of the animals present. During the focal sample, we recorded the animal's activity state, body position and height within the environment at the beginning of each 2-minute interval (observation sheets are in Appendix H). To determine the dominance hierarchy, we recorded mandrills that avoided and were avoided by the focal animal. We also recorded all other submissive, aggressive, sexual and affiliative behaviours. We recorded the occurrence of calls, and the self-directed behaviours yawn, scratch, groom, touch and shake. The instantaneous scan sample included the animal's height, body position, activity and proximity to other mandrills. Staff used a simplified sheet (Appendix I) which did not include self-directed behaviour.

There were typically 2-3 observers per shift. At the beginning of each shift the observers created a list with all of the mandrill's names. Each observer verified with the other observers that the order in their list was different from that of the other lists. The lists did not have a systematic method for their generation but discouraged repeat observation of the same or easiest animals to view. After the observer finished observing one animal they moved to the next animal on their list. If the next animal was out of sight they moved on to the next animal on the list. Unfortunately, the staff ($n \geq 24$) frequently changed as the result of scheduling, making achieving reliability difficult. As a result, I only used my own observations in analyses. The local staff presence was primarily to assure the animals received their supplemental food, to employ local stake holders and to maintain a conservation presence in the release area.

2.16 Post-release monitoring

After releasing group 1, we allowed the animals access to the pre-release enclosure for approximately one week so they could return to the enclosure if they wished. We then brought the adult male back into the enclosure from the surrounding forest for a controlled reintegration with Group 2. This involved opening the cage door and allowing him to walk in. We then shut the cage door to force his proximity with the second release group. We also allowed Group 2 access to the enclosure for one week after we released them, after which we only allowed the animals into the enclosure for medical procedures. Towards the end of the study we trained animals wearing GPS collars to enter and exit the cage because we wanted to be able to safely sedate the animals in the enclosure to remove the collars if they did not drop off automatically. The training involved luring the individuals into the cage with food.

Upon release the animals used the forest surrounding the cage freely and received ~2 kg of supplementary food each, twice per day, for the first two months post-release. We did not decrease the feeding until after the last group was released. After all of the mandrills had been released, we used the supplemental food primarily to guide the mandrills to key food resources in the area as post-release training. We decreased the food in 10% increments over the course of the study, based on the animals' condition and behaviour, rather than set time periods.

2.17 Ethical approvals and research authorisations

Durham University granted this study ethical approval (Appendix J) and MEF provided the research authorisations (Appendix K; Appendix L). Authorisation for the international transfer faecal samples from Congo to the United States of America was provided by CITES (Appendix M). Permission to conduct the mandrill release was provided by the Congolese government, the Jane Goodall Institute, and the WCS park

director Hilde VanLeeuwe. Moreover, there was also a cultural requirement to receive approval from the local village chiefs and the forest mermaids. Forest mermaids are a part of local mythology and are believed to look after specific sections of land (Drewal, 2008). Local customs require the ritual consumption of alcohol by a local mystic at specific location where the mermaid is believed to live. According to local lore the mermaid also requires the donation of alcohol to the local villagers and the distribution of golden pennies in the river (Drewal, 1988).

Detailed methods on the stationary GPS collar testing, assumptions tested, and statistical tools used in the analyse are included in the methods sections of Chapters 3 and 4. Faecal sampling protocols, assumptions tested, and statistical analysis are included in the methods section of Chapter 5.

Chapter 3: Testing GPS collars in preparation for a primate release

3.1 List of authors and affiliations:

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Authorship Contribution Statement:

I conceived the aim, designed the study, collected and analysed data then wrote the chapter, under the supervision of R.A. Hill and J.M. Setchell. R. Atencia provided access to staff and resources vital to the project. D. Cox instigated the project, selected the collars and oversaw much of the fundraising and logistics for the project.

3.2 Abstract

Global Positioning System (GPS) telemetry collars are widely used in wildlife field studies and the International Union for Conservation of Nature recommends using telemetry in release projects to monitor animals after release. Animal behaviour, topography, and vegetation can affect the ability of a collar record its three-dimensional (3D) location and acquire a successful fix (fix success). When behaviour and environmental factors systematically affect fix success these factors may introduce bias into the data obtained. Collars operate optimally when they are upright with unobstructed access to satellites. Many animals live in dense forest and their behaviours place collars in orientations and locations that can reduce fix success rates. GPS collars are also designed to function fitted to an animal. Fitting the collars to animals may increase fix success rates because the animal's body may provide additional ground plane for the GPS antenna and enhance its performance. This is important because some collar studies are performed with empty collars and others use various substrates to simulate an animal in the collar. In preparation for a release of a group of semi-terrestrial mandrills (*Mandrillus sphinx*) into a dense tropical forest in the Republic of Congo, we tested eight GPS and two Argos collars under conditions as close as possible to those the collars would be deployed in. We measured the effects of fitting the collars to a simulated animal, then using collars fitted to simulated animals we measured the effect of collar orientation, and collar height in the forest structure on fix success rates. Presence of a simulated animal and collar orientation did not have a significant effect on 3D fix success rates, but presence of the simulated animal significantly increased the time required to acquire a fix. Collars placed in trees took significantly less time to acquire fixes and had a significantly higher proportion of successful 3D fixes than collars placed 50 cm from the ground. Researchers

using GPS collars should test for a similar affect in fix success in their study area to control for any height-related bias that might exist in the collar data.

3.3 Introduction

Global Positioning Systems (GPS) are widely used to remotely gather information on wildlife. In primate field studies most of these tracking devices are on collars fitted to the animal's neck (Trayford and Farmer, 2012). GPS collars acquire and store (fix) their location by triangulating the collar's location in relationship to multiple satellites (Sager-fradkin et al., 2007, Bêlant, 2009). Collars can acquire 2D fixes where three satellites are used to acquire the location or 3D fixes where 4 or more satellites are used to acquire the location (Rempel et al., 1995). 3D fixes are more spatially accurate and thus higher quality than 2D fixes. When and how frequently a collar attempts to acquire a fix is determined by the researchers and the collars typically arrive pre-programmed by the manufacturer. Fix success is defined as when a collar acquires a 2D or 3D fix and the fix success rate is the percentage of successful fixes in relation to scheduled fixes (Sager-fradkin et al., 2007).

Collars perform best when upright with the antenna pointed towards the sky and with an unobstructed view of satellites (D'Eon and Delparte, 2005). Collars are best suited for studying large terrestrial animals such as white-tailed deer (*Odocoileus virginianus*, (Merrill et al., 1998, Bowman et al., 2000), moose (*Alces alces*, Remple et al. 1995; Edenius, 1997; Dussault et al., 1999) and caribou (*Rangifer tarandus*, Craighead and Craighead, 1987) who regularly graze in open spaces and whose neck positions places the collar in an oriented upright. Tilting the collar away from zero degrees can have a negative effect on fix success (D'Eon and Delparte, 2005, Heard et al., 2008, Bêlant, 2009) and some animals have behaviours that frequently take the collar's orientation away from zero degrees. For example, in bears, resting or nursing young can

tilt the collar away from zero degrees (Moen et al., 1996, Obbard et al., 1998, D'Eon et al., 2002).

One of the benefits claimed for GPS collars is they remove observer bias from field studies where observations are more likely to occur in places observers can easily access (Sprague et al., 2004). However, GPS collars are more likely to acquire successful fixes under favourable habitat conditions and so introduce their own bias into ranging data. To understand and account for these potential biases in data, researchers have conducted tests on stationary collars in various conditions. Such tests show that topography (Gamo & Rumble, 1999), habitat (Di Orio et al. 2013), collar orientation (D'Eon and Delparte, 2005), weather, and animal behaviour (Rempel et al., 1995, Schwartz and Arthur, 1999, Heard et al., 2008, Jiang et al., 2008) all affect fix quality.

Battery life is a major concern in collar studies (Trayford and Farmer 2012; Campbell et al. 2010). Researchers can limit the amount of time the collars spend looking for satellites to acquire a fix (hereafter referred to as time to fix) to extend battery life (Addessi et al., 2007). The amount of time between fixes (fix interval) affects collar performance because short time intervals allow collars to benefit from information about the satellite locations from the previous fixes (Janeau et al., 2004). For example, fixes less than 15 minutes apart benefit from the previous fix but those more than 1 hour apart do not benefit from the previous fix and are less likely to be successful (Forin-Wiart et al., 2015). Larger batteries weigh more but can increase collar life and number of fix attempts. Increasing the collar weight may not be possible with small animals thus, it is important to consider the trade-offs between collar weight and the functionality needed (Sprague et al., 2004).

Collars are made to be used on animals. Because animals are largely water, fitting the collar to an animal may increase fix rate by attenuating with the animal (pers comm. Telonics representative). Telonics is the most frequently used radio collar manufacturer in

primate release (Trayford and Farmer, 2012) and they use collars fitted to simulated animals fashioned from paper towels soaked in salt water then placed in a plastic bag for quality assurance tests. Some studies have found collars perform best on an object that simulates the ground plane of an animal, such as a water balloon filled with saline solution (Frair et al., 2010) or a plastic bottle filled with salt water (Janeau et al., 2004, Forin-Wiart et al., 2015), while others conduct tests with empty collars (Agouridis et al. 2004). However, the body of the animal in the collar may also negatively affect fix success rates. For example, one study found that as the circumference of a bear's neck increases, the fix success decreases (Graves and Waller, 2006).

Collars tend to perform better in stationary tests than when deployed on animals in the same habitats (Biggs et al., 2001, Lewis et al., 2007) and thus stationary tests do not perfectly account for lost fixes on deployed collars (Moen et al., 1996). This is probably because stationary tests measure a single variable and do not account for an animal's behaviour in a varied habitat. Some researchers have implemented mobile collar tests in an attempt to create a more directly applicable account of how collars function on animals in natural habitats. Some of these tests included collars worn by dogs or humans or placed on top of moving cars (Janeau et al., 2004). For example, collars fitted to dogs performed worse than empty stationary collars (Cargnelutti et al., 2007). However, stationary tests isolate variables that affect collar function, are applicable across species and can be replicated in most study sites (Frair et al., 2010). A better understanding of what influences fix success is important as researchers continue to expand the use of GPS collars to new species and into new habitat types. Stationary tests isolate factors that inform models but do not directly predict the performance of collars fitted to animals and perfectly correct for missed fixes prior to analysis.

Trees, terrain and vegetation density can also negatively affect GPS collar fix rates and time to fix by reducing access to satellites (Moen et al., 1996, Obbard et al.,

1998, Gamo and Rumble, 2000, D'Eon et al., 2002, Di Orio et al., 2003, Lewis et al., 2007, Bêlant, 2009, Recio et al., 2011). Forests with tall, densely packed trees with large diameters interfere with collar performance more than forests with shorter, less densely spaced trees with smaller diameters (Janeau et al., 2004). Different habitats affect fix success rates and time to fix differently so GPS collar tests should be performed in habitats relevant to those they will be deployed in to most accurately estimate collar performance (Lewis et al., 2007).

GPS collars are widely used in primate field studies, but most primates live in forested areas (Clutton-Brock and Harvey, 1977) and GPS collars tend to have low fix success rates in closed canopy primate habitat (Finn, 1998, Phillips et al., 1999, Sprague et al., 2004, Sánchez-Giraldo and Daza, 2019). Olive baboons in open arid habitat have higher fix success rates (Markham and Altmann, 2008) than species with densely forested areas in their home range such as macaques (*Macaca fuscata*, Sprague et al., 2004) and white-footed tamarins (*Saguinus leucopus*, Sánchez-Giraldo and Daza, 2019).

Most primates are arboreal or semi-terrestrial, although some species such as the patas monkey (*Erthrocebus patas*), gelada (*Theropithecus gelada*), hamadryas baboon (*Papio hamsdryas*) and olive baboon (*Papio anubis*) are mostly terrestrial (Milton and May, 1976). Arboreal and semi-terrestrial animals travel both horizontally and vertically through space and GPS collar fix success rates and location error are assumed to improve with increased height because there is less obstruction between the collar and satellites (Adams et al., 2013). Even small changes in height of 33-66 cm can introduce location error in VHF collars (Grovenburg et al., 2013). However, this effect has not been tested for GPS collars. In stationary tests, collars are sometimes set to approximate the height of the study species or simulate a specific behaviour (Gamo and Rumble, 2000, Grovenburg et al., 2013). In tests measuring the effect of collar orientation, topography and forest density on GPS fix rates, the collars were all <2 m from the ground (at 1.5 m: Dussault et

al., 1999; Rempel and Rodgers, 1997; 1.35 m: Di Orio et al., 2003; 1 m: Blackie, 2010; Cargnelutti et al., 2007; D'Eon et al., 2002 et al. 2002; Lewis et al., 2007; 50 cm: (D'Eon and Delparte, 2005); "otter height": Boitani et al., 2012; ground level Bêlant, 2009); or the height is not mentioned (Cain et al., 2005). Thus, these tests may not be generalisable to semi-terrestrial, arboreal or flying animals, who can range 30+ m on the vertical axis in the environment. Trees are frequently located in ravines creating a confound of forest density and topography.

The aim of this study is to understand how the use of a simulated animal, collar orientation, collar height in a forest, and collar height in a ravine affect GPS collar performance. We measured performance as the number of 3D fixes achieved during each test mode and the time to it took to achieve those fixes. We tested the following hypotheses and predictions.

1: If the presence of a simulated animal in the collar improves collar performance through additional ground plane, then collars fitted to simulated animals will have more 3D fixes and a shorter time to fix than empty collars.

2: If orienting collars away from zero degrees negatively affects collar performance, then collars that are not upright will have fewer 3D fixes and an increased time to fix in comparison to upright collars.

3: If forest density negatively affects collar performance, then collars in forests that are less dense will have significantly more 3D fixes and significantly shorter time to fixes than collars in more densely forested areas.

4: If vegetation density affects collar performance, then collars placed higher in a tree will have more 3D fixes and a lower mean time to fix than collars placed in dense undergrowth beneath the same tree. Here we use the height of the collar as a proxy for vegetation density.

5: If height in trees located in a ravine affects collar performance, then collars placed higher in a tree will have more 3D fixes and a lower mean time to fix than those closer to the ground with more topographical obstruction.

3.4 Methods

We performed five field tests on eight Telonics GPS radio collars and two Argos collars in two protected areas in the Republic of Congo (Congo). We intended to perform all tests in the habitat surrounding the release site, but logistical complications made this impossible. We conducted Tests 1, 2 and 5 at Tchimpounga Reserve, ~61 km south west of the release site in habitat types found at the release site (UTM 32 M 814303 9500175). We conducted Tests 3 and 4 in the forest surrounding the release site in Conkouati-Douli National Park (Conkouati) (UTM 32 M 774300 9567971). The park has a long rainy season (February to May) and a short rainy season (October to November) and a long dry season from (June to September) and short dry season from (December to January, Le Hellaye et al., 2010). We conducted the tests between March 2012 and January 2014, during both the rainy and dry seasons.

The GPS collars were pre-programmed to stop trying to acquire a fix (time out) after 120 s. The Argos collars were pre-set to timeout after 180 s. Pre-deployment manufacturer simulations suggested that the mean time to fix was 75 seconds for both types of collars. We used the factory setting test mode for all tests. This mode attempts one fix 5 s after the collar is activated, then one fix each hour for 3 hours (total 4 fixes at 1 h intervals). Test mode measures fix success or failure, time to acquire fix, latitude, longitude, altitude, speed, heading and satellite count. We ran the collars through two tests each day and rotated each collar to a new location on the test platform between the tests to minimise any possible effect related to location on the platform. We tested all collars in each condition to ensure we tested the effect of the condition and not variation between collars. We aimed to start the first test between 08:00 h and 10:00 h and adhered

to this schedule as closely as possible. GPS data from the collars was downloaded using Telonics Data Converter software.

We made simulated animals by rolling strips of towel 18 cm wide, binding them in string, soaking them in water collected from the ocean and wrapping them in a plastic bag to replicate tests performed by the manufacturer. Telonics drills holes in the collar bands to enable fitting to the release subjects and collars function optimally when these holes are used (Telonics manual). We shaped the simulated animals to touch all sides of the collar in its middle setting to simulate a properly fitted animal. The collars were different sizes, so the simulated animals also varied in size, so that it simulated a properly fitted collar with the antenna facing the sky when the collar was oriented vertically at 0o. We re-saturated the towels between tests of each hypothesis to account for potential evaporation. We excluded the first of the 4 fixes from analysis to control for the effect caused by variation in fix interval attributable to the cold start.

3.4.1 Test 1: Presence of a simulated animal

We conducted Test 1 in a savanna at the top of a large hill to eliminate any effect related to vegetation and topographical obstruction. We placed all collars on a wooden platform approximately 50 cm off the ground to simulate the approximate height of a mandrill (Figure 3.1). We then fitted four GPS and two Argos collars on simulated animals and positioned four GPS collars without a simulated animal. We ran the collars

through 5 test cycles with simulated animals and 5 cycles without simulated animals, resulting in a total of 279 fix attempts in March 2013, during the long rainy season.

Figure 3.1 Empty collars and collars fitted to simulated animals placed upright on a platform in open savanna.

3.4.2 Test 2: Collar orientation

We conducted Test 2 in a savanna at the top of a large hill to eliminate any effect related to vegetation and topographical obstruction. We used eight GPS collars and two Argos collars placed on a wooden platform approximately 50 cm off the ground (Figure 3.2). For this study, 0° means the antenna is pointed directly at the sky and 180° means

Figure 3.2 Collars fitted to simulated animals, placed on platform in open savanna. The collars were oriented at 0°, 45°, 90°, 135° and 180° to test for an effect caused by collar orientation.

the antenna is pointed directly at the ground. We placed the collars at 0°, 45°, 90°, 135° and 180°, and ran all collars through two tests in the same platform location and with the same collar orientation each day, giving us six replicates per collar (three per test cycle) and 60 fix attempts between the collars at each angle (Figure 3.16). We used the collar IDs to track and counterbalance the order of the collars on the platform and their orientation. We conducted Test 2 in March 2013, during the long rainy season.

3.4.3 Test 3: Habitat type

For Test 3, we selected four points in representative forest types in the area around the release site (riparian, secondary forest, forest fragment, savanna, figure 3.3). We built platforms at each site and rotated the collars through each habitat. We placed the collars on the platforms oriented at 0◦ and reset them once at midday. We obtained six fix attempts per collar per day for six days, giving 36 attempted fixes per collar and 360 fix

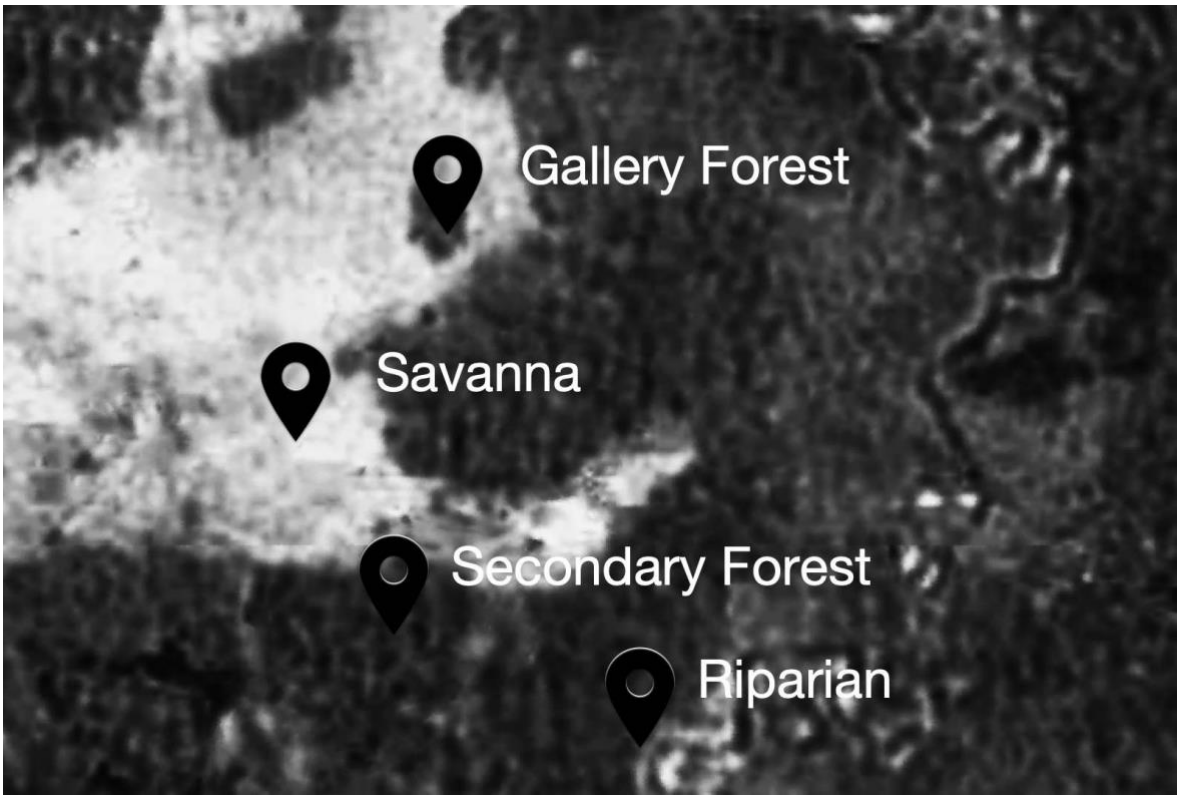

Figure 3.3 Location of the four platforms located in representative forest types (riparian, secondary forest, forest fragment, savanna) found in the release area. The pre-release enclosure was next to the riparian platform location.

attempts per location. We used the collar IDs to track and counterbalance the order the

collars on the platforms and across the four locations. We estimated canopy coverage as the percentage of visible sky (0-25%, 26-50%, 50-75%, 76-100%) when standing at the platform. We conducted Test 3 between 4 October 2013 and 9 October 2013, during the short rainy season.

3.4.4 Test 4: Height in the canopy

We conducted Test 4 in a forest with ~50% canopy coverage and very dense undergrowth providing 100% coverage. The test site was near the release site and within the expected home range of the animals. We fitted the collars to simulated animals and placed them at 0.5 m and 18.8 m in a tree. We selected 18.8 m because it was the highest point the collars could reasonably be placed in the tree. The mandrills would need to pass this location to forage in the tree. We used the collar IDs to track and counterbalance the order of the collars on the platform and at each location. We conducted Test 3 between 29 September 2013 and 2 October 2013, during the short rainy season.

a)

b)

c)

d)

Figure 3.4 Collars fitted to simulated animals and placed upright on platforms at a) 18.8 m and b) 0.5 m to test for the effect of height on collar performance. The platform at 18.8 m was located directly above the platform at 0.5 m. Forest structure in much of the release area had c) sparse secondary growth with d) dense marantaceae undergrowth. In c) I am at ~5 m directly below the collar platform; d) was taken at ~1.5 m.

3.4.5 Test 5: Height in a ravine

We conducted Test 5 in a tree in a large ravine in forest with ~75% canopy coverage and no marantaceae undergrowth (Figure 3.5). We placed the collars at 0.5 m to approximate the height of a mandrill on the ground, at a mid-point in the tree (21.7 m was the lowest point we could reasonably place the collars), and at the highest reasonably accessible point in the tree and approximately level with the surrounding savanna (28.3 m) (Figure 3.6). We fitted the collars to simulated animals and each collar attempted six fixes per day. We conducted Test 5 between 21 December 2013 and 6 January 2014, during the short dry season.

Figure 3.5 The forested ravine used for Test 5 (height in a ravine). The tree used in the test is highlighted with a white rectangular box

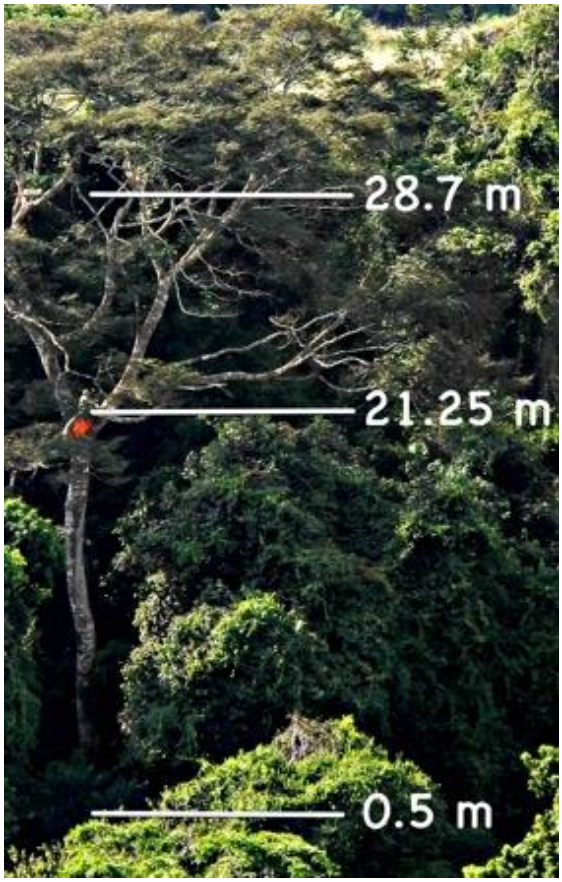

Figure 3.6 Collars placed at three heights in a tree located in a ravine

3.5 Data analysis

We conducted all statistical analysis in SPSS using mixed models to account for repeated measures using the same 10 collars. We tested the influence of predictor variables (simulated animal, orientation, habitat type, height) on the presence/absence of a 3D fix using General Linear Mixed Models with a binomial outcome. We tested the influence of predictor variables on the time to fix using Linear Mixed Models. We plotted the presence/absence of a 3D fix as the percentage of fixes that were successful for each collar in each condition, and time to fix as the mean +/- for each collar in each condition. We did not have enough Argos collars for statistical analysis but show their values separately in the tables and figures for each test.

3.6 Results:

3.6.1 Test 1: Presence of a simulated animal

We found no significant effect of the simulated animal on the number of 3D fixes acquired ($F_{1,277} = 0.001$, $p = 0.981$; Table 3.1; Figure 3.7). However, the simulated animal had a significant effect on increasing the time to fix ($F_{1,273} = 6.834$, $p = 0.009$; Table 3.2; Figure 3.8). Empty collars had a slightly shorter mean time to fix than collars fitted to simulated animals (Table 3.2).

Table 3.1 3D fix rates for empty collars (Empty) and collars fitted to simulated animals (SA). Collars placed on a platform in an open space with no obstruction of sky

Collar group	Empty or with SA	n	Mean	CI(m)	SD	SE (m)
Combined	SA	10	0.99	0.98-1.10	0.082	0.007
Combined	Empty	10	0.99	0.98-1.10	0.086	0.007
GPS	SA	8	0.99	0.97-1.01	0.101	0.010
GPS	Empty	8	0.99	0.98-1.01	0.09	0.008
Argos	SA	2	1.00	1.00-1.00	0.000	0.000
Argos	Empty	2	1.00	1.00-1.00	0.000	0.000

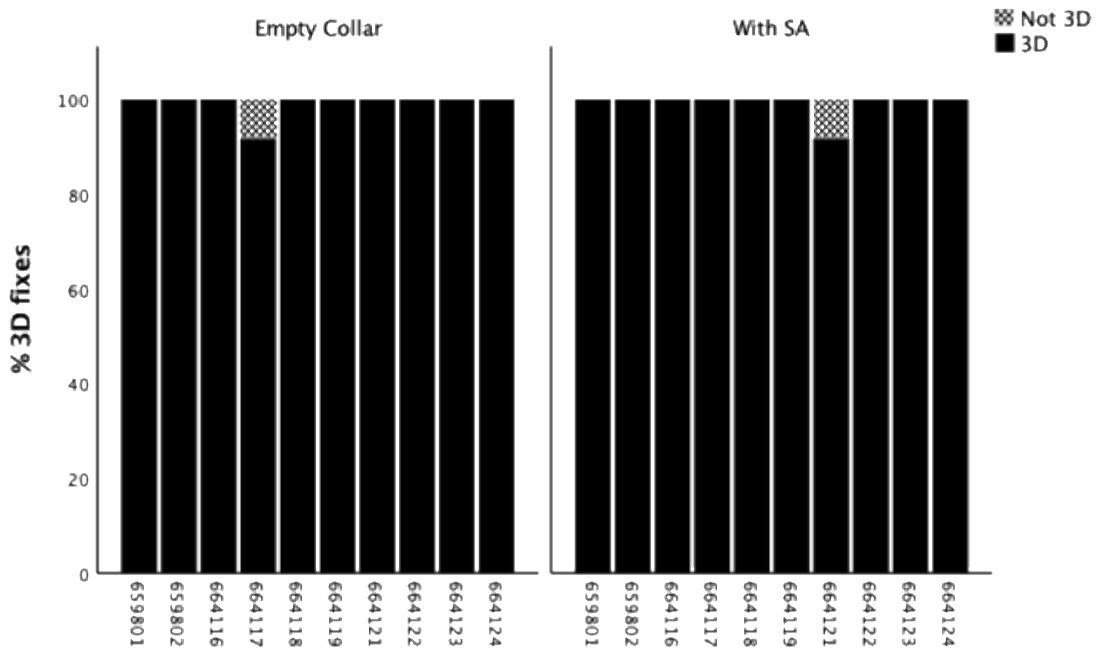

Figure 3.7 3D fix percentage for empty collars and collars fitted to simulated animals (SA). Collars were on a 0.5 m platform in open savanna. Collars 659801 and 659802 on the left of each column panel are Argos collars, others are GPS-only collars.

Table 3.2 Time to fix for empty collars (Empty) and collars fitted to simulated animals (SA). Collars placed on a platform in an open space with no obstruction of sky

Collar group	Empty or with SA	n	Mean (s)	Range (s)	SD	SE (m)
Combined	SA	10	41.61	38.71-43.54	16.79	1.376
Combined	Empty	10	37.84	35.37-40.81	14.31	1.222
GPS	SA	8	41.62	38.60-44.63	15.11	1.518
GPS	Empty	8	38.39	35.54-41.24	15.99	1.442
Argos	SA	2	40.13	35.47-44.28	14.30	2.063
Argos	Empty	2	35.00	24.46-45.36	16.31	4.708

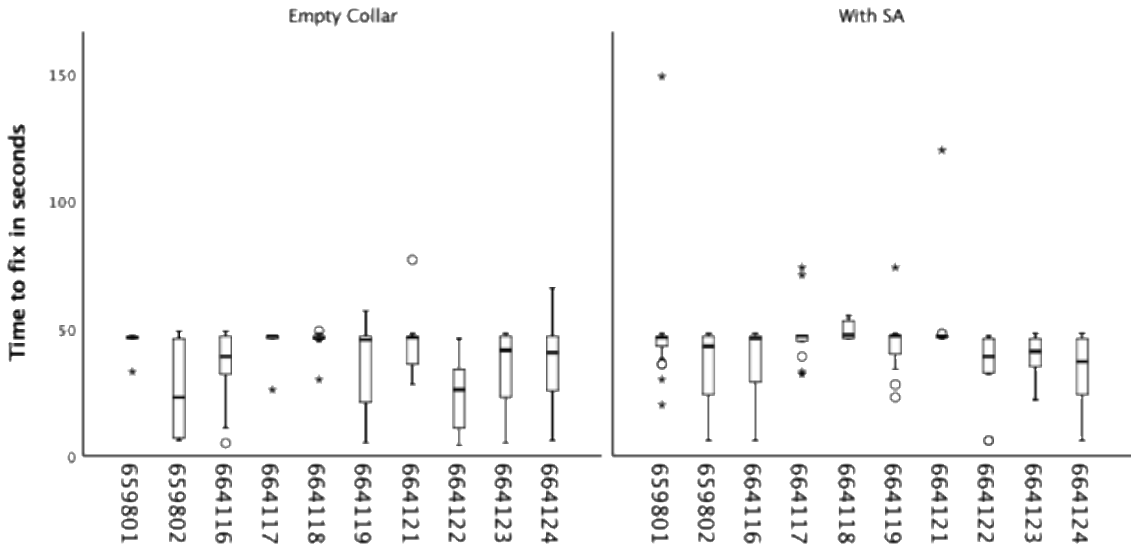

Figure 3.8 Mean time to fix for empty collars and collars fitted to simulated animals (SA). Boxplots show the median (black bar) and the first and third quartiles (boxes). Whiskers show the maximum and minimum values excluding outliers (points). Collars were on a 0.5 m platform in open savanna. Collars 659801 and 659802 on the left of each column panel are Argos collars, others are GPS-only collars.

3.6.2 Test 2: Collar orientation

We found no significant effect of collar orientation on the number of 3D fixes acquired ($F_{1,286} = 0.439$, $p = 0.508$; Table 3.3; Figure 3.9) or time to fix (($F_{4,278.23} = 2.044$, $p = 0.088$; Table 3.4; Figure 3.10). There was little variation in the mean time to fix across the angles (range: 37.8-44.3 s; Table 3.4).

Table 3.3 3D fix rates for collars fitted to simulated animals (SA) and placed at 0° (upright), 45°, 90°, 135°, 180°. Collars placed on a platform in an open space with no obstruction of the sky.

Collar group	Angle	n	Mean (s)	CI(s)	SD	SE(s)
Combined	0	10	0.98	0.95-1.02	0.131	0.017
Combined	45	8	1	0.00-1.00	0.000	0.000
Combined	90	10	1	0.00-1.00	0.000	0.000
Combined	135	10	1	0.00-1.00	0.000	0.000
Combined	180	10	1	0.00-1.00	0.000	0.000
GPS	0	8	.98	0.93-1.02	0.147	0.022
GPS	45	6	1	0.00-1.00	0.000	0.000
GPS	90	8	1	0.00-1.00	0.000	0.000
GPS	135	8	1	0.00-1.00	0.000	0.000
GPS	180	8	1	0.00-1.00	0.000	0.000
Argos	0	2	1	1.00-1.00	0.000	0.000
Argos	45	2	1	1.00-1.00	0.000	0.000
Argos	90	2	1	1.00-1.00	0.000	0.000
Argos	135	2	1	1.00-1.00	0.000	0.000
Argos	180	2	1	1.00-1.00	0.000	0.000

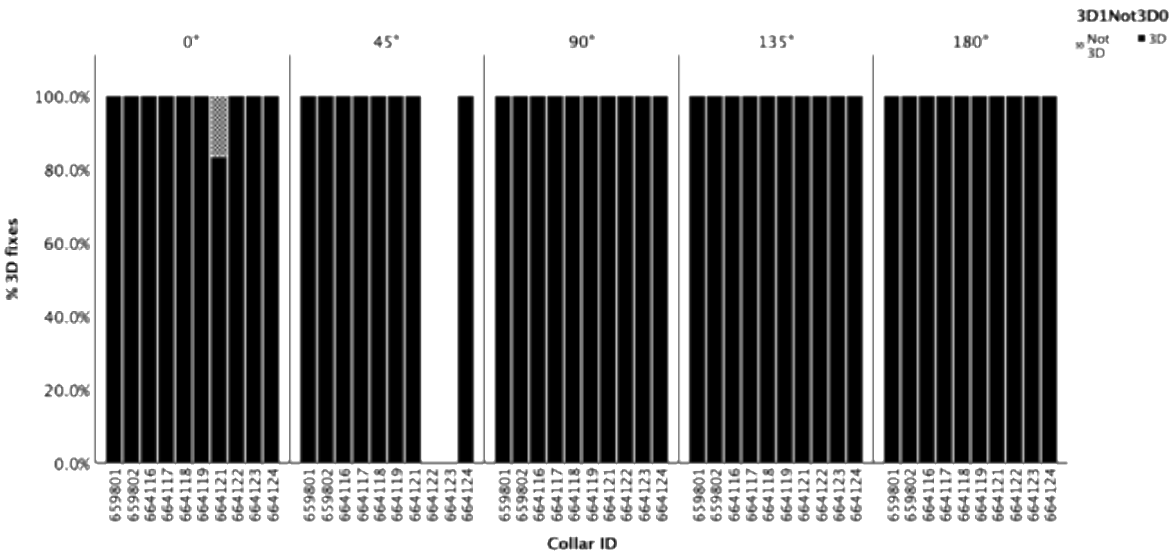

Figure 3.9 Mean 3D fix percentage for collars fitted to simulated animals and positioned at 0o, 45o, 90o, 135o, 180o away from upright. The collars were on a 0.5 m platform in open savanna. Collars 659801 and 659802 on the left of each column panel are Argos collars; others are GPS-only collars.

Table 3.4 Time to fix for collars fitted to simulated animals and placed at 0° (upright), 45°, 90°, 135°, 180°. Collars placed on a platform in an open space with no obstruction of the sky.

Collar group	Angle	n	Mean (s)	CI(s)	SD	SE(s)
Combined	0	10	41.28	35.98-46.19	20.23	2.548
Combined	45	8	44.31	42.15-46.47	7.44	1.074
Combined	90	10	41.73	37.97-45.50	15.07	1.884
Combined	135	10	43.20	38.11-47.92	18.98	2.450
Combined	180	10	37.81	34.08-41.54	14.83	1.868
GPS	0	8	42.50	36.36-48.64	20.64	3.049
GPS	45	6	45.11	42.51-47.71	7.69	1.282
GPS	90	8	43.58	39.49-47.66	14.67	2.035
GPS	135	8	44.06	38.19-49.94	20.24	2.921
GPS	180	8	38.56	34.55-42.56	14.66	1.995
Argos	0	2	35.67	27.60-43.73	12.70	3.665
Argos	45	2	41.92	37.39-45.94	6.33	1.828
Argos	90	2	33.75	24.38-43.12	14.75	4.259
Argos	135	2	38.83	30.87-46.80	12.54	3.620
Argos	180	2	33.33	21.11-45.56	15.91	5.302

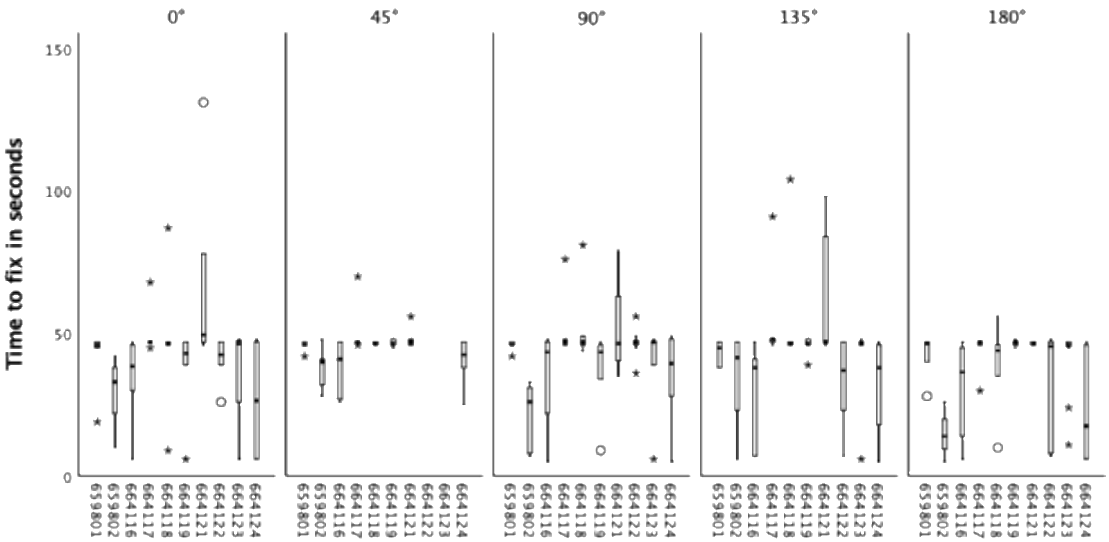

Figure 3.10 Mean time to fix for collars fitted to simulated animals and positioned at 0o, 45o, 90o, 135o, 180o away from upright. Boxplots show the median (black bar) and first and third quartiles (box). Whiskers show the maximum and minimum values excluding outliers (points). The collars were on a 0.5 m platform in open savanna. Collars 659801 and 659802 on the left of each column panel are Argos collars; others are GPS-only collars.

3.6.3 Test 3: Habitat type

We found a significant effect of habitat on 3D Fix success ($F_{3,356} = 22.69$, $p < 0.001$; Table 3.5; Figure 3.11) and time to fix ($F_{3,351.066} = 37.640$, $p < 0.001$; Table 3.7; Figure 3.12). Post-hoc comparisons showed significant differences between each of the locations except the riparian and the forest fragment (Table 3.8).

Table 3.5 Time to fix for collars fitted to simulated animals and placed in four different habitat types

Forest type	*n*	*Mean (s)*	*Range (s)*	*SD*	*SE (m)*
Riparian	10	88.81	20-180	47.81	5.039
Secondary Forest	10	113.28	20-180	41.50	4.050
Savanna	9	49.17	6-180	37.33	3.925
Forest Fagment	9	87.89	16-180	52.32	6.042
Riparian	8	81.62	72.18-91.6	36.54	4.717
Secondary Forest	8	104.92	98.01-111.83	32.43	3.477
Savanna	7	41.79	36.17-47.41	23.93	2.820
Forest Fagment	7	73.72	63.20-84.24	39.65	5.252
Riparian	2	103.2	79.65-126.75	63.07	11.514
Secondary Forest	2	153.67	125.85-181.48	55.93	13.183
Savanna	2	78.67	48.35-108.99	60.97	14.371
Forest Fagment	2	132.78	101.60-163.79	62..70	14.777

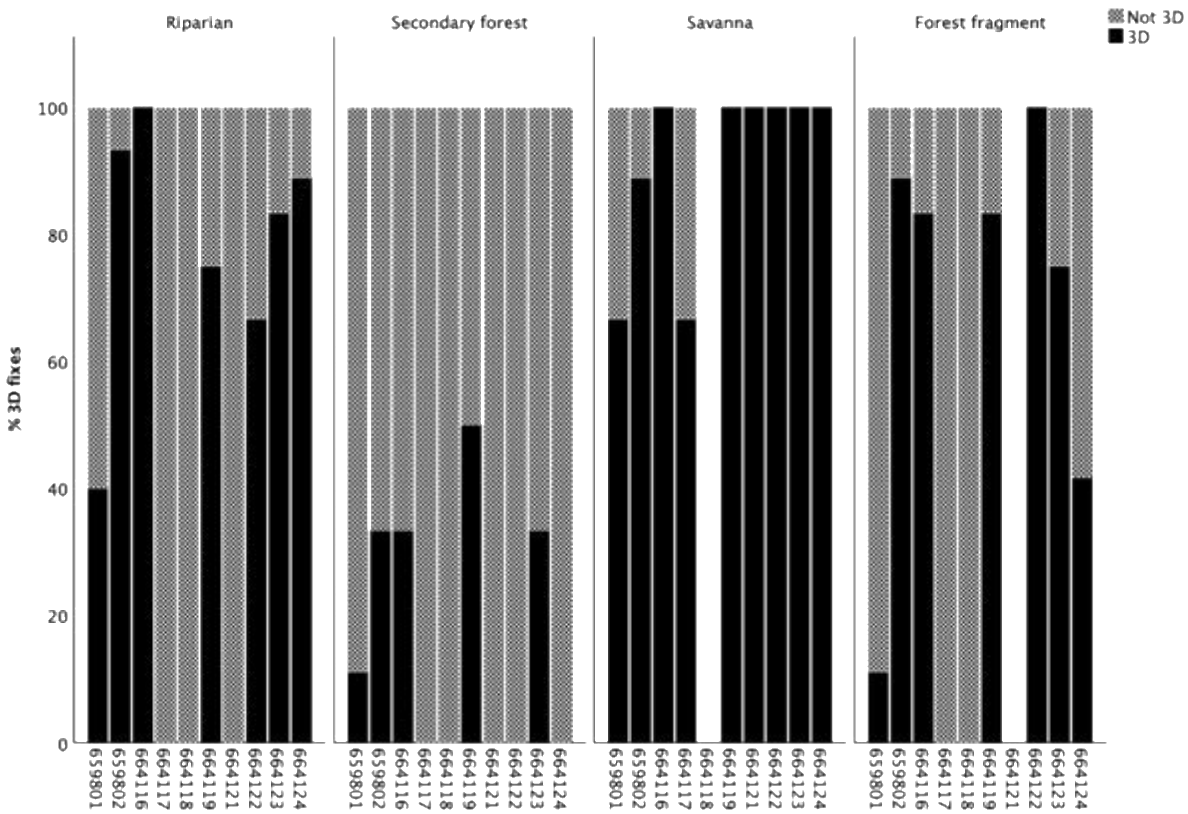

Figure 3.11 Mean 3D fix percentage for collars fitted to simulated animals and placed in four forest types (riparian, secondary forest, savanna, forest fragment). Collars were on a 0.5 m platform in open savanna. Collars 659801 and 659802 on the left of each column panel are Argos collars; others are GPS-only collars.

Table 3.7 Fix *rates for GPS radio collars fitted to simulated and placed in four forest types (riparian, secondary forest, savanna, forest fragment). Collars were on a 0.5 m.*

Collar group	Forest type	n	Mean	CI	SD	SE (m)
Combined	Riparian	10	0.60	0.05-0.70	0.24	0.052
Combined	Secondary Forest	10	0.12	0.06-0.19	0.11	0.032
Combined	Savanna	9	0.93	0.88-0.99	0.06	0.026
Combined	Forest Fagment	9	0.52	0.40-0.64	0.25	0.058
GPS	Riparian	8	0.57	0.44-0.70	0.50	0.065
GPS	Secondary Forest	8	0.10	0.04-0.07	0.31	0.033
GPS	Savanna	7	0.93	0.93-1.01	0.17	0.020
GPS	Forest Fagment	7	0.53	0.39-0.66	0.50	0.067
Argos	Riparian	2	0.67	0.49-0.85	0.48	0.088
Argos	Secondary Forest	2	0.22	0.01-0.43	0.43	0.101
Argos	Savanna	2	0.78	0.57-0.99	0.43	0.101
Argos	Forest Fagment	2	0.50	0.24-0.76	0.51	0.121

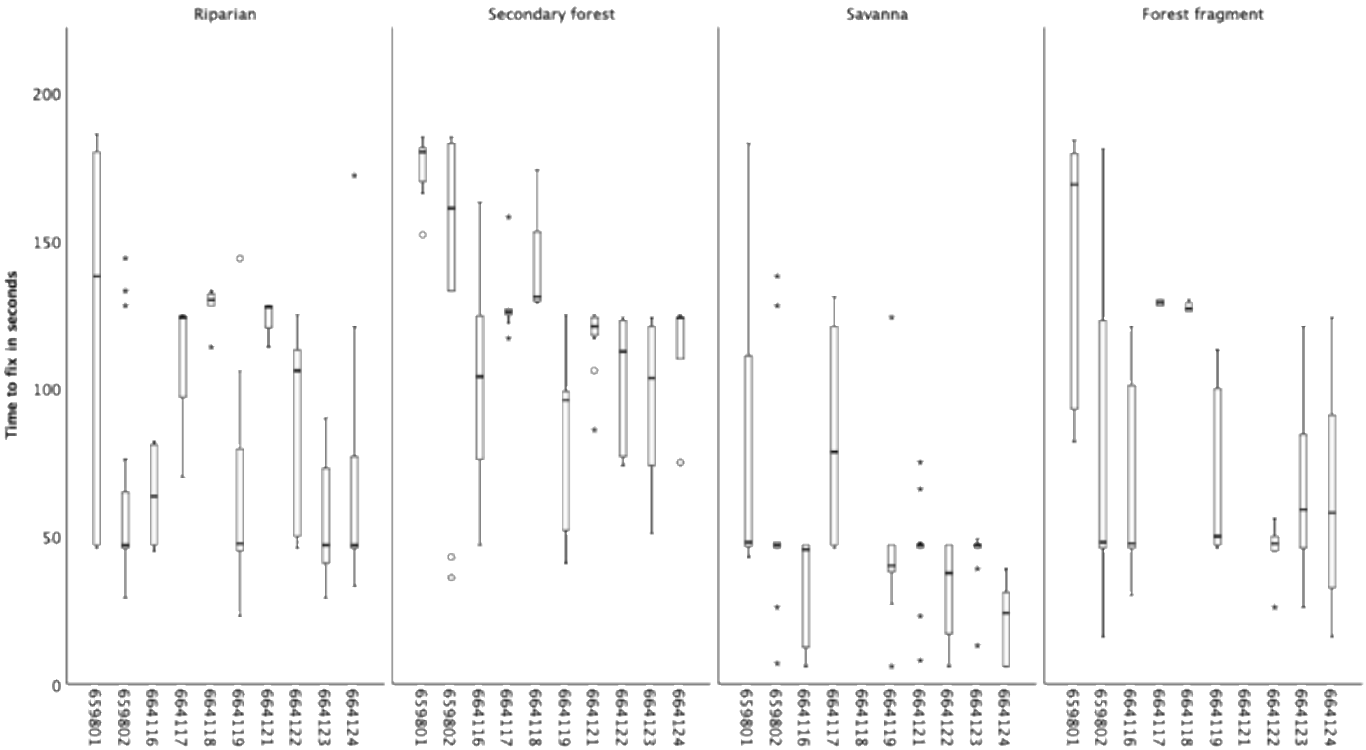

Figure 3.12 Mean time to fix for collars fitted to simulated animals and placed in four forest types. Boxplots show the median (black bar) and first and third quartiles (box). Whiskers show the maximum and minimum values excluding outliers (points). Collars were on a 0.5 m platform in open savanna. Collars 659801 and 659802 on the left of each column panel are Argos collars; others are GPS-only collars.

Table 3.8 Post hoc pairwise comparison of the mean difference in time required to obtain fixes for collars fitted to simulated animals and placed in four forest types. Collars were on a 0.5 m platform in open savanna.

Location	Location	Mean Difference (s)	SE	df	P
Riparian	Secondary Forest	27.7	5.85	352.01	<0.001
	Forest Fragment	3.2	6.19	399.01	0.609
	Savanna	33.7	5.94	349.72	<0.001
Secondary Forest	Forest Fragment	24. 6	6.21	352.80	<0.001
	Savanna	24. 6	6.21	351.30	<0.001
Forest Fragment	Savanna	36.9	6.32	351.39	<0.001

3.6.4 Test 4: Height in the canopy

The 3D fix rate success increased significantly with height ($F_{1,1,899} = 75.278$, $p < 0.001$; Table 3.5; Figure 3.13). Collars also spent significantly more time searching for fixes at 0.5 m than they did at 18.8 m ($F_{1,205} = 9.020$, $p = 0.003$, Table 3.6; Figure 3.14).

Table 3.5 3D fix rates for GPS radio collars fitted to simulated animals and placed on platforms at two heights (0.5 m,18.8 m) in a secondary forest with dense undergrowth.

Collar group	Height (m)	n	Mean	Range	SD	SE (m)
Combined	0.5	10	0.32	0.23-0.41	0.47	0.046
Combined	18.8	10	0.77	0.69-0.85	0.42	0.042
GPS	0.5	8	0.25	0.14-0.35	0.47	0.052
GPS	18.8	8	0.76	0.67-0.84	0.42	0.044
Argos	0.5	2	0.47	0.30-0.64	0.51	0.084
Argos	18.8	2	1.00	1.00-1.00	0.00	0.000

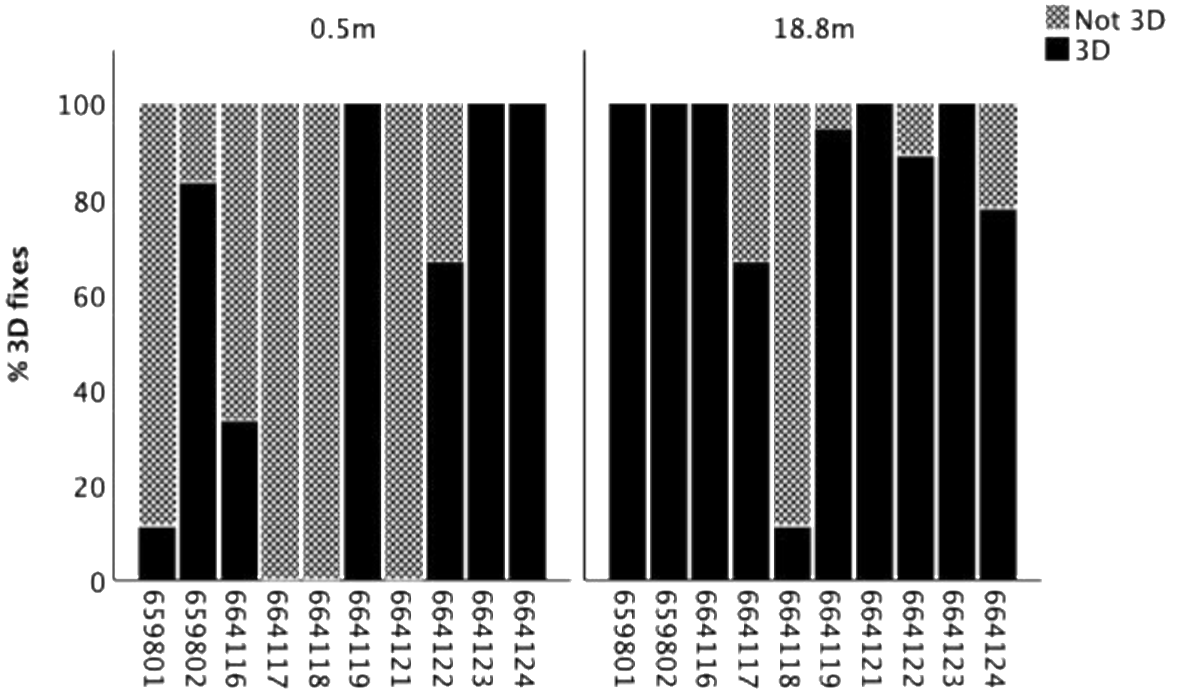

Figure 3.13 Mean 3D fix percentage of collars fitted to simulated animals and placed at two heights in a secondary forest with dense undergrowth. Collars 659801 and 659802 on the left of each column panel are Argos collars; others are GPS-only collars.

Table 3.6 Time to fix for collars fitted to simulated animals and placed on platforms at two heights in a secondary forest with dense undergrowth

Collar group	Height	n	Mean (s)	Range (s)	SD	SE (m)
Combined	0.5m	10	109.33	100.21-118.64	47.14	4.601
Combined	18.8m	10	67.35	60.53-74.17	35.07	3.439
GPS	0.5m	8	103.39	95.78-111.00	31.66	3.812
GPS	18.8m	8	67.41	60.41-74.24	34.47	3.482
Argos	0.5m	2	120.72	98.15-143.30	66.73	11.121
Argos	18.8m	2	67.67	17.61-117.72	47.70	19.472

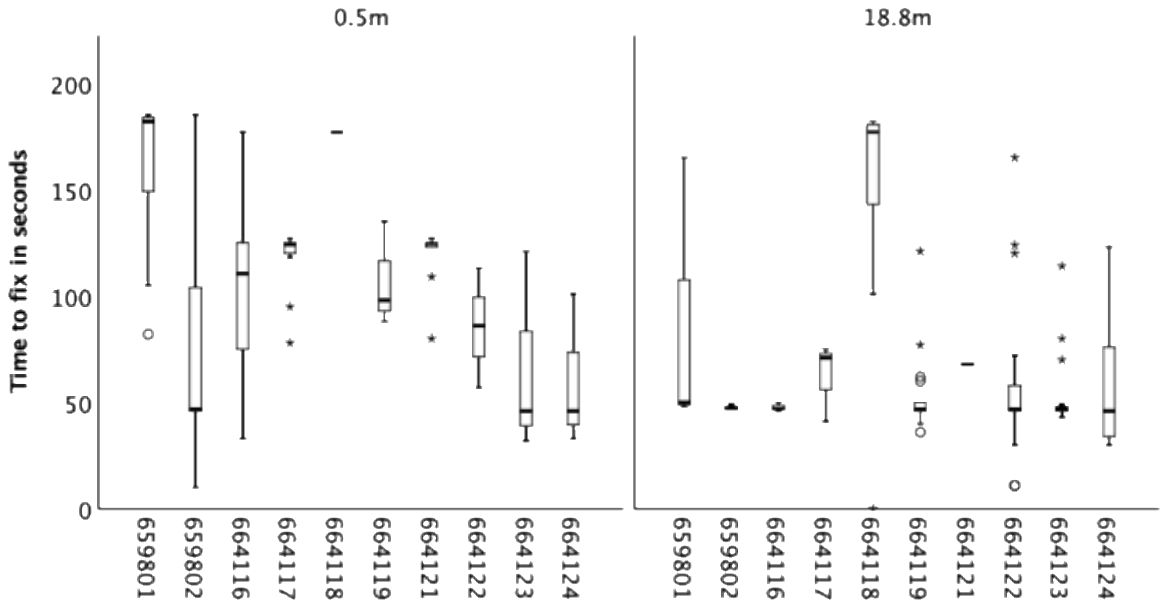

Figure 3.14 Mean time to fix for collars fitted to simulated animals and placed at two heights in a secondary forest with dense undergrowth. Collars 659801 and 659802 on the left of each column panel are Argos collars; others are GPS-only collars.

3.6.5 Test 5: Height in a ravine

Increased height was associated with increased 3D Fix success ($F_{2,419.136} = 44.231$, $p < 0.001$, Table 3.10, Figure 3.15) and a significant decrease in the amount of time the collars spent searching for satellites ($F_{2,419.178} = 39.779$, $p < 0.001$, Table 3.11; Figure 3.16). Post hoc pairwise comparisons showed these differences were significant between all three heights (Table 3.12, Table 3.13).

.10 3D fix rates for collars fitted to simulated animals and placed on platforms at three heights in a secondary forest located in a ravine

Collar group	Height (m)	n	Mean	Range	SD	SE (m)
Combined	0.5	10	0.45	0.37-0.53	0.51	0.042
Combined	21.25	10	0.62	0.54-0.71	0.50	0.040
Combined	28.7	10	0.85	0.79-0.91	0.36	0.030
GPS	0.5	8	0.45	0.35-0.54	0.50	0.046
GPS	21.25	8	0.63	0.54-0.72	0.49	0.046
GPS	28.7	8	0.83	0.76-0.90	0.37	0.035
Argos	0.5	8	0.46	0.24-0.67	0.51	0.104
Argos	21.25	2	0.61	0.43-0.78	0.50	0.086
Argos	28.7	2	0.90	0.79-1.01	0.31	0.056

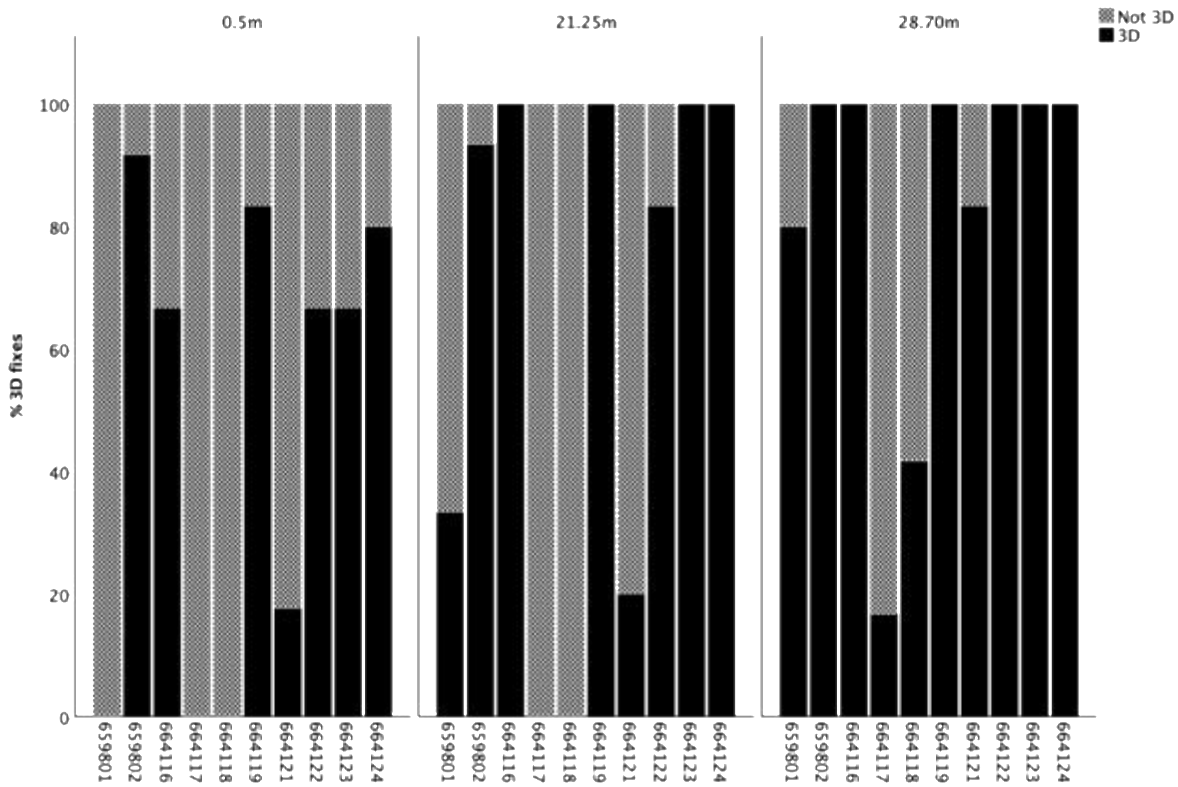

Figure 3.15 Mean 3D fix percentage for collars fitted to simulated animals and placed at three heights in a secondary forest located in a ravine. Collars 659801 and 659802 on the left of each column panel are Argos collars; others are GPS-only collars.

Table 3.11 Time to fix for collars fitted to simulated animals and placed on platforms at three heights in a secondary forest located in a ravine.

Collar group	Height (m)	n	Mean (s)	Range (s)	SD	SE (m)
Combined	0.5	10	91.13	88.46-102.40	42.16	3.525
Combined	21.25	10	72.66	71.19-86.97	47.89	3.991
Combined	28.7	10	58.35	53.66-65.60	36.24	3.020
GPS	0.5	8	91.13	84.88-97.37	34.41	3.155
GPS	21.25	8	72.66	65.60-79.71	37.58	3.561
GPS	28.7	8	58.35	52.21-64.49	22.11	3.101
Argos	0.5	2	116.75	88.98-144.52	65.77	13.425
Argos	21.25	2	100.67	76.13-135.20	69.19	12.045
Argos	28.7	2	64.50	47.09-81.91	46.63	8.512

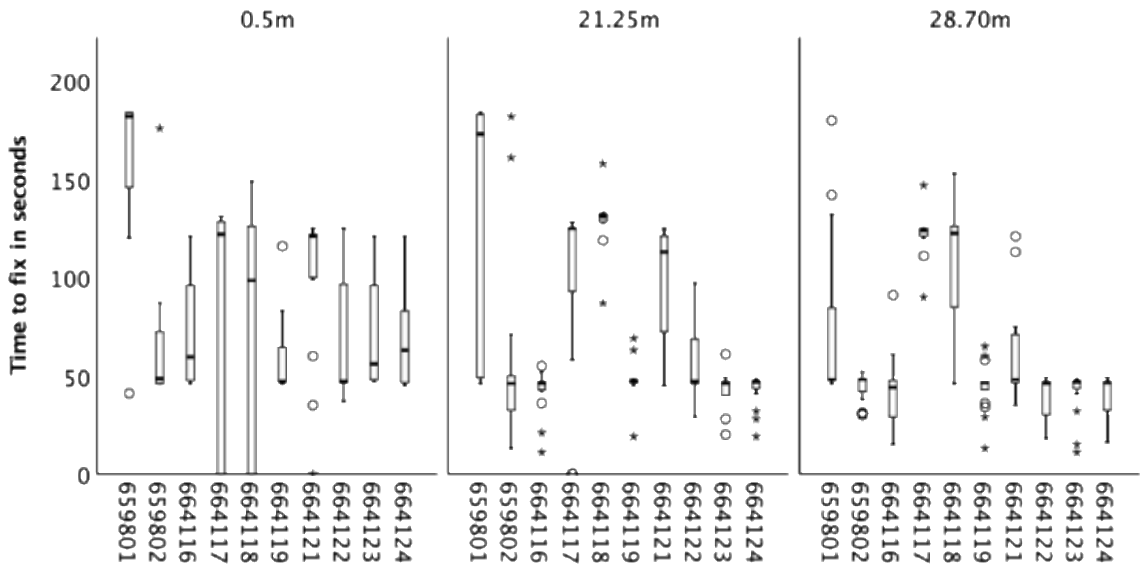

Figure 3.16 Mean time to fix for collars fitted to simulated animals and placed at three heights in a secondary forest located in a ravine. Boxplots show the median (black bar) and first and third quartiles (box). Whiskers show the maximum and minimum values excluding outliers (points). Collars 659801 and 659802 on the left of each column panel are Argos collars, others are GPS-only collars.

Table 3.12 Post hoc pairwise comparison of 3D fix and non-3D fixes for collars on three platforms.

Height 1 (m)	Height 2 (m)	Mean Difference (m)	Std. Error	df	P
0.05	21.25	0.155	0.36	419.137	< 0.001
	28.7	0.343	0.36	491.172	< 0.001
21.25	28.7	0.187	0.36	419.097	< 0.001

Table 3.13 Post hoc pairwise comparison of the time to fix in seconds for fixes for collars on three platforms.

Height 1 (m)	Height 2 (m)	Mean Difference (m)	Std. Error	df	p
0.05	21.25	16.154	3.603	419.18	< 0.001
	28.7	32.178	3.608	419.224	< 0.001
21.25	28.7	16.024	3.592	419.13	< 0.001

3.7 Discussion

3.7.1 Test 1: Presence of a simulated animal

We predicted that the presence of simulated animals would lead to an increase in fix success rates and reduced time to fix for collars in comparison to when a collar is empty. The collars were 100% successful in gaining fixes both with and without a simulated animal, suggesting no major influence of the presence of a simulated animal on fix success. Placing the collars in less than optimal conditions, such as in a forest, however can reveal effects that are not detected under optimal conditions as we used here

(Yamazaki et al., 2008). To understand if the presence of a simulated animal has an effect on fix success rates it may be necessary to conduct such tests under less optimal conditions.

Contrary to our prediction, collars fitted to a simulated animal had a longer time to fix than empty collars. These results suggest that the simulated animal interfered with collar access to satellites. In bears, larger neck circumferences correlated with reduced success rates (Graves and Waller, 2006). Studies have used different substrates for simulated animals including towels soaked in water, water bottles or water balloons (Frair et al., 2010, Forin-Wiart et al., 2015), so our results are not directly comparable to others (Janeau et al., 2004). The density of the towel in comparison to a bottle or balloon filled with water may produce different results and further study is required to determine which simulated animal is most appropriate for testing collar performance.

3.7.2 Test 2: Collar orientation

We found no influence of collar orientation on collar performance. As for the simulated animal test, this may be due to the otherwise ideal conditions of the test. The effect of collar orientation may be more pronounced under more difficult conditions, such as dense forest or topographical obstruction (Yamazaki et al., 2008). Ideally, this test would be conducted on both empty collars and collars fitted to simulated animals in various habitat types.

3.7.3 Test 3: Habitat type

Collars had a higher 3D fix and reduced time to fix in locations that were less densely forested and had more sky availability, supporting our prediction, and consistent with findings of previous studies (Gamo and Rumble, 2000). The collars preformed best in the savanna where mandrills spend little time and worst in the secondary forest which typifies the release area and is where the mandrills spent most of their time. The collars

also performed better in the forest fragment than the secondary forest, presumably because forest with smaller mass produced less obstruction. This effect is likely to be even more pronounced when the same collars are deployed on monkeys. For example, a study of free-ranging Japanese macaques (*Macaca fuscata*) found almost all failed fixes occurred when the animals were in a forested habitat (Sprague et al., 2004).

3.7.4 Test 4: Height in the canopy

Collar height influenced fix success rates. This outcome is probably because the amount of vegetation interfering with the collar's access to satellites decreased with the collars increased vertical position in the forest. This has implications for studies using GPS collars on arboreal, semi-terrestrial or flying animals with systematic sex, age class or individual differences in use of forest strata. In mandrills, for example, males spend most of their time foraging on the ground and females and juveniles spend much of their time foraging in trees (Jouventin, 1975). Because collars are more likely to get fixes in trees, females and juveniles may appear to spend more time in forested areas and adult males more time in lightly wooded areas, forest edges and savanna. Neck circumference negatively correlated with fix success rates in bears (Graves and Waller, 2006) and in mandrills a similar effect may exist. If this were the case the smaller neck circumference of the females in comparison to an adult male may also lead to females having slightly higher fix success rates. The choice to place the collars at 0.5 m to simulate the approximate neck height of a mandrill may have reduced the magnitude of the effect and collars placed directly on the ground would have been exposed to an additional 0.5 m of obstruction.

3.7.5 Test 5: Height in a ravine

Collar height also influenced fix success rates when we tested the collars in a ravine. This effect may be due to either the topography, vegetation, or a combination of

both. The combination of these two variables would be present in most forest with undulating terrains and characterised the area surrounding the release site. The collars were vertically aligned, and all three heights were exposed to the same amount of forest density and topical obstruction from a 2-D perspective. This effect is likely caused by increased height leading to a decrease in the amount of topographical and vegetative obstruction interfering with the collars ability to access satellites. Our findings are relevant to species which live in habitat types where the study species can use trees or other objects to move vertically among obstructions. GPS collars on flying animals who live amongst obstruction which reduces their collar's access to satellites would likely experience a similar effect.

As wildlife habitat is increasingly repurposed by humans, tests 4 and 5 also have potential implications for studies in urban environments. Human-made structures in urban environments may affect GPS collar fix success rates by reducing sky availability (Rose et al., 2005, Adams et al., 2013) and studies may need to account for an animal's use of 3D space. It may not be possible to rely on GPS radio collars to give an accurate description of habitat use in forests. GPS data may be biased by the amount of time the animals spent at various heights within the forest structure independent of forest density. Objects such as telephone poles, fences, satellite towers, bridges and buildings may bias results if they systematically decrease a study subject's access to satellites in parts of their home range.

3.8 Conclusion

We found that forest density, the height of a collar in the forest at the time of a fix and topographical obstruction affect collar performance. Future tests measuring the usefulness of simulated animals in collar testing should test various substrates that are appropriate proxies for wildlife to use as the ground plane. Tests measuring the effect of fitting the collar to a simulated animal should be conducted under conditions such as on a

forest floor, so any effect is more pronounced. Under our conditions collar orientation did not have an effect on collar performance and future tests should be conducted under stressed conditions.

The results for tests 4 and 5 are not directly comparable because the collar heights are different, and topographical obstruction was present in test 5. Under both conditions, increased height increases collar performance, presumably because it reduces the amount of obstruction between the collar and satellites. As a result, animals which are primarily arboreal may be more likely to have successful fixes than animals that are primarily terrestrial. Animals who have a three-dimensional relationship with their environment may have systematic differences in fix success rates caused by the amount of time spent at various heights in the forest canopy. Thus, studies planning to retrospectively correct for biases in collar data caused by forest density may also need to account for the animal's 3D relationship with that vegetation. This is particularly important in species where there may be systematic differences in forest strata usage amongst age groups, sexes and individuals.

A key finding is that the collars with IDs ending in 01, 17, 18 and 21 performed consistently worse than the other collars. Had we analysed these data prior to fitting the collars to animals we would not have used those collars. This highlights the usefulness of performing quality assurance testing under field conditions prior to fitting the collars to the animals. Collars should be tested by the researchers in the environment where the collars will be used before fitting the collars to the animals. I address this topic further in Chapter 4. Finally, stationary tests tend to overstate a collar's ability to attain 3D fixes, so these results probably understate the magnitude of the effects on GPS collars fitted to animals. Thus, disparities in collar function found in stationary tests may be cause to contact the manufacture for technical support or to have the collars replaced where possible.

3.9 Acknowledgements

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Chapter 4: Height bias in GPS Collar Studies: a post-release study of mandrills

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Authorship Contribution Statement:

I conceived the aim, designed the study, collected and analysed data then wrote the chapter, under the supervision of R. A. Hill and J. M. Setchell. L. Pinetea extracted the raw tree height and forest density estimates in GIS for all GPS locations. R. Atencia provided access to staff and resources vital to the project. D. Cox instigated the project, selected the collars and oversaw much of the fundraising and logistics for the project.

4.2 Abstract

Tree height, vegetation density, topographical obstruction and animal behaviour affect GPS fix success rates and may cause biases in GPS collar data. Models to correct for habitat related bias in GPS data assume habitat has a predictable and correctable effect on collar data. However, these models do not consider that the obstructive effect of vegetation, topography and human-made structures is relative to the animal's height from the ground. In this study we aimed to examine whether systematic height-related differences in habitat use across individuals undermines the predictable effect of habitat on GPS collars. We further explore data from the height in the canopy (Section 3.4.4) and height in a ravine (Section 3.4.5) tests from Chapter 3 with the home range analysis tools in the ZoaTrack platform. We then compare the findings from the stationary height test to data retrieved from the GPS collars fitted to a group of mandrills released in the Republic of Congo. Observers collected behavioural data regarding the animal's height and handheld GPS points. The stationary tests showed collar height affects fix success and fix accuracy independently of vegetation or topographical obstruction. We found that mandrills with a larger body mass spent more time on the ground and less time at heights <5 m than animals with a smaller body mass. The mandrills were almost always within 100 m of one another and the observers, but the collar data suggests that the individual animals had very different relationships with their environment. Our findings suggest an animal's three-dimensional position within a habitat will bias data independent of data bias introduced by the habitat type and can undermine the predictable nature of the effect habitat has on GPS collars. GPS collar studies may need to account for the animal's height in its habitat at the time of the fix. Not doing so may introduce height-related biases into home range estimates and misidentify critical habitat boundaries in conservation management plans.

4.3 Introduction

GPS radio collars have lower fix success rates in dense forests with closed canopies and in the presence of topographical obstructions (Gamo and Rumble, 2000, Camp et al., 2016). Chapter 3 explored the effect of tilting the collar away from a vertical orientation (collar position), forest density and height in a forest on fix success rates. Under ideal conditions collar position did not have a significant effect on fix success rates but habitat type and the collar's height in the habitat both had a significant effect on fix success rates and time to fix. However, stationary collar tests, tend to overstate a collar's ability to acquire a fix when compared to deployed collars in the same habitats (Moen et al., 1996). This makes it important to test the effect of height on collar data when worn to better understand the implications of habitat for wildlife studies.

4.3.1 Habitat introduces systematic bias in GPS collar data

Forest density, forest height and topographical obstruction have largely been considered from the two-dimensional perspective in GPS collar studies. However, animals who climb and fly have a three-dimensional relationship with their environment, making the amount of obstruction affecting their collar's performance relative to and dependant on their height at the time of fix.

The three-dimensional distribution of food resources and variation in mammalian body size result in complex foraging behaviour and different habitat use between and within taxa (Bakker and Kelt, 2000). Arboreal, flying and semi-terrestrial animals may have systematic differences in how they use vertical space between sex and age classes, social groups and individual animals. For example, adult black and gold howler monkeys (*Alouatta caraya*) are more likely to travel and spend time on the ground than juveniles and infants who spend more time feeding in, and traveling on, smaller branches higher off the ground (Freeland, 1980). Species who are largely arboreal have a three-dimensional

range. Mangabeys (*Cercocebus albigena*), for example, spend the majority of their time off the ground but move freely within the height range of 6.1-36.58 m (Freeland, 1980). Social dynamics and reproductive strategies can also affect how individuals and groups use their environment. Southern flying squirrels (*Glaucomys vikans*) are more likely to nest in and use habitat without related individuals (Cannan et al., 2011) while in another species of flying squirrels (*Glaucomys volans*) males have significantly different home range areas to each other, but females do not (Taulman and Smith, 2004). Taxa can also have seasonal differences in the amount of time they spend on the ground and in various heights within a forest structure (Miller, 2002), which could cause a seasonal height bias in data. Likewise, bird species can have systematic differences in habitat use within micro-climates within their distribution (Holmes and Robinsoni, 2016). It is therefore important to explore datasets to identify patterns in fix success rates related to species specific behavioural patterns (Aguado et al., 2017).

Mandrills live primarily in dense continuous forests, forage on the ground and in the lower parts of the canopy and sleep in trees (Hoshino, 1985, Lahm, 1986, Norris, 1988). They spend much of their time on the ground digging, tearing apart fallen tree trunks and sifting through leaf litter (Lahm, 1986). Their foraging behaviours place them in many of the same positions that cause decreased fix success rates in bears (Obbard et al., 1998). Young mandrills spend more time in trees and are more likely to escape to trees than adult males (Lahm, 1986). Although Norris (1988) found females spent more time foraging on the ground than males, this is likely to be because there were no adult males in his sample, and in reality, adult males are likely to spend more time on the ground than females.

GPS collar studies also include semi-terrestrial animals living in urban environments (Berentsen et al., 2004, Floyd and Underhill-Day, 2013, Klegarth et al., 2017). Human-made structures reduce sky availability and have a similar negative affect

on fix success rates and data accuracy to vegetation (Adams et al., 2013). Forest fragmentation is increasingly forcing semi-terrestrial and arboreal species to use the ground between trees more frequently and over greater distances (Dale et al., 1994, Prates and Bicca-Marques, 2008). Thus, it may be important to account for the proportions of time animals spend at various heights within their environment in all GPS collar studies with animals who are not entirely terrestrial.

4.3.2 Models for correcting bias

Collars deployed on wild animals in the same habitat can have very different fix success rates as a result of individual differences in habitat use and behaviour (Johnson et al., 2002). Failed fixes and fixes with poor accuracy are more likely to happen in the densely-forested areas where many primate species live (Sprague et al., 2004). It is argued that GPS collar error is largely predictable and can be corrected for under multiple sampling designs through incorporating the collar brand, forest structure, season, terrain and time of day (Frair et al., 2004). GIS technology allows us to compare corrected GPS data with environmental data and is widely used to explore animals' use of an environment (Miller et al., 2004, Moscovice et al., 2010) and to make tree height and density estimations (Rempel et al., 1995, Gamo and Rumble, 2000) that can be used in computer modelling. GIS estimates canopy density by measuring the canopy cover to ground ratio as seen from the air and the forest height by measuring from top of the tree to the ground with Lidar (ESRI, 2016). These estimations can provide detailed environmental data, but they do not account for the animal's height within the environment during a fix attempt.

Our initial objective for this study was to use GPS radio collars to monitor the ranging patterns of a group of mandrills post-release. In viewing the data in point form on a map we found the data acquired by the collars was inconsistent between the collared individuals and understated the use of densely forested areas even though the animals

almost always travelled together and should have had very similar ranging data. Because increased height significantly increased fix success rates in stationary tests (Chapter 3) and stationary tests tend to understate the effect in a deployed environment we decided to focus on height as a possible explanation for the differences in these data. To achieve this, we tested a) for a systematic difference in forest height use between the mandrills and b) whether height affected the point spread in stationary (test) collar data used in the previous chapter. We tested the following hypotheses and predictions.

4.3.3 Hypothesis 1: body mass and behaviour

If body mass has a systematic effect on the amount of time mandrills spend at various heights in their environment, then larger animals will spend more time on the ground and in the lower strata than smaller animals.

4.3.4 Hypothesis 2: collar accuracy and height above the ground

If collar location accuracy decreases with decreased height amongst obstruction, then stationary collars located closer to the ground will have a larger spread of fixes than collars higher off the ground.

4.3.5 Hypothesis 3: fix success and body mass

If fix success rates increase with height, and body mass has an inverse relationship with the amount of time mandrills spend at greater heights within their environment, then smaller animals will have more frequent successful fixes than animals with a larger body mass, given they spend most of their time in dense forests.

4.3.6 Hypothesis 4: fix success and time of day

If collars perform better higher in the forest and when stationary than on the ground and moving, then the percentage of successful fix attempts will be higher at night when mandrills are more likely to be stationary and high off the ground. The adult male

in the release group slept on the ground but was more likely to be stationary than during the day.

4.3.7 Hypothesis 5: fix success and habitat

If habitat has a predictable and correctable effect on GPS collars, then collared animals from the same group should show similar ranging patterns and range size estimates.

4.3.8 Hypothesis 6: collars and handheld GPS data

If collars accurately capture mandrill ranging patterns, then these data should be similar to the handheld GPS data collected by the observers.

4.4 Methods

The Jane Goodall Institute conducted a soft release of 14 wild born mandrills confiscated in Republic of Congo then released into the Conkouati-Douli National Park. We fitted 5 mandrills with store-on-board GPS radio collars and two mandrills with Argos collars (Table 2.2; p 39). We selected mandrills to wear the collars based on their inclusion in the release program and the mean collar weight being less than 5% of their body mass (range 1.71-4.69%; Table 2.2; p 39). We selected the two largest males to wear the Argos collars based on the outcomes of the first published release of mandrills where adult and sub-adult males were the most likely to leave the group immediately, become peripheral or (after infants) die within the first year (Peignot et al., 2008). We had originally hoped to collar all of the animals, but the collars were too heavy and bulky to fit on the younger monkeys.

Argos collars both transmit and store the collar's coordinates making it possible to track the collar's location from any location with access to the internet. GPS collars store the animal's location on the collar and emit a VHF signal that can only be tracked

over short distances. Because the Argos collars were more expensive, we could only afford to put them on the animals that were most likely to leave VHF range. In Chapter 3 we found an effect of collar height on fix success rates. To better understand this effect, we conduct additional analysis on these data here; then explore how the effect may influence fix success rates in radio collars fitted to seven mandrills involved in the release programme (Table 4.1).

We set the collars to attempt either 4 (Argos) or 7 (GPS) fixes every 24 h. The GPS Collars attempted fixes at with seven fixes during daylight hours between 06:30 h and 17:30 h and two night-time fixes. The Argos collar attempted five daytime fixes between 08:00 h and 17:30 h and two night-time fixes. At the selected times the collars attempted to acquire and record the GPS coordinates and location error for fix. We conducted 20-minute focal observations each followed by a scan sample of the animals present between 06:30 h and 18:30 h on observation sheets (Appendix H; Appendix I). We sought to follow these schedules but field conditions caused us to deviate from the behavioural observation schedule regularly. We collected height data every 2 minutes on the focal animal. After each focal observation, we collected a hand-held GPS point from our observation position then conducted a scan sample of the animals we could locate and identify within 4 minutes. We categorised the animal's height as: on the ground; 0-5 m; 5-15 m; 15-30 m; and >30 m. New staff received multiple days of training on height assessment using a measuring tape laid out on the ground. Markers were placed at various points on the measure and the individuals estimated the distance. Existing staff who were present on those days also participated in the trainings to assure they were still assessing the distance categories accurately. Ideally, we would have conducted training with a vertically oriented measure, but it was not feasible to set up and maintain such an apparatus and ground training was the next best option. We did not perform formal

reliability tests. We also had difficulty funding the batteries for the GPS units and often had to conduct the observations without them.

4.5 Analysis

We used a Spearman analysis in SPSS to test for correlations between mandrill mass and percentage of time spent on the ground. We conducted home-range and density estimations in the ZoaTrack platform which has tools for processing GPS collar data and then converts the outputs into KLM files for Google Earth or Shape files for analysis in GIS software packages. We analysed the data from the height in the canopy and height in a ravine tests from Chapter 3 with the home range analysis tools in the ZoaTrack platform. We used two popular tools used to estimate and analyse an animals 2-D home range: kernel density and local convex hull estimators. Kernel density (KD) estimators can be used to produce non-parametric estimates of nearly any shape and are wildly used in home range analysis in wildlife (Erran and Powell, 1996). Local convex hull (LCH) estimates are argued to be superior to kernel densities because they exclude unused areas of the animals range and can be used to more accurately measure the movements of animals where their habitat usage is influenced by natural boundaries such as rocky outcrops, rivers or cliffs (Getz et al., 2007). We processed the date in ArcGIS and QGIS and conducted statistical analysis in SPSS. JGI provided forest height and canopy density estimations extracted from GIS to compare with GPS coordinates. We created the local convex hull with 95% of the 3D fixes, Neighbours: 30. We placed the collars at 0.5 m, 21 m and 29 m.

We used a paired sample t-test in SPSS to compare the percentage of 3D fixes acquired at night and during the day. To test for differences in the number of satellites available during daytime and nighttime fix attempts we used a one-way ANOVA.

4.6 Results

4.6.1 Hypothesis 1: body mass and behaviour

We collected 301 height samples from focal observations and 1376 height observations from scan samples during the study. We found a significant effect of mandrill body mass on the amount of time spent at different levels of the forest strata ($r = 0.807$, $p = 0.003$, $n = 11$). Heavier mandrills spent more time on the ground (Fig 4.1).

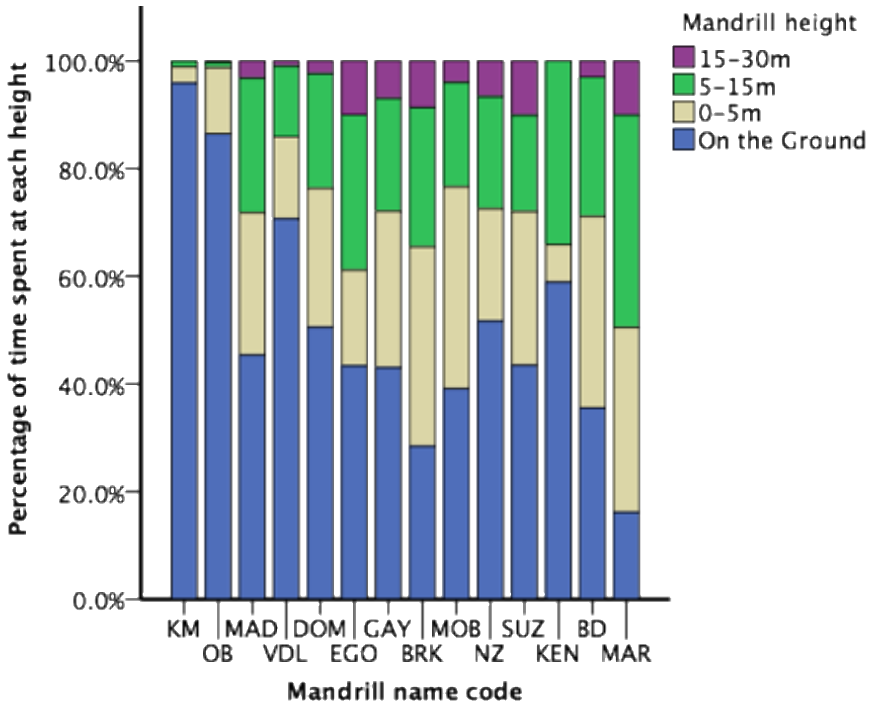


Figure 4.1 The percentage of time each mandrill spent in each of four height categories post-release. Name codes are ordered by the animal's body mass with the largest mandrill on the left and the smallest on the right.

4.6.2 Hypothesis 2: collar accuracy and height

Height had a significant inverse relationship with the point distribution using both methods in both tree height tests (Table 4.1, LCH: area $r_s = -0.975$, $p = 0.005$, Figure 4.2, circumference $r_s = -0.975$, $p = 0.005$, Figure 4.3; KU: area $r_s = -0.975$, $p = 0.005$, Figure 4.4, circumference $r_s = -0.975$, $p = 0.005$, Figure 4.5). The results for the two methods are correlated to the point of redundancy.

The spread of the points decreased with increased height. The LCH analysis of the height in the canopy test (Figure 4.2) and height in a ravine test (Figure 4.3) showed the 3D points collected near the forest floor were more broadly distributed than the points collected higher up in the tree. The results from the KU analysis for the height in the ravine test (Figure 4.4) and height in the canopy test (Figure 4.5) followed the same pattern.

Table 4.1 Point spread perimeter and area for the height in a ravine and height in the canopy tests calculated using Local Convex Hull and Kernel Distribution tools in ZoaTrack

	Local Convex Hull		**Kernel Distribution**		
Height (m)	Perimeter (km)	Area (m^2)	Perimeter (km)	Area (m^2)	
0.5	1.72	79,833	1.84	232,006	
21	0.26	3,361	0.49	15,526	
29	0.16	1,325	0.23	3,887	
0.5	0.67	14,843		0.9	51,637
18.8	0.27	4,287		0.52	20,015

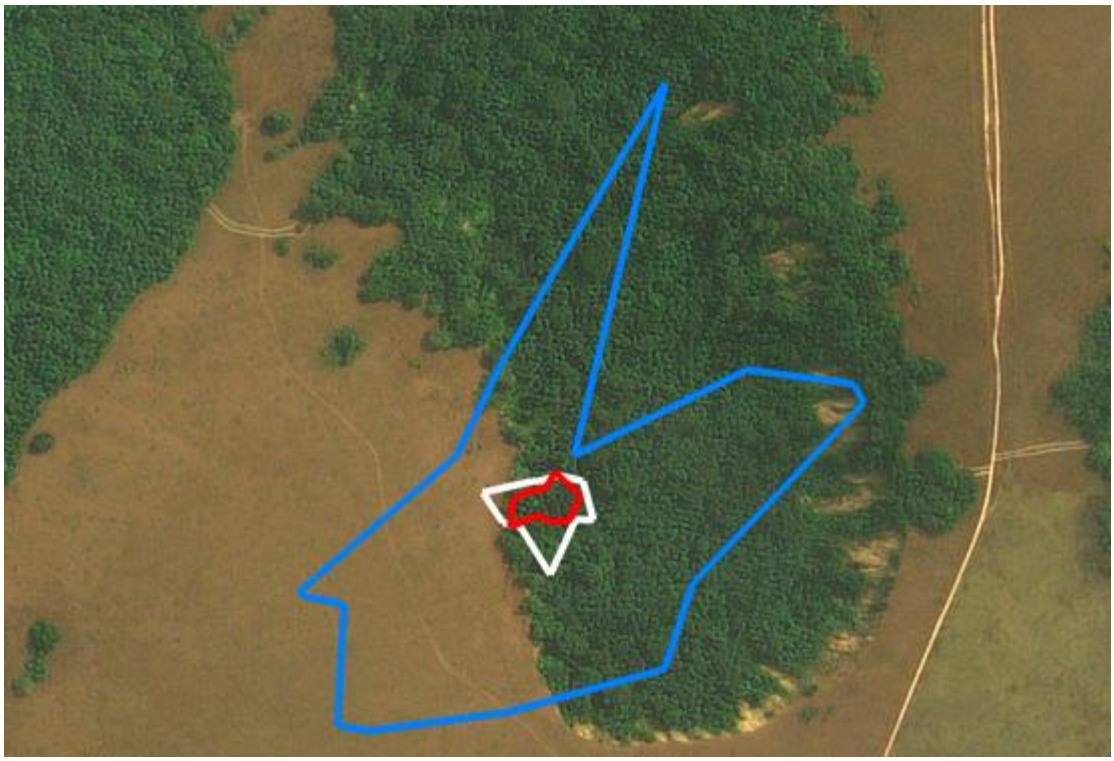


Figure 4.2 Local Convex Hull (95%, Neighbours: 30) of GPS collars in the stationary collar test. The blue polygon was created using the 3D points collected at 0.5 m, the white polygon was created using 3D points collected at 21 m and the red polygon was created using 3D points collected at 29 m.

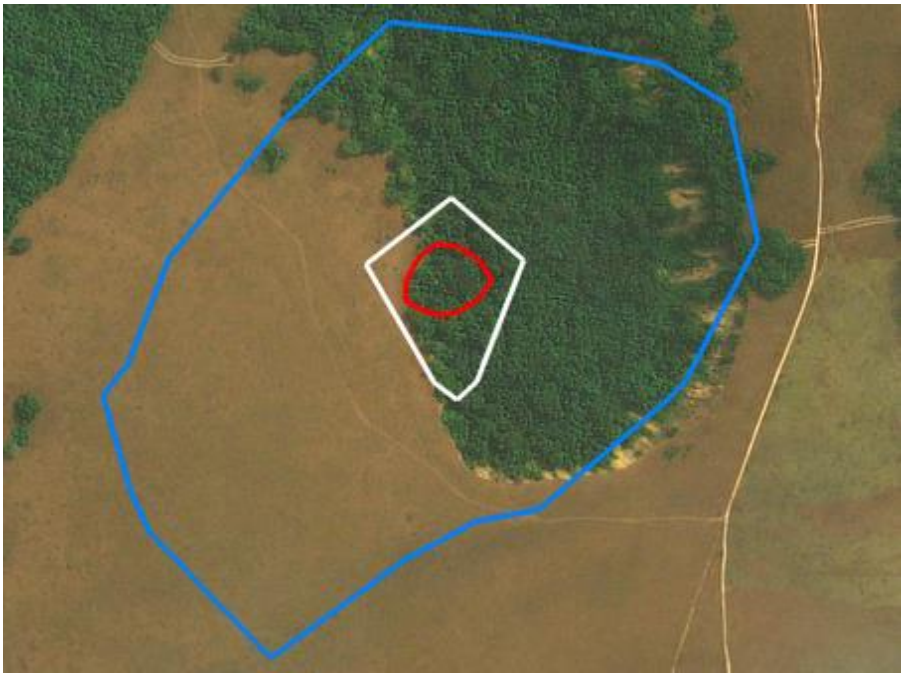


Figure 4.3 Kernel Utilisation Distribution Density of GPS collars in the stationary collar test. The blue polygon was created using 3D points collected at 0.5 m. The white polygon was created using the 3D points collected at 21 m and the red polygon was created using 3D points collected at 29 m.

Figure 4.4 Kernel Utilisation Distribution Density of the height in the canopy test. The blue polygon was created using 3D points collected at 0.5 m. The red polygon was created using 3D points collected at 18.8m.

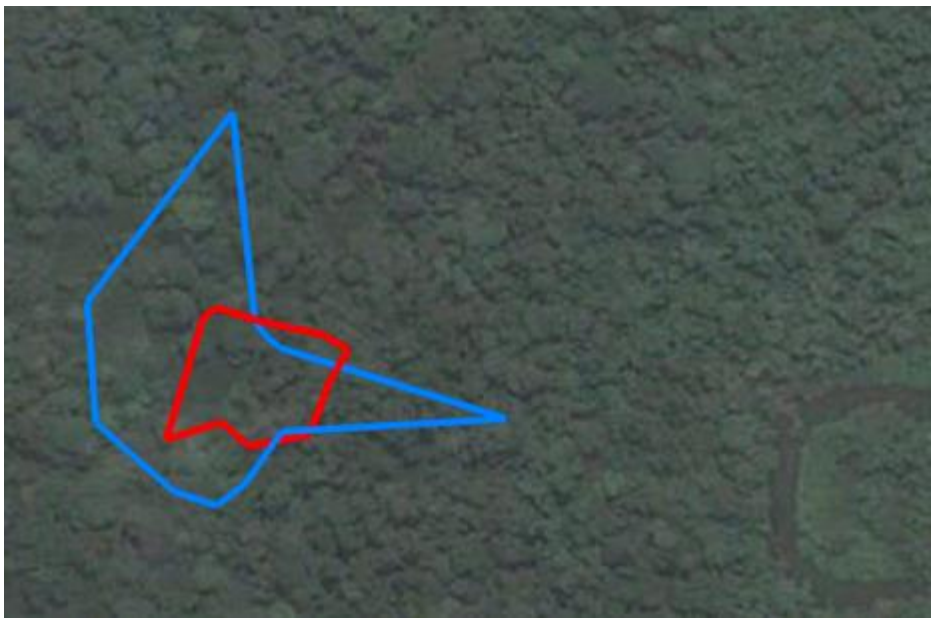


Figure 4.5 Local Convex Hull (95%, Neighbours: 30) density of GPS collars in height in the canopy test. The blue polygon was created using 3D points collected at 0.5 m. The red polygon was created using 3D points collected at 18.8 m.

4.6.3 Hypothesis 3: fix success and body mass

We know from Chapter 3 that three of the collars did not work appropriately. The animal weighing 9.3 kg in the first row of (Table 4.2) and shown in the first column of Figure 4.6 was wearing one of those collars which accounts for its disproportionally low percentage of 3D fixes in comparison to the other animal weighing 9.3 kg. With the broken collar included in the Spearman's correlation, mass had a weak and non-significant relationship with the percentage of 3D (r_s = -0.200, p = 0.352), 2D (r_s = 0.207, p = 0.347), resolved (r_s = -0.657, p = 0.078), unresolved (r_s = -0.600, p = 0.104), and failed fixes (r_s = -0.200, p = 0.352). Removing the broken collar from the analysis resulted in a non-significant but inverse relationship between mass and the percentage of 3D fixes (r_s = -0.700, p = 0.094), a perfectly inverse and highly significant relationship between mass and the percentage of resolved fixes (r_s = -0.100, p < 0.001), a strong and significant inverse relationship with the percentage of unresolved fixes (r_s = -0.900, p = 0.019), and an inverse non-significant relationship with the percentage of failed fixes (r_s = -0.600, p = 0.142).

Table 4.2 Mandrill mass and percentage of collar fixes at each quality level

Mass (kg)	3-D (%)	2-D (%)	Resolved (%)	Unresolved (%)	Failed (%)	Totals
9.3	21	0	617	20	11	669
9.3	940	2	2541	64	69	3616
12.0	772	0	2369	76	126	3343
16.5	24	0	988	50	463	1525
20.0	2	0	105	3	44	154
34.0	95	2	0	0	0	97
Totals	1854	4	6620	213	713	9404

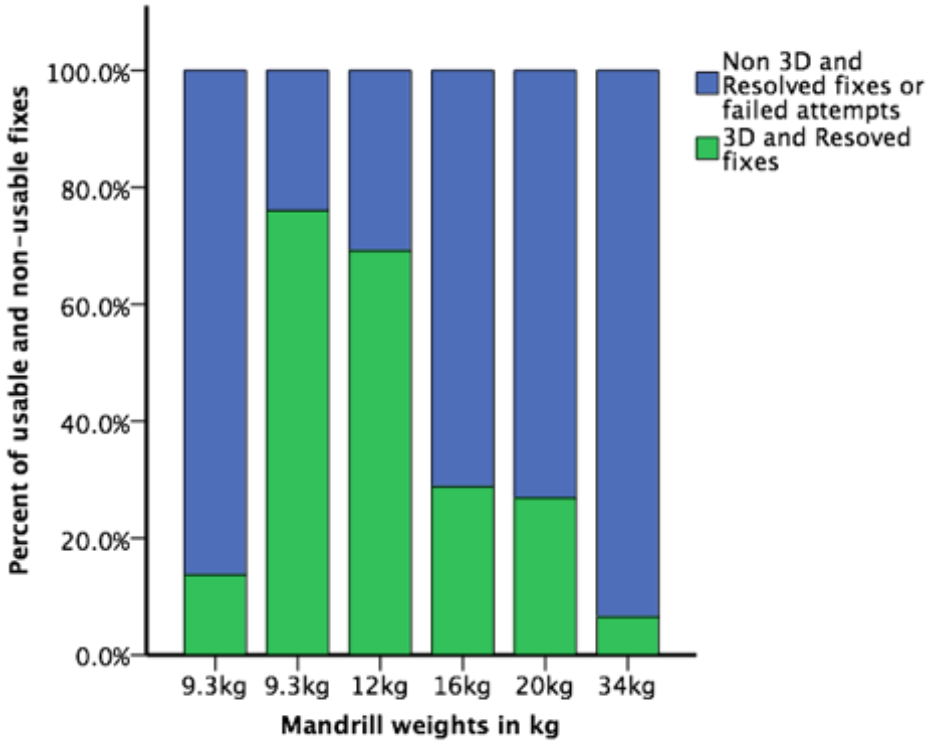


Figure 4.6 Percentage of 3D and resolved fixes by body mass. The collar on the left was faulty.

4.6.4 Hypothesis 4: fix success and time of day

Approximately 92% of nighttime fixes (n = 2284) and 55.8% of the daytime (n = 6254) fixes were accurate within 30 m (Figure 4.7). Although the collars only attempted 26.12 % of the fixes at night, they acquired ~62% of the total 3D fixes and 35.6% of all usable fixes at night. The collars acquired a significant portion of their 3Dfixes at night

when only 26.12 % of the total fix attempts occurred. There were significantly more 3D fixes at night than during the day (X_2 (1) = 1810.50, $p < 0.01$).

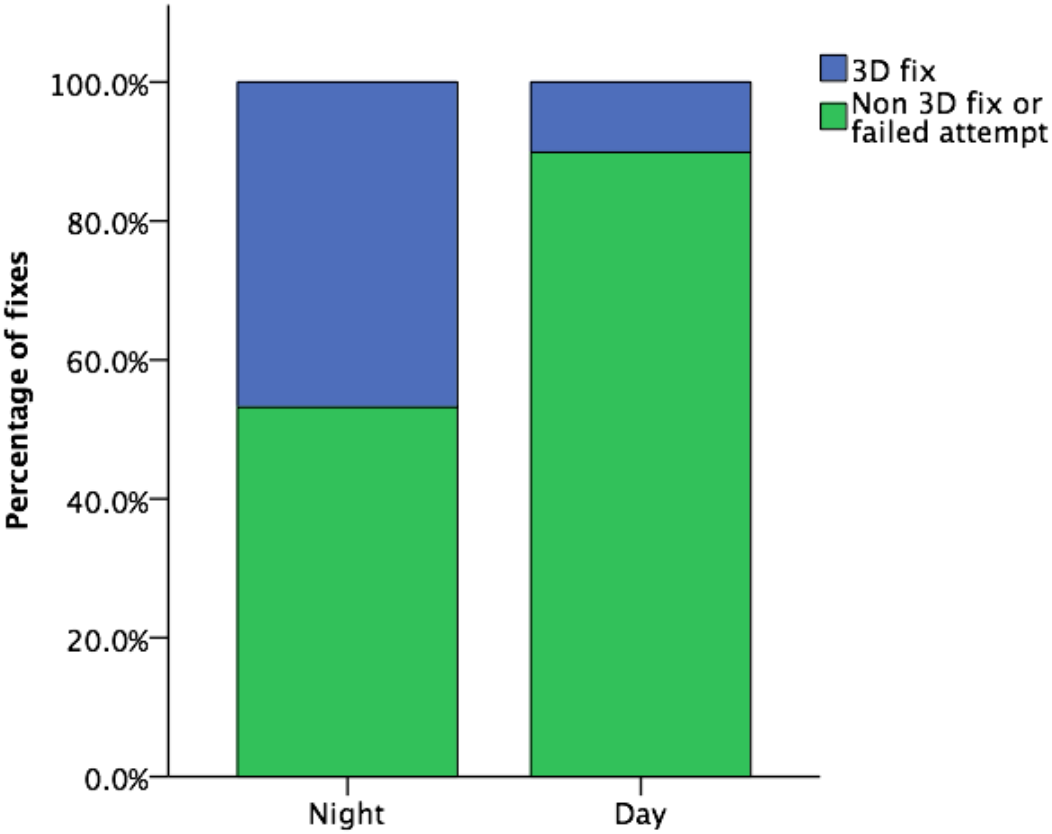


Figure 4.7 Percentage of 3D fixes and Non 3D fixes split by night and day attempts.

The collars made 13,663 fix attempts during the 15-month study. Of the fix attempts, 2,467 were at night and 11,202 were during the day. The percentage of successful 3-D fixes (~ 2-15 m accuracy) was low (9.7%), but less accurate resolved fixes (≤ 30 m) were relatively abundant (34.7%). Resolved uncertain fixes (≤75 m) made up 1.1% of the total fix attempts, unresolved (within several hundred meters) made up 3.7% and 50.6% provided no data.

On average the collars acquired a higher percentage of 3D fixes at night (mean = 25.18%, SE = 11.44%) than during the day (mean = 5%, SE = 2.31%). This difference

was significant (t_5 = 2.21, p = 0.039) with a large effect size (d = 4.04). There were significantly more available satellites ($F_{1, 14696}$ = 56.25, p=<.001), during daytime fix attempts (mean = 5.10, SE = 0.023) than during nighttime fix attempts (mean = 4.75, SE = 0.023).

4.6.5 Hypothesis 5: Fix success and habitat

The mandrills spent most of their time under canopy cover. Analysis of the fix locations against forest density estimations in GIS showed 77% of the combined 3D and resolved fixes occurred in areas with a 89-91% density and 83.2 % occurred in areas with a mean tree height of 14-17 m. A majority of the 3D fixes (88.9%) occurred in areas with a forest density of 89-91% and 92% occurred in areas with an estimated tree height of 14-17 m.

The animals travelled together on most days except when one animal remained in camp. Because the animals were in the same areas at the same time their points should look the same. However, when we used all 3D and resolved fixes for each animal to construct the LCH distribution, the results suggest differences in habitat use that did not actually exist (Figure 4.8).

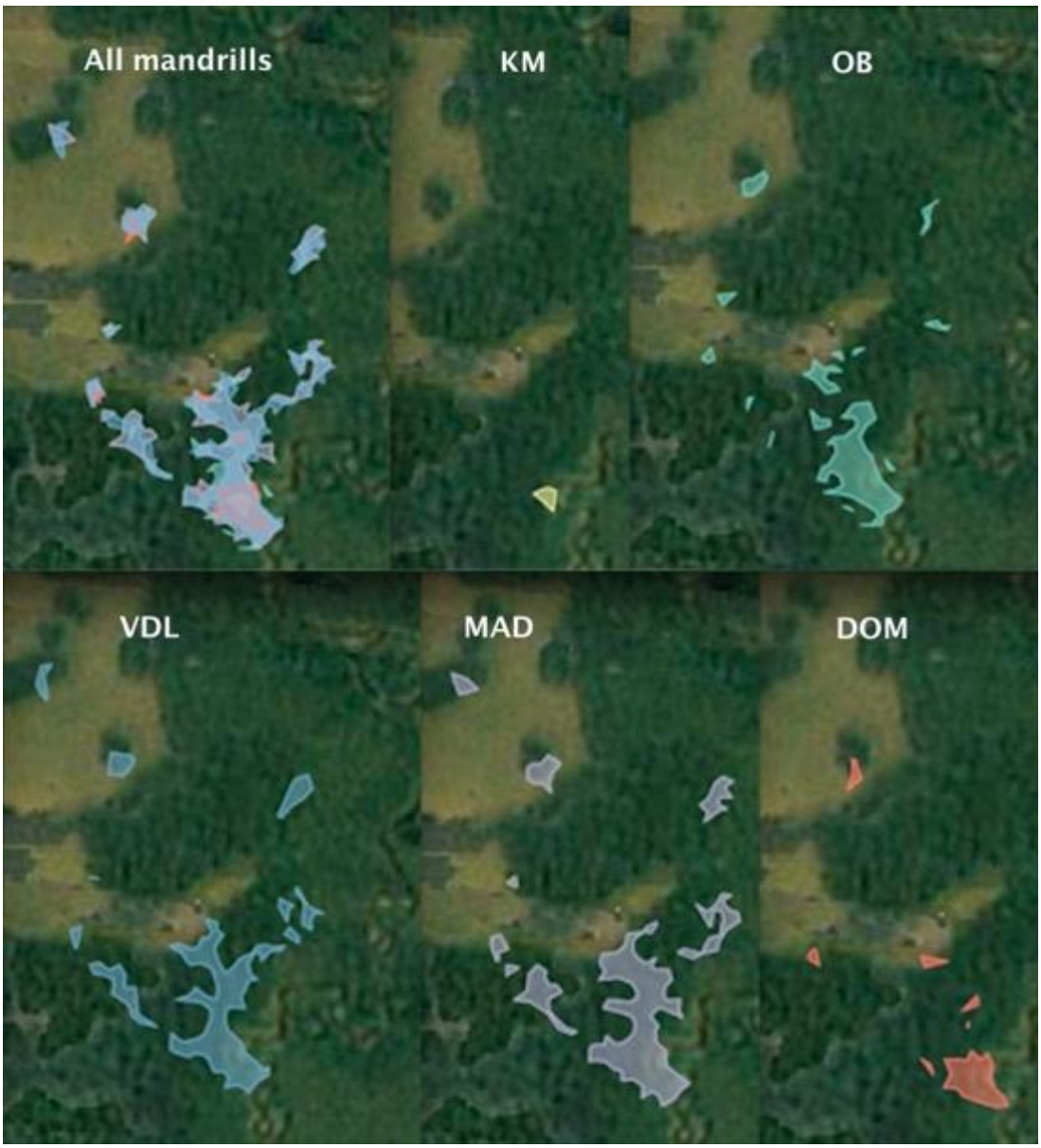


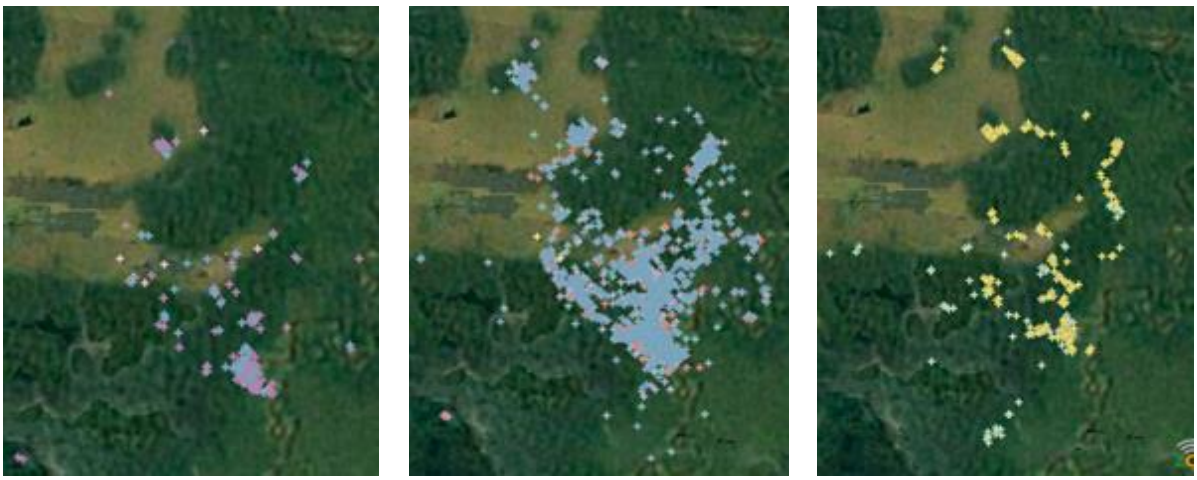
Figure 4.8 Local Convex Hull (95% 40 m) for 3D and resolved daytime fixes. The image on the top left is a compilation of all of the ranges overlaid, followed by mandrill KM in yellow, OB in Green, VDL in blue, MAD in purple and DOM in red

Figure 4.9 shows the distribution of fixes in point form. Two animals only had one 3D daytime fix during the study: the female with the faulty collar (DOM) and the other was the adult male who vary rarely left the ground (KM).

Figure 4.9 Successful daytime 3D fixes. The image on the top left is all 3D daytime fixes from all collars, followed by mandrill KM in yellow, OB in Green, VDL in blue, MAD in purple and DOM in red.

4.6.6 Hypothesis 6: Collars and handheld GPS data

We could not measure the difference between fix data from the GPS collars and the hand-held GPS units because the handheld fixes were not synchronised with the GPS collar fix attempts and two of the collars had too few points for analysis. However, we could compare data for the same animals over the same time period (Figure 4.10). The 3D daytime fixes show limited use of the denser parts of the forested area (Figure 4.10a). 3D and resolved fixes show broader use of the area including points on both sides of the river (Figure 4.10b). The hand-held points show use of the forest around the paths used by the team (Figure 4.10c).



a) 3D daytime fixes *b) 3D and resolved daytime fixes* *c) Handheld GPS Data*

Figure 4.10 Point distribution of GPS collar data (a, b) and hand-held GPS data (c)

4.7 Discussion

The positive correlation between the amount of time spent on the ground and body mass is similar to the findings in other primate species (Freeland, 1980) and aligns with previous observations of mandrills (Lahm, 1985). In mandrills adult females weigh

~1/3 the mass of adult males (Setchell, 1999) and would thus be expected to spend more time off the ground than adolescent and adult males, making them better candidates for collaring than their larger male counterparts. We discontinued observations when we could no longer see the animals which biased the observations away from the highest portions of the canopy in both the focal and scan sampling. In this study, the small sample size and the bias towards observations closer to the ground probably underestimated the amount of time the females and juveniles spent in the higher parts of the canopy.

The location accuracy of the 3D GPS fixes increased as the collar height in the forest increased. This is similar to findings in radio-telemetry where location error significantly increased in dense forests with changes in height <1 m (Grovenburg et al., 2013). In GPS collars, changes in forest density affected fix acquisition and location error (D'Eon et al. 2002). From a 2D perspective forest biomass is relative to the collar's vertical position within that biomass. Home range size and distribution of points in a home range may vary in relation to the amount of time an animal spends in a particular level of the forest strata.

During the data analysis for Chapter 3 we found one of the two of the collars did not function appropriately. Unfortunately, we did not know that before the release and fitted that collar on one of our females. As the result her collar has a disproportionate percentage of failed fixed in comparison to the other 9.3 kg mandrill who had a functioning collar. The results of the ravine test in Chapter 3 showed us that this collar had no successful fixes at 0.5 m and 21.25 m but did have successful fixes at 28.7 m. As we know the collar only received fixes when in the higher strata, the fact that this collar had any fixes at all when on the mandrill may indicate that this animal spent a great deal of time high in the trees.

Because a majority of the fix attempts took place in dense forest with a mean height of 14–17 m smaller animals may have acquired more fixes because they spent

more time in the trees. The increased height gave their collars more access to satellites than those of the larger animals. The height profiles for the two mandrills weighing ~9 kg was very similar, and their fix rates are likely to have been similar if collar 7 had worked. This highlights the importance of redundancy when collaring group-living primates. These two monkeys travelled together daily and spent similar amounts of time foraging off the ground but without behavioural observations the data would have given the impression they had very different and separate relationships with the area. This also highlights the importance of validating measures prior to data collection (Setchell 2019).

The collars had higher fix success rates at night when the animals were more likely to be stationary and off the ground than during the day. It is not possible to say if height, lack of movement or a combination of the two variables was responsible for the effect because the test confounds height and movement. These findings are useful for planning collar studies with species who have regular diurnal patterns in relation to sky availability and movement. In animals who sleep in conditions with greater sky availability than during their waking hours, researchers can expect higher fix success rates and a decreased point spread at night than during waking hours. The percentage of 3-D fixes was greater at night while the animals were more likely to have been stationary and in the canopy. The adult male was an exception and typically slept on the ground in the centre of the camp against one of the exterior walls of the pre-release enclosure. This area provided a relatively large amount of sky availability in comparison to the surrounding forest.

Home range patterns using collar data were different between animals because the number of usable fixes varied between the individuals. The combined point set was more representative of the animal's movements as captured by the handheld units but favoured points near open and less dense portions of the study area and missed areas of the release site the mandrills regularly used.

The handheld GPS data was more representative of the group's daily ranging activities than the GPS collar data. In densely forested areas collar studies should be limited to answering questions that do not require high levels of fix success and accuracy.

4.7.1 Implications for wildlife collar studies

Collar studies conducted with species who have a 3D relationship with objects in their environment should consider the effect of height-related bias on their data. Correcting for bias in GPS collar data using only environmental factors such as tree height and forest density misses out on systematic bias introduced by individual behaviour. In Chapter 3 we found the height of a collar in a forest and forested ravine affected fix success rates and time to fix. The predictability of GPS collar error relating to environmental factors is therefore dependent on understanding the collar's 3D position at the time of the fix attempt. Thus, in environments where increased height leads to increased sky availability, a GPS collar's 3D relationship with that environment affects its fix success rates, time to fix, and fix accuracy.

Individuals in this group of mandrills had systematic differences in the amount of time they spent at various heights in the forest and those differences corelated with fix success rates. These behaviour-dependant differences between individuals led to substantially different records for individual ranges and the habitat types they frequented during the release program.

It is unfortunate that collars perform poorly in the forested habitats that many species of wildlife inhabit. This poor performance could be dangerous if the bias in the data is not accounted for prior to using it to inform management decisions. It has been suggested that collaring a single animal in group-living monkeys can provide collar data that is representative of the movements of the whole group (Stark et al., 2017). Our

results indicate collaring one animal in this group would have produced entirely different accounts depending on which animal we collared (Figure 4.9).

In mandrills, adult females may be the best candidates for collaring in long-term studies because they are not growing and spend much of their time in the trees. One consideration with collaring adult females is collars with large battery packs, wide bands, or are that are fitted too tightly may obstruct the mother's view of and ability to groom dependant young. In short-term studies where the collars have an automatic release function, young adolescent males may also be an appropriate choice because they are large enough to carry a collar and small enough to spend a lot of time off the ground. The study would need to ensure the collars self-released before they became tight around the animal's neck due to growth. Mandrill growth rates have been well documented (Setchell et al. 2001) and those studies can be used to estimate a reasonable timeframe for the automatic release function.

4.8 Potential areas of research to understand and reduce height bias.

Some GPS collars provide proximity data for other animals present (Prange et al., 2006). In group-living species, collaring a sample of animals across age and sex classes may provide the opportunity to reduce error in home range distribution caused by height-related bias in data. Proximity sensors may provide a mechanism to reduce height-related bias in collar data for group-living animals. The sensors in the collars would account for animals who are present but whose collars could not acquire a fix because of their position in the forest strata. Proximity sensors may be a less useful solution in species with high fission- fusion dynamics such as chimpanzees where long periods of time may pass between contact with other members of their community. Further study is required to understand if proximity sensors are a practical, cost effective method for correcting height related bias in collar data.

In environments where fix success rates are predicted to be low, building research questions around behavioural patterns associated with increased sky availability may be useful in reducing height related bias in data. Chimpanzees nest off the ground at night, predictably increasing their collars' sky availability and likelihood of fix success and decreasing the magnitude of height-related bias in data collected at that time. Some bird species also tend to sleep in trees at night and forage on the ground during the day (Ayala-Guerrero et al., 2003). Primarily arboreal nocturnal species such as the koala (*Phascolarctos cinereus*), however, sleep in trees during the day and are more likely to be on the ground at night (Hasegawa and Carrick, 1995).

These systematic behavioural patterns may be useful in taxa where collars are less likely to acquire fixes during periods of the day spent terrestrial. There is a trade-off between the number of fixes a collar can attempt and battery life. In this study the fix attempts were separated from the previous fixes by multiple hours in an attempt to collect ranging data over a longer period. However, collars are more likely to acquire a successful fix within 15 minutes of the previous fix because they can benefit from the stored satellite locations from the previous fix (Adams et al., 2013). The collars deployed in this study acquired very few fixes. Prior to deployment it may have been useful to test if making two consecutive fix attempts in the release area increased the likelihood of achieving the second fix. The first attempt could be removed in the cleaning process and the second attempt used in the analysis. This doubling of fixes would consume battery life but may result in more usable fixes over the study. It is important to understand and then operate within the optimal bounds of the equipment used rather than attempt to stretch functionality to reach unrealistic ideals.

Chapter 5: Faecal glucocorticoids as a biological measure of welfare during captive transfers and primate release

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Authorship Contribution Statement:

I conceived the aim, designed the study, collected samples, analysed data and wrote the chapter, under the supervision of J.M. Setchell and R.A. Hill. I also oversaw and participated in the collection and processing of the faecal samples, along with G.T. Woodruff who oversaw the veterinary staff and project while I was out of the country for several weeks. R. Atencia specified the release protocol. D. Cox instigated the project and oversaw much of the fundraising and logistics. The Disney Animal Kingdom partners conducted the assay validation and the hormone analysis. C.J. Wheaton and S.R. Lavin validated the endocrine procedures used in this study, processed the samples used in this study and provided critical feedback on the methods of the study.

5.2 Abstract

Primate sanctuaries are increasingly planning to release animals into the wild for animal welfare reasons and to relieve overcrowding. Stress associated with release and translocation is a major cause of failure in release programs. Faecal glucocorticoid metabolites (FGCMs) are a noninvasive proxy for the stress response in wildlife. We measured FGCMs at each stage of a release of 15 confiscated, orphaned mandrills (*Mandrillus sphinx*) into Conkouati-Douli National Park, Republic of Congo. The mandrills were initially housed at Tchimpounga Chimpanzee Rehabilitation Sanctuary, then transferred to a pre-release enclosure in the National Park, and finally released into the surrounding forest. We predicted that FGCMs would increase after transfer to the pre-release enclosure, then decrease as animals habituated to their conditions. We predicted the same pattern of increase then decrease after the animals were released. We collected 1143 faecal samples from known individuals and quantified FGCMs using an enzyme-immunoassay for 11-β-hydrozyaetiocholanolone based on biological validation in zoo mandrills. Transfer to the pre-release enclosure caused an increase in FGCM values and those values decreased over the following four weeks; however, FGCM values did not increase significantly post-release and did not decrease significantly over the first four weeks post-release. These results show that non-invasive measures of stress physiology are useful to monitor the physiological stress response during primate release programs.

5.3 Introduction

Primate populations are being depleted globally by human pressures, including habitat destruction (Morgan and Sanz, 2007), illegal hunting (Pourrut et al., 2011), and the illegal pet trade (Mack and Mittermeier, 1984). These pressures have created an abundance of illegally traded and kept primates, many of which are later confiscated by

government agencies. Primate sanctuaries have arisen in response to the need to care for confiscated primates. These sanctuaries are rapidly reaching, or have exceeded, their carrying capacity (Faust et al., 2011). Limited capacity and the belief that release is good for animal welfare has made the release of primates into the wild a goal for many primate sanctuaries (Trayford and Farmer, 2012). If an animal is released under suitable circumstances and adapts to life in the wild its welfare may improve in comparison to its life at the sanctuary, however many animals suffer and die as the direct outcome of being released into the wild (King et al., 2011, Guy et al., 2012). It is thus important for sanctuaries to measure whether release into the wild improves animal welfare.

In addition to welfare releases conducted by sanctuaries, conservation projects also attempt to fortify depleted wild populations by introducing captive bred and reared animals or translocating wild animals from other areas (e.g., gorillas *Gorilla gorilla*; King *et al.*, 2009; golden lion tamarins *Leontopithecus rosalia*; Soorae, 2010). Moreover, animals also need to be translocated from areas where humans are intending to or have destroyed the habitat (Soorae and Baker, 2002). With approximately 60% of non-human primate species at risk of extinction and approximately 70% of populations in decline (Estrada et al., 2017), the welfare and survival of the remaining populations, including translocated and reintroduced animals, is more important than ever.

The stress associated with release into the wild and translocation is a major cause of failure in release programs (Teixeira et al., 2007). Stressors are environmental disturbances that disrupt the body's physiological homeostasis; the stress response is the body's attempt to return to physiological homeostasis after it has been disrupted (Sapolsky, 1987). The stress response activates the hypothalamic-pituitary-adrenal releasing hormones including glucocorticoids which attempt to return the animal to homeostasis (Sapolsky et al., 2000). An acute stress response can help an animal escape a stressor and an increase in glucocorticoids indicates the animal is coping physiologically

in response to stress. However, pathological effects such as impaired cognition, growth, reproduction and immunity are associated with chronic stress, which can negatively influence animal health, cognitive processes and behavioural competence (Sapolsky et al., 2000), and ultimately affect the fate of a released or translocated animal.

The International Union for Conservation of Nature (IUCN) is a union of government agencies, states, and non-governmental organisations from around the world that address conservation issues at local, regional, and global levels (IUCN, 2002). Within the IUCN, the Reintroduction Specialist Group is a network sharing lessons from past release programs and providing recommendations to release project managers wishing to use release to address the loss of biodiversity (IUCN, 2010). IUCN has produced Guidelines for Nonhuman Primate Reintroductions as a practical set of methods for release project managers to follow during the planning, execution and assessment of a release project (Soorae and Baker, 2002). These guidelines separate release projects into two categories: 1) soft release, where animals receive pre-release training, are housed temporarily in a pre-release enclosure at the release site, and receive post-release supplementary food and training (Scott-Brown et al., 1986); and 2) hard release, where animals do not receive training or support before or after the release (Kleiman, 1989). Soft release is thought to be preferable to hard release, as it helps the animals adjust to the new environment (Soorae and Baker, 2002) and thus to reduce stress.

During the release process animals may be immobilised or held in isolation during transfer (Soorae and Baker, 2002). They may experience disruptions to their social structure (Teixeira et al., 2007) and changes in the staff working directly with them. They also experience changes in their physical environment and may be exposed to novel sounds and smells. These stimuli create a situation that includes both uncontrollable and unpredictable circumstances, making the term “stressful” appropriate to describe their experiences (Koolhaas et al., 2011). The physiological effects of stressors in release

projects can be cumulative (Aguilar-Cucurachi et al., 2010) and are likely to take the animals into a state of chronic stress (Dickens et al., 2010). However, we do not have a good understanding of how long it takes animals to physiologically recover from stressors at various stages of release into the wild (Fischer and Lindenmayer, 2000).

Both behavioural and physiological methods can be used to measure stress in primates. Self-directed behaviour (SDB) has been used as a non-invasive indicator of emotional states (Maestripieri et al., 1992) and anxiety levels in primates (Manson & Perry 1999). SDBs include auto-grooming, yawning, body shaking and scratching, and are associated with stress in primates (Shino 1988). Faecal glucocorticoid cortisol metabolites (FGCMs) are a biomarker of the state of an animal's wellbeing (reviewed in Touma & Palme 2005). Glucocorticoids are metabolised in the liver and their conjugates are excreted into the gut where they are then metabolized by microbial flora (reviewed in Touma & Palme 2005). FGCM analysis is useful because faeces can be collected noninvasively and provides an integrated measure of hormonal activity over a period of hours or days rather than point samples with large daily fluctuations as in blood saliva or urine (Whitten et al., 1998a, Heistermann, 2010).

FGCMs may be more appropriate than SDBs to measure the cumulative physiological effects of stress associated with failure in releases. Behavioural measures of stress do not correlate significantly with FGCMs in Old World primates. For example, FGCMs and rates of SDB were not correlated in female wild olive baboons (*Papio hamadryas anubis*) (Higham et al., 2009). In captive baboons, social crowding lead to an increase in salivary cortisol but not reliably elevated levels of SDB (Pearson et al, 2015). SDBs may be a better indicator of anxiety than of physiological stress and should be used with caution as an indicator of stress or anxiety in general because they can be driven solely by an animals temperament (Maestripieri, 2000).

Several studies have examined the effects of disturbance, translocation and mixing of groups in captive environments on an animal's FGCM values. For example, a captive female gorilla exposed to many of the potential stressors involved in the release process - transfer, a novel environment, isolation from conspecifics and exposure to new human caretakers - showed an elevated glucocorticoid response (Jacobs et al., 2014). When the female was introduced to a male, both gorillas showed increased glucocorticoid levels (Jacobs et al., 2014). Several species of felids showed significant increases in glucocorticoids during 24 weeks of environmental disturbance caused by zoo enclosure renovations compared to pre-construction values (Chosy et al., 2014). The values remained elevated for the 13 week sampling period post transfer into the new enclosure, indicating the animals were either still stressed by the renovations or did not habituate to the new habitat in that timeframe (Chosy et al., 2014).

Mandrills (*Mandrillus sphinx*) are large-bodied, sexually dimorphic, forest dwelling, semi-terrestrial, social primates, found in Cameroon, Equatorial Guinea, Gabon and Republic of Congo (Grubb, 1973). They live in habitats subject to human exploitation. Mandrills are most threatened in the Republic of Congo where the main reason for their risk status is heavy hunting for bushmeat (Oates and Butynski, 2008). Two groups have released mandrills to the wild. The International Center for Medical Research in Franceville, Gabon, released mandrills into Lekedi Park, Gabon, with 33% mortality in the first 8 weeks of the initial 12 month study (Peignot et al., 2008). The animals were tracked by radio-collar triangulation only for the first 8 weeks and the exact cause of the deaths is unknown. The authors attributed the deaths to environmental stress and malnutrition because the remaining animals were thin and instituted an supplemental feeding programme as a result (Peignot et al. 2008). The authors recommend future releases do not make assumptions about the benefits of pre-release ecological or social experience and highlighted that ecological adaptation was the largest challenge their

release group faced. They also highlight that hard releases and releasing pregnant females or those with dependant young should be avoided for mandrills (Peignot et al., 2008).

In 2009, The Jane Goodall Institute Tchimpounga Chimpanzee Rehabilitation Centre (Tchimpounga) conducted releases of 1-4 mandrills in Conkouati-Douli National Park, Republic of Congo. They transferred the mandrills to a release site in pet carriers and then released them in an area where supplemental food was readily available. Data sheets with the exact timing, methods and group dynamics of the releases were lost. The mandrills left the release site separately within hours or days and were not seen again.

Together these mandrill releases suggest that the first 8 weeks post-release are critical, that supplemental feeding is required and a habituation period at the release site is needed. Building on these experiences, Tchimpounga decided to include a pre-release enclosure at the release site to allow the animals to acclimatise to the new location in this study. This created the opportunity to study the animals for an extended period of time in three phases of the release process: at the sanctuary, in the pre-release enclosure and post-release.

Our objective in this study was to use Tchimpounga's second mandrill release effort to improve release procedures for non-human primates by measuring glucocorticoids at each stage of the release process to identify how long it takes an animal to overcome the stressors associated with each phase of the release. To achieve this we validated non-invasive, field-friendly methods used successfully in other primate species to measure faecal glucocorticoids in mandrills. Then, we a) measured FGCM levels of mandrills housed at the sanctuary, in a pre-release enclosure, and when released into the forest, and b) explored the magnitude and duration of changes in FGCMs caused by the release process. We tested the following hypotheses and predictions:

1: If the events associated with translocations are stressful for the mandrills, then transfer to the pre-release enclosure will lead to an increase in FGCMs in comparison to their FGCM values at the sanctuary.

2: If it takes time for the mandrills to acclimatise to their new surroundings, then the FGCM response associated with the transfer will decrease over a period of weeks as the animals overcome the stress related to transfer and habituate to their new environment.

3: If release from a pre-release enclosure is stressful for the mandrills, then release will lead to an increase in FGCM values.

4: If it takes time for the mandrills to acclimatise to the release environment, then the FGCM response associated with the release will decrease over a period of weeks.

5: If the mandrills have habituated to the release environment in the pre-release enclosure the magnitude of the glucocorticoid response at release will be lower than at transfer to the pre-release enclosure.

6: If the release environment is less stressful than the sanctuary, then, after an acclimation period, FGCM values will be lower post-release than in the sanctuary.

7: If there is a sex difference in response to the release process, then, there will be a significant difference in the magnitude of the response between the males and females.

5.4 Methods

5.4.1 Study site

We conducted fieldwork in two protected areas in the Republic of Congo (Congo). We first received, rehabilitated and housed mandrills at Tchimpounga in the Tchimpounga Reserve in southern Congo (UTM 32 M 814303 9500175). We then transferred the animals to Conkouati-Douli National Park (UTM 32 M 774300 9567971).

We conducted the study in three locations in these protected areas: the mandrill enclosures at Tchimpounga; a pre-release enclosure in Conkouati; and the forest directly surrounding the pre-release enclosure, into which we released the animals.

5.4.2 Study animals

The mandrills who participated in the study were bushmeat or pet trade orphans confiscated by, or with the approval of, the Congolese environmental law enforcement agency, the Ministère de l'Economie Forestière. After confiscation, they were transferred to Tchimpounga for long-term care. Tchimpounga was responsible for 15 confiscated mandrills (10M/5F; Table 2.1 p. 24) and two animals born during the study(1M/1F). Only the animals born during the study are known to be related to other members of the group. The initial study group was composed of seven mandrills housed together in a stable group for over a year, aged approximately 4-11 years old. We did not include an adult male from the original group because he was aggressive towards staff. During the course of the study we received two additional animals we deemed inappropriate for release because one was less than a year old and the other had trouble walking and was aggressive towards observers.

We released the first seven mandrills in two groups (Table 2.1 p. 24) to ensure we had sufficient resources to track the animals before dealing with a larger number. The first five mandrills (Group 1) remained near the cage so we released the second group (Group 2) seven days later. The adolescent males in Group 1 and the adolescent male in Group 2 fought. To avoid further wounding the male in Group 2 was returned to the sanctuary. The dominant female in Group 1 was aggressive towards observers and also removed prior to the release of Group 2 (March 2014). We released a further five new arrivals (Group 3) in January 2015.

5.4.3 Tchimpounga enclosure

At Tchimpounga the mandrills were housed in three outdoor enclosures with corrugated tin roofs, walls 3 m high, chain-link sides, dirt floors and a 1 m concrete brick foundation around the perimeter (Figures 2.5-2.9). The enclosures were divided into two separate areas by a chain link fence above a 1 m brick foundation and a sliding door. Each enclosure had diagonal structural elements passing through the centre and fire hoses or hammocks as enrichment, and platforms made with planks and perches in the corners to allow the mandrills to leave the ground. Only two of the enclosures were constructed at the time of faecal sampling at the sanctuary and these were connected by a chain-link corridor passing through the space where Enclosure 3 was later built. The mandrills in Groups 2 and 3 had access to all three enclosures. The total area of Enclosure 1 was approximately 30 m^2 and the total area of Enclosures 2 and 3 approximately 43 m^2.

For all release groups we tried to maintain a consistent diet by providing the mandrills with leafy greens, fresh branches, tree limbs and entire aframomum plants at Tchimpounga and in the pre-release enclosure. We did this as training, to aid their transition and avoid large differences in dietary fibre between captivity and post-release, which has been shown to affect FCGMs (Von Der Ohe et al. 2004). JGI staff fed the captive mandrills approximately 2 kg per animal per day. After release the mandrills received a combination of seasonal fruit, rice, and sweet potatoes every morning and afternoon. JGI staff shut the sliding gate between the two sides of each enclosure during feeding to allow low-ranking animals access to food. Food was placed on the foundation wall around the enclosure and on two feeding platforms approximately 40 x 70 cm each.

5.4.4 Pre-release enclosure

The pre-release enclosure was constructed at the release site from the same materials and in a similar fashion to the Tchimpounga enclosures: a 1 m foundation and a

slider door, diagonal structural elements, firehoses or hammocks for enrichment, platforms and perches. The three compartments had a total area of ~58 m^2 of covered space. Construction of the third compartment was completed after Group 1 was transferred to the release site. We included two chain link outdoor runs without roofs that allowed the mandrills to forage in the open air. For most of their lives the mandrills had been under a tin roof and did not have to look above them for opportunity or danger. The runs allowed the mandrills to become accustomed to looking directly above them for food and predators and to experience rain and direct sun prior to being released. They also offered greater access to wild foods and the ability to forage in the leaf litter.

5.4.5 Release

After releasing Group 1, we allowed them access to the pre-release enclosure for approximately one week. We then brought the adult male back into the enclosure for controlled integration with Group 2. We also allowed Group 2 access to the enclosure for one week after we released them, after which we only allowed the animals in the enclosure for medical procedures. An adolescent male who left the release area was recaptured and held in the enclosure during the habituation period and after the release of Group 3, so we could not give Group 3 access to the enclosure post-release because it was occupied.

In May 2015 we trained animals wearing GPS collars to enter and exit the cage because we wanted to be able to safely sedate the animals in the enclosure to remove the collars if they did not come off automatically. The training included luring the designated animal into the enclosure with supplemental food then closing the cage. The cage was then reopened, and the animal left. The animals used the forest surrounding the cage freely and the animals received ~2 kg of food each twice per day for the first two months post-release. The food was carried in buckets to pre-defined feeding spots and then scattered to encourage foraging behaviour and allow low ranking animals access to the

supplemental foods. We decreased the amount in 10% increments over the study, based on the animals' condition and behaviour. The animals were still receiving cup of cooked rice daily at the end of the study.

5.4.6 Release methods

We based the release processes on the IUCN Guidelines as far as possible. We worked closely with governmental and local authorities during the preparation process and release. All animals were quarantined for >30 days and screened for communicable diseases. We conducted pre-release behavioural observations to document self-directed and social behaviours for a related study (unpublished data). We conducted a survey of the release area consisting of 54, 1 km transacts covering 300 km² of the park (unpublished data). We selected the release location based on findings from the survey and constructed the pre-release enclosure in the forest we intended to release the animals into. We then sedated and performed health checks on Group 1 and transferred them to the pre-release enclosure in August 2013 (Table 5.1). We transferred Group 2 to the pre-release enclosure in two sub-groups in February 2014 (Table 5.1). We transferred Group 3 to the pre-release enclosure in two sub-groups in November-December 2014. In preparation for each release we fitted mandrills large enough to wear a collar (total n = 7) with artificial collars to habituate them to wearing collars, then later replaced the artificial collars with radio collars. In accordance with the accepted standard set by the American Society of Mammologists committee (American Society of Mammalogists, 1998), collars were less than 5 % of the animal's body mass. We originally intended to house the animals at the release site for 3 months. However, circumstances outside our control and project timeline constraints caused some animals to be held for much longer and some animals to be held for a much shorter time. For example, a local village experienced social unrest after we transferred Group 1 to the release site which forced us to delay the release of Group 1 for several months. The disruption also caused us to reduce the

amount of time Group 2 had in the pre-release enclosure. Group 1 was held in the pre-release enclosure for 197 days, Group 2 for 19-21 days and Group 3 for 56-76 days prior to release (Table 5.1).

Table 5.1 Individual transfer and release dates with numbers of sampling days and faecal samples collected

Mandrill ID	Number of sampling days				Number of samples			
	sanctuary	pre-release enclosure	post release	total	sanctuary	pre-release enclosure	post release	Total
George	88	197	5	290	16	49	1	66
Dominque	88	197	362	647	26	56	56	138
Kiki Mpaka	88	197	362	647	12	46	106	164
Obia	88	197	362	647	22	61	56	139
Madol	88	197	362	647	19	56	84	159
Kiki Tchiali	88	17	-	105	24	7	-	31
Gagaga	88	21	57	166	15	10	26	51
Gayard	-	21	355	376	-	7	127	134
Mobote	-	19	355	374	-	3	55	58
Veiu de Loin	-	19	355	374	-	10	88	98
Suzo	-	76	33	109	-	18	2	20
Nzelly	-	76	33	109	-	13	3	26
Kento	-	57	33	81	-	2	6	8
Brek	-	57	33	81	-	8	12	20
Egeuo	-	57	33	90	-	15	16	31
Mean	88	92	121	316	19	24	46	76

5.4.7 Faecal sampling collection and processing

We collected 1143 faecal samples between 22 May 2013 and 4 March 2015, with a mean of 72 samples per individual (Table 5.1). Samples were not evenly distributed across the study and among the mandrills and we were unable to collect sufficient samples for analysis (0-3) for the two animals born during the study. The staff was in the field with the released animals and were not available to collect Tchimpounga samples from some of the animals in Group 2 or any of the animals in Group 3. Although it would have been ideal for the hormone study, it was impractical to move staff and financial resources from the field operation to collect pre-release samples from the animals still at the sanctuary.

At Tchimpounga, a keeper observed the animals between 08:00 h and 18:00 h 5-6 days per week and collected faecal samples from identified individuals opportunistically. Previous studies of mandrills (Setchell et al., 2008) and western lowland gorillas (Shutt et al., 2012) found no diurnal variation in FGCMs so we collected samples throughout the day. We processed the samples using a validated field-friendly method for hormone extraction (Shutt et al., 2012). We discarded samples contaminated with urine. We removed debris such as sticks, or hay from the exterior, then homogenised the sample with a fresh stick, weighed 1 g of faeces, and placed this in a 15 ml falcon PP tube with 10 ml 90% ethanol within 5 minutes. We shook the sample by hand for 5 minutes then let the sample rest on a table for a minimum of 4 h before transferring 1ml of the supernatant to an Eppendorf tube. We then placed the tubes in a drying rack in a yogurt cooker where they dried within 24 h.

For samples collected in the pre-release enclosure and post-release, the staff collected faecal samples from identified individuals opportunistically using a clean dry marantaceae stick and placed the sample on a clean dry marantaceae leaf. The observer wrote the name of the mandrill and the time the sample was produced on the leaf and left it with the on-duty nurse, approved staff or me. The sampling team processed the samples using the same methods used at the sanctuary. Drying time was slower in the field because of increased humidity and a lack of electricity. We heated the samples on a gas stove in an aluminium Dutch oven. We lined the base of the Dutch oven with salt to disperse the heat through the oven. Approximately every 20 minutes we turned the burner on and heated the oven until it was warm to the touch. We then removed the oven from the heat and let it rest. To reduce drying time, we dried 1 ml of supernatant in two separate 1.5 ml micro-centrifuge tubes, containing 0.5 ml each. We placed the dried tubes in a plastic bag with desiccant and stored them at ambient temperatures in the dark until shipping them to the USA where they were frozen. Time in the oven ranged 1-14 days,

but the range of drying time was smaller. We assessed the effect of drying time on the samples and applied corrections to our analysis (see Section 5.5.1 Accounting for the effect of drying time).

The research department at Disney Animal Kingdom's Department of Animals, Science and Environment (DAKDASE) conducted all laboratory work in North America. We shipped the samples in two batches to DAKDASE where they were stored in a freezer upon receipt. They selected an appropriate assay for mandrills, conducted field method validations, and analysed the samples.

5.4.8 Validation of the enzyme immunoassay:

Cortisol is metabolised differently in the liver in different species and between sexes within species so careful biological and physiological validation is necessary for each new species (Touma and Palme, 2005, Möstl et al., 2005, Palme, 2005, Ziegler and Wittwer, 2005). In non-human primates it may be more useful to measure cortisol metabolites than faecal cortisol because some species secrete virtually no faecal cortisol (Bahr et al., 2000). In Old World primates, group-specific antibodies are more likely to detect changes in GC production than those designed to measure corticosterone or cortisol because they have a higher biological sensitivity and are more likely to work cross-species (Heistermann et al., 2006). Moreover, in large-bodied mammals, gut passage time causes a lag of days between a rise in circulating hormones and levels in the faeces (Whitten et al., 1998b Palme et al., 1996). Peak excreted hormone values vary greatly between primates and understanding this lag time for the study species is essential to link hormone and behavioural data (Heistermann et al., 2006). For example, in baboons, an Old World primate of a similar size to mandrills, there is a 40-50 h lag between hormone injection and peak faecal concentrations (Wasser et al., 1993). In contrast, radio-labelled faecal cortisol values peaked at 24 h in a chimpanzee (*Pan troglodytes*) (Bahr et al., 2000).

The gold standard for validation of an enzyme immunoassay is an injection of adrenocorticotrophic hormone (Heistermann et al., 2006), which causes a rapid rise in adrenal activity over a period of hours which can then be used to test whether an assay captures the metabolite activity accurately (Wasser et al., 2000). However, this method cannot always be used due to lack of availability or ethical reasons (Wasser et al., 2000). An alternative is to use potentially stressful events opportunistically to validate hormone assays biologically (Davenport et al., 2006, Setchell et al., 2008, Shutt et al., 2012). We, therefore, used faecal samples from 2 female and 1 male mandrills collected 1 day before and for 5-7 days after routine health checks to validate our FGCM assay, as described in (Lavin et al., 2019), and summarised here.

DAKDASE collected samples from two female mandrills before and after a routine health check and used these to test eight assays: cortisol (R4866;cortisol HRP), corticosterone (CJM006; corticosterone; HRP), cortisol metabolite 69a (Frigerio et al., 2004) 5-β-androstane-3α,11β-di-ol-17-one-CMO:BSA (Ak3138/6/99) 5b-androstane-3a,11b-di-ol-17-one-CMO-biotinyl-LC (EL 69) and cortisol metabolite 72t (Möstl et al., 2002) 11-oxoaetiocholanolone-17-CMO:BSA (Ak 3199/6/96) 11-oxoaetiocholanolone-17-CMO-biotinyl-3,6,9-trioxaundecanediamin (EL 71). The methods used for the assays are covered in detail in (Lavin et al., 2019) and are described briefly in (Appendix n). They found that antibody 69a was the most appropriate measure of FGCM present in the samples because it captured the greatest peak and change (Figure 5.1). The 69a values peaked 24-48 h after the procedure (Lavin et al., 2017). These findings are similar to those of Setchell et. al (2008) where FGCM values in 13 female mandrills significantly increased one day after a stressful experience. We adjusted our data for this time-lag by comparing behavioural observations and events with FGCMs 24 h later.

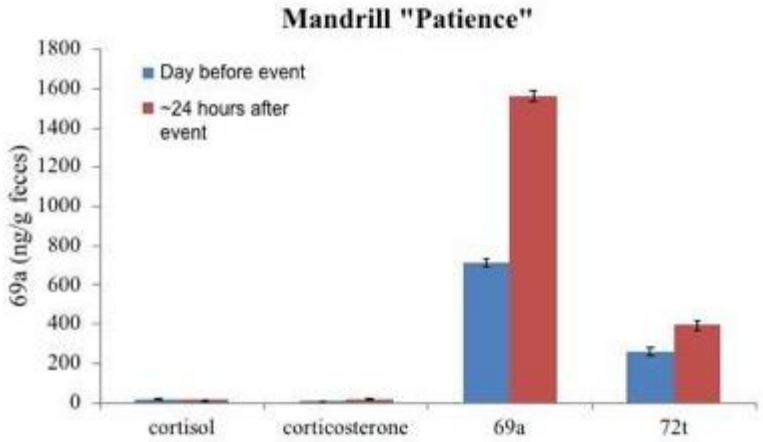

Figure 5.1 Results of assays testing the reactivity of antibodies to cortisol, corticosterone and cortisol metabolites 69a and 72t using faecal samples from a female mandrill before and after a routine health check. Samples were collected immediately from known individuals in the morning before the health check and approximately 24 hours after the health check.

Having established the assay, DAKDASE ran each sample in duplicate. We included four controls on each plate: high and low concentrations made with a 69a stock solution (Coefficient of Variation, CV: 8.63 and 14.3) and two mandrill samples at different concentrations (CV: 15.06). A spike recovery test yielded 98 ± 1.5% indicating a very low signal to noise ratio in the assay. The assay sensitivity was 5.12pg/well. We used the mean of duplicates in all analyses.

5.4.9 Statistical analysis

We tested our predictions using linear mixed effect models (LMMs) in SPSS 20. We logged the FGCM values prior to analysis to achieve normally distributed residuals. We included subject ID as a random factor in all analyses to account for repeated measures from individual animals, and included sex in each analysis, to account for potential sex differences. We did not include transfer group ID as a factor in the analyses because the sample size became too small for analysis. To account for this limitation data in the results section are coloured by release group. Our limited sample size also meant that we could not control for age in analysis. However, our matched design (matching individuals across treatments) mitigates these limitations.

To test Prediction 1, that transfer to the pre-release enclosure would lead to an increase in FGCM values, we compared values during the final month of sampling at the sanctuary with values during the first week in the pre-release enclosure. To test Prediction 2, that FGCM values will decrease over a period of weeks following transfer to the pre-release enclosure, we tested for a relationship between FGCM values and time during weeks 1-4 in the pre-release enclosure. To test Prediction 3, that release will lead to an increase in FGCM values, we compared values for the final month of sampling in the pre-release enclosure with values for the first week post-release. The number of fixes for Prediction 3 was too small for statistical analysis, so we provided data for visual comparison. We excluded samples from the first 30 days after transfer to the enclosure to allow for habituation. This meant that we did not have data for animals that spent less than a month in the pre-release enclosure (Table 5.1). To test Prediction 4, that the FGCM response caused by the release will decrease over a period of weeks, we compared the mean pre-release enclosure values during the last month of sampling in the enclosure with values during the first month of the release. We compared the last month of samples in the enclosure because 1) we did not have samples from all of the animals in the final week and 2) the final health checks and fitting some of the animals with their real collars during the last week lead to an increase in FGCM values that did not represent of values in the enclosure. To test Prediction 5, that the magnitude of the GC response at release will be lower than that at transfer to the pre-release enclosure, we compared pre-release enclosure and post-release values. We excluded samples collected during the first 4 weeks post-release to allow for habituation. To test Prediction 6, that the mandrills will have lower FGCM values in the forest than in the sanctuary, we compared sanctuary values with post-release values beginning four weeks post-release to allow for habituation.

5.4.10 Accounting for the effect of drying time

We recreated our field methods at the Disney lab to test whether differences in drying time between samples collected at Tchimpounga and in Conkouati influenced our FGCM results (Lavin et al., submitted). We collected an identifiable faecal sample, homogenised it and extracted 4x 1 g samples, placing them in four separate containers filled with 10 ml of 90% methanol. We then delayed drying for 4 h, 24 h, 48 h and 96 h to simulate drying times in the field.

Mean faecal extraction of 69a changed with drying time (Lavin et. al, 2017). Faecal extraction of 69a decreased 2.8% between 4 and 24 h but increased 36.7% between 24 h to 48 h, then increased only 4.5% between 48 and 96 h. All samples at Tchimpounga dried in <24 h, but only 17.9% of samples in the field dried in <24 h. We, therefore, added 36.7% to samples that dried in <24 h, to account for the effect of drying time in the field and re-ran our models. We refer to these numbers as "adjusted" in the results section and include them where we compare data based on two different methods.

5.5 Ethical Note

This study received approval from the Animal Welfare Ethical Review Board at Durham University. All transfers of hormone extracts followed international CITES regulations (CITES permit number: 1125666), and national requirements for transfer of faecal extracts between the Republic of Congo and the United States of America. We only sedated animals to perform health checks or conduct processes necessary for the release, including health screening, transfer to the release site, and fitting of collars. All sedation was conducted by a qualified veterinarian or human nurse.

5.6 Results

5.6.1 Prediction 1: Transfer to the pre-release enclosure will lead to an increase in FGCM values.

We found evidence to support Prediction 1. FGCM values increased in all mandrills on transfer to the pre-release enclosure (Figure 5.2). This increase was significant ($F_{1,54.84} = 96.7$, $p < 0.001$; adjusted: $F_{1,54.84} = 51.13$, $p < 0.001$), and the mean increase was 204% (range: 130-251%). With a correction for drying time, the mean increase was 122% (range: 68-157%). There was no influence of sex on FGCM values ($F_{1,3.21} = 0.70$, $p = 0.46$; adjusted: $F_{1,3.21} = 0.84$, $p = 0.46$).

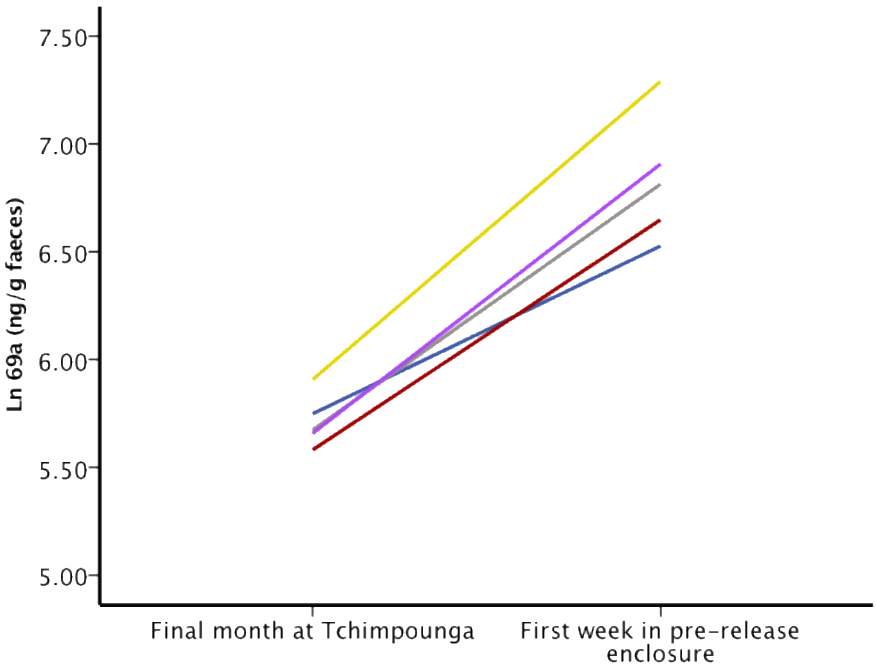

Figure 5.2 Comparison of FGCMs in mandrills during their final month at the sanctuary and their first week in the pre-release enclosure. Each line represents one individual.

Prediction 2: The FGCM response associated with the transfer to pre-release enclosure will decrease over a period of weeks

We found some evidence to support Prediction 2. Mean FGCM values decreased significantly over the first four weeks in the pre-release enclosure (days from transfer:

$F_{1,117.09} = 8.3$, $p = 0.005$; Figure 5.3) and again there was no sex difference ($F_{1,11.41} = 1.7$, $p = 0.218$). There are breaks in the data because we could not able to sample all animals each week. Individuals varied in their response, possibly because Groups 2 and 3 did not have a full month in the pre-release enclosure and were exposed to enclosure construction and new mandrills during their time there. When I restricted the data to Group 1, who were not subject to these confounds, the pattern was clearer (days from transfer: $F_{1,58.64} = 13.34$, $p = 0.001$; green lines in Figure 5.4), and again there was no sex difference ($F_{1,2.74} = 1.8$, $p = 0.280$).

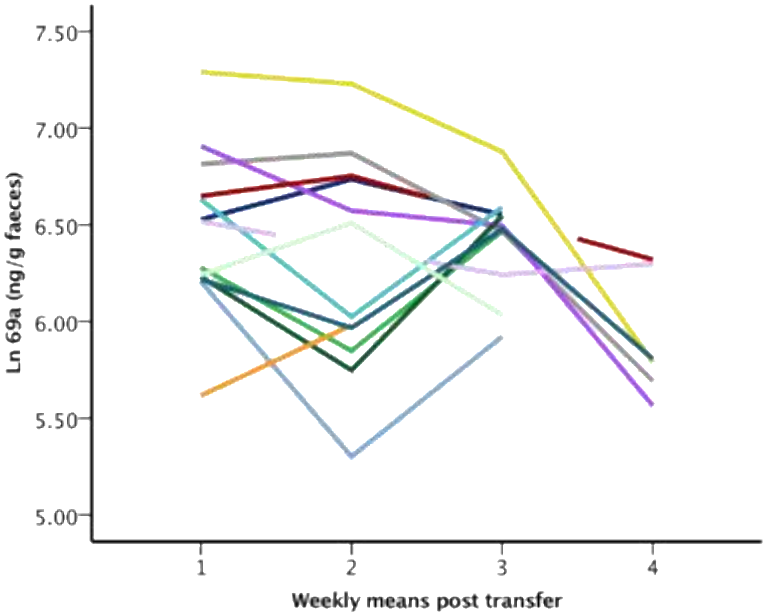

Figure 5.3 Weekly FGCM values for mandrills during the first four weeks post transfer to the pre-release enclosure. Each line represents one individual

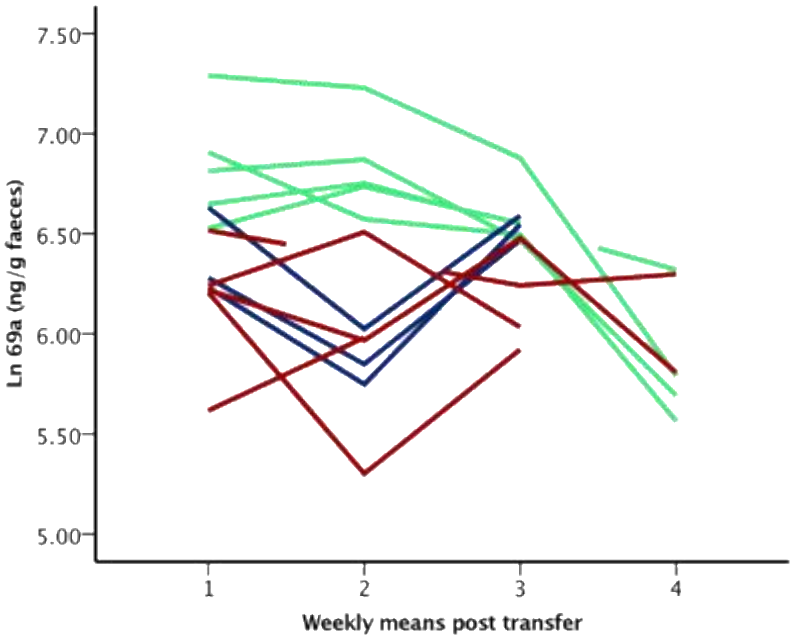

Figure 5.4 Weekly FGCM values for mandrills in Groups 1-3 during the first four weeks after transfer to the pre-release enclosure. Each line represents an individual. Green indicates Group 1, dark blue Group 2 and red Group 3.

5.6.2 Prediction 3: Release will lead to an increase in FGCM values.

We found no statistically significant support for Prediction 3. Release did not lead to a significant increase in FGCM values when compared to values in the pre-release enclosure ($F_{1,96.37} = 3.07$, $p = 0.083$), although values increased for 5/9 individuals (Figures 5.5 and 5.6). There was no sex difference ($F_{1,5.84} = 0.16$, $p = 0.706$).

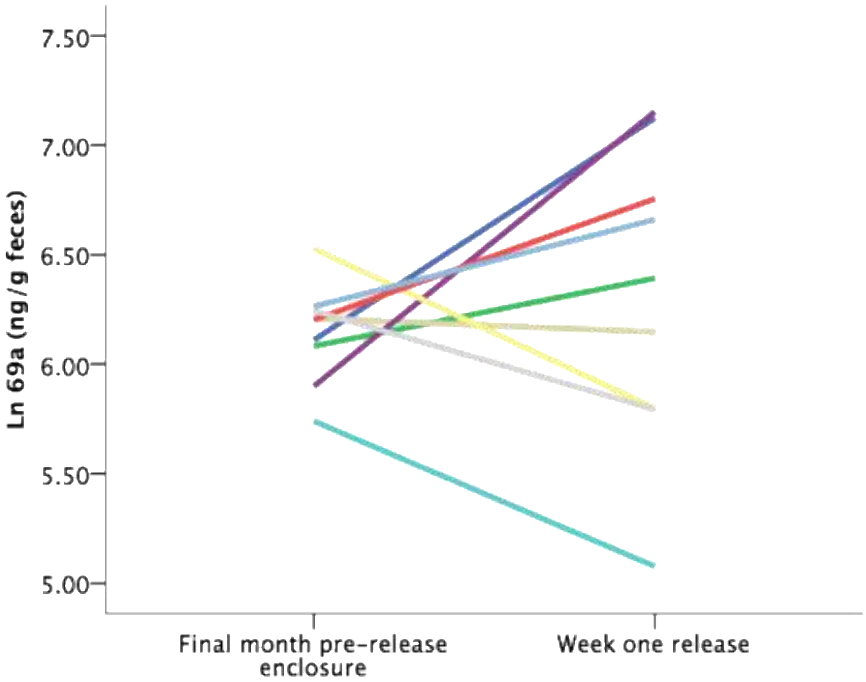

Figure 5.5 Comparison of FGCMs in mandrills during their final month of sampling while housed in the pre-release enclosure and during their first week post-release. Each line represents one individual

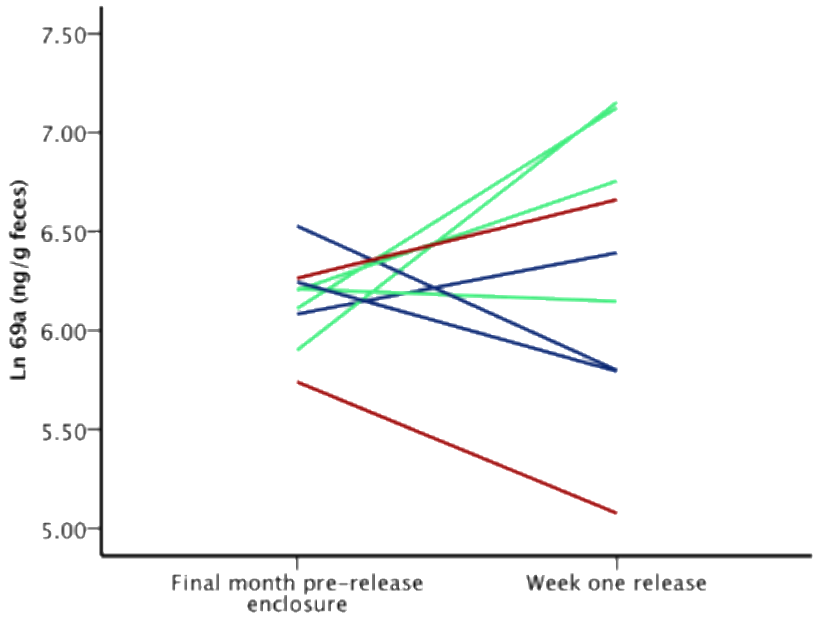

Figure 5.6 Comparison of FGCMs in Groups 1-3 during the final month in the pre-release enclosure and the first week post-release. Each line represents an individual. Green indicates Group 1, blue Group 2, crimson Group 3.

5.6.3 Prediction 4: The FGCM response associated with the release will decrease over a period of weeks.

We found no statistically significant support for Prediction 4. Mean FGCM values did not decrease over weeks 1-4 post-release ($F_{1,57.77} = 3.16$, $p = 0.081$; Figures 5.7 and 5.8) As before, there was no significant influence of sex ($F_{1,15.41} = 0.24$, $p = 0.63$).

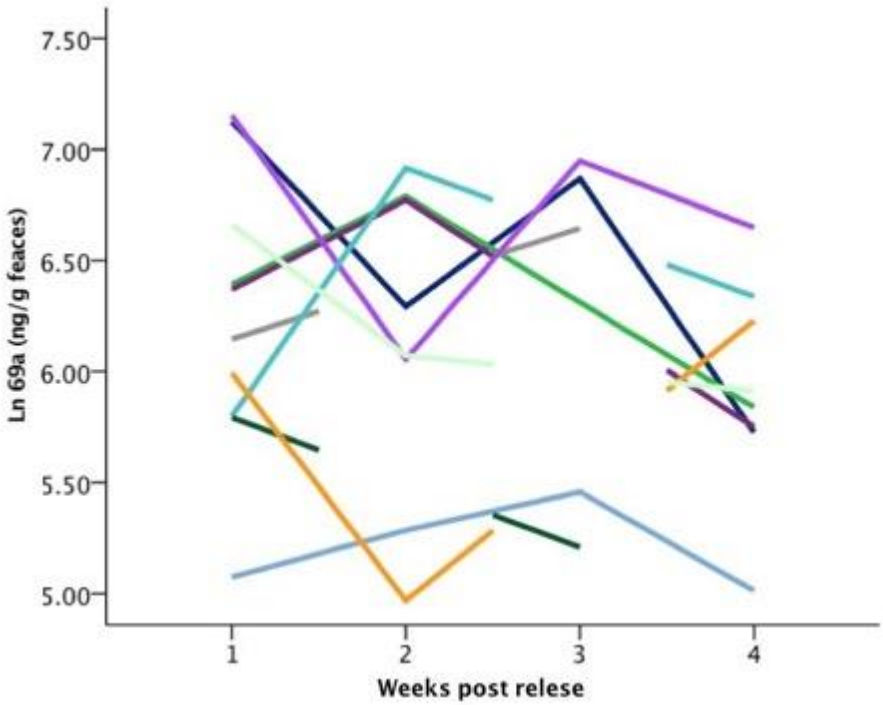

Figure 5.7 Weekly FGCM values of mandrills during the first four weeks post-release. Each line represents one individual.

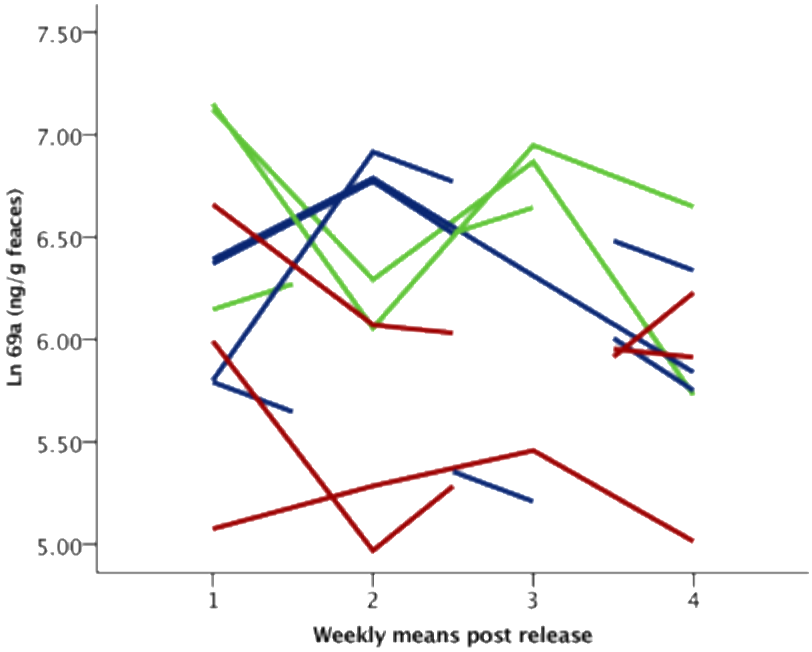

Figure 5.8 Weekly FGCM values for mandrills in Groups 1-3 during the first four weeks post-release. Each line represents an individual. Green indicates Group 1, blue Group 2, red Group 3.

5.6.4 Prediction 5: The magnitude of the glucocorticoid response at release will be less than at transfer to the pre-release enclosure.

Although FGCM values decreased for 6/11 mandrills, we found no statistically significant support for Prediction 5. FGCM values were not significantly lower during the first week of the release than they were during the first week of the transfer to the pre-release enclosure ($F_{1,96.37} = 3.07$, $p = 0.083$; Figures 5.9 and 5.10). There was no significant influence of sex ($F_{1,5.84} = 0.157$, $p = 0.706$).

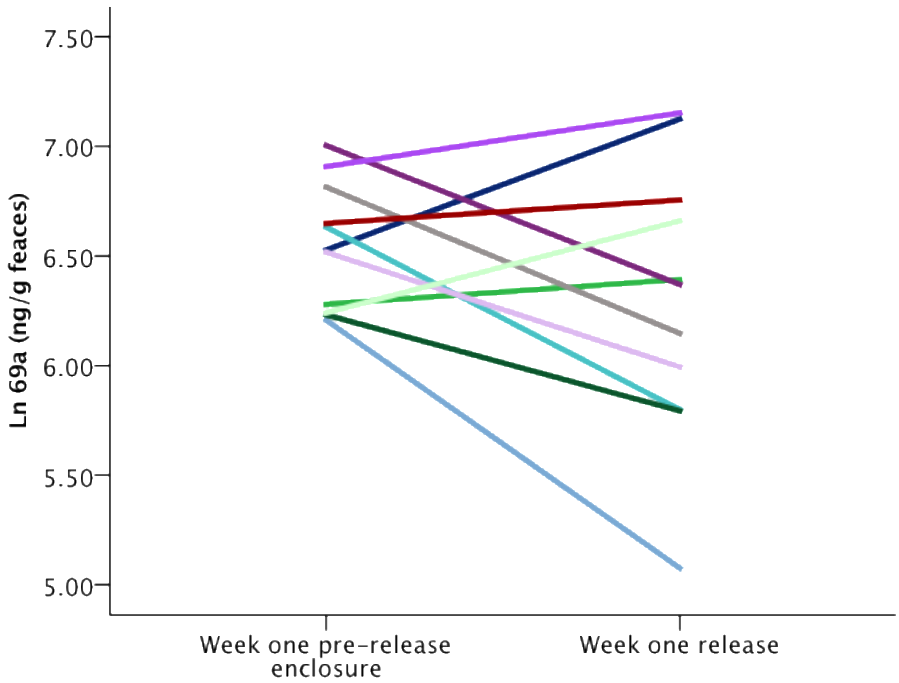

Figure 5.9 FGCM levels for mandrills in the pre-release enclosure and during the first week post-release. Each line represents one individual.

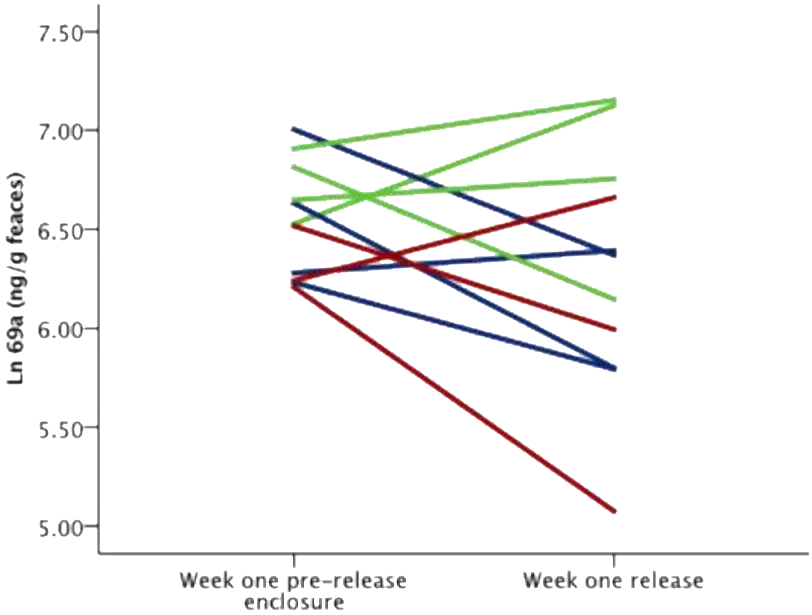

Figure 5.10 FGCM levels for mandrills in the pre-release enclosure and during the first week post-release. Each line represents one individual. Green indicates Group 1, blue Group 2, red Group 3.

5.6.5 Prediction 6: FGCM values will be lower post-release than in the sanctuary.

We did not find statistically significant support for Prediction 6. The uncorrected FGCM values were significantly lower post-release than they were in the sanctuary for the four mandrills for whom we had samples in both locations ($F_{1,370.54} = 5.53$, $p = 0.019$; Figure 5.11a. However, reanalysis with values adjusted for the possible effect of drying time showed no significant effect $F_{1,371.64} = 2.61$, $p = 0.107$; Figure 5.11b. There was no significant sex difference ($F_{1,3.23} = 0.05$, $p = 0.546$; adjusted: $F_{1,3.24} = 2.61$, $p = 0.556$).

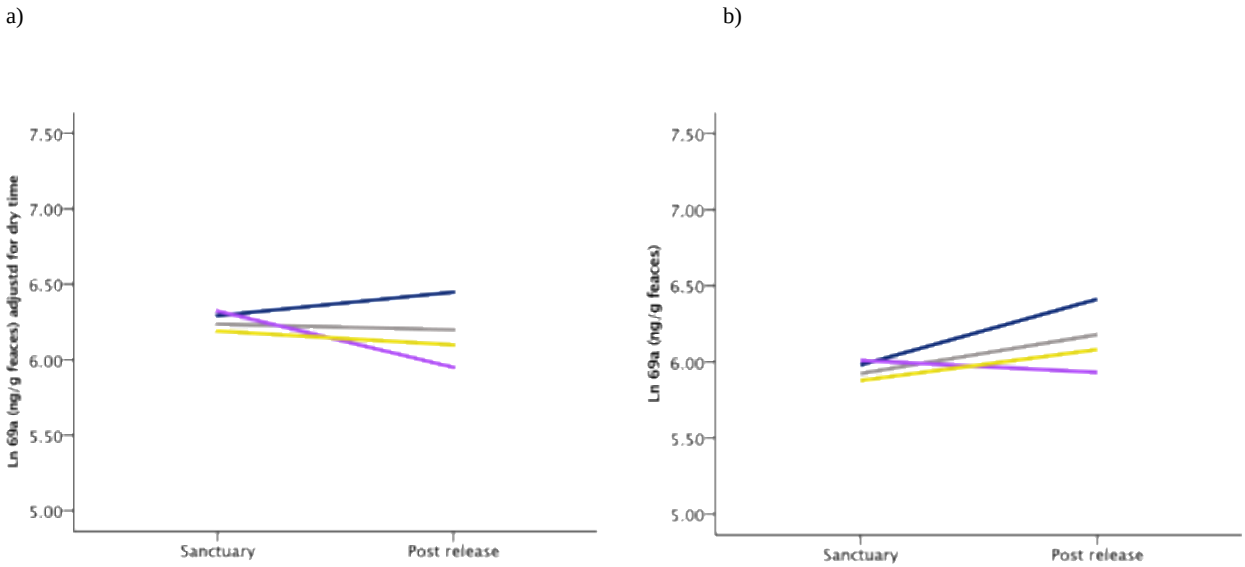

Figure 5.11 FGCM levels for mandrills in the sanctuary and after release. Each line represents one individual. a: unadjusted values. b: values adjusted for drying time

5.7 Discussion

We successfully monitored the biological response of a group of mandrills to the stages of a soft release. Transfer elicited a significant physiological response, which decreased over several weeks. In contrast, release into the wild did not cause a significant increase in FGCMs. The pre-release enclosure appears to have been useful to decrease the cumulative effects of stress associated with the release process in this species. The lack of increase post-release may also indicate the animals habituated to their surroundings and did not find the release as stressful as the initial transfer. Realtime hormone analysis was not possible during this project and the results arrived after the release was complete. Although the hormone data could not be used to inform this release, future mandrill release projects now have a biological reference point to inform their decision to use a pre-release enclosure and the duration of time that the animals should spend in it.

5.7.1 Prediction 1: Transfer to the pre-enclosure will lead to an increase in FGCMs

We found evidence to support this prediction, as the adjusted FGCM values increased significantly with a mean increase of 123% across the animals, after transfer to the pre-release enclosure. The increase is similar to the effect of a medical procedure in the female mandrill used in the validation, whose FGCM values increased 119%. Although sex did not have a statistically significant effect on FGCM values, the subordinate female had the highest FGCM values in the group during the final month at Tchimpounga and during the first week in the pre-release enclosure. Being subordinate is, but is not always (Setchell et al., 2008), associated with increased cortisol values and the corticoid response to social stressors varies greatly among individuals (Abbott et al., 2003). In addition to the physiological indicators of stress, behavioural indicators suggest that the animals found the transfer process stressful. The animals were agitated during the darting process and the dominant male exhibited an abnormal vocalisation pattern during the transfer, continuously alternating between a two-part groan and teeth grinding.

Our result is similar to findings for translocations of Grevy's zebra (*Equus grevyi*) (Franceschini et al., 2008), white rhinoceros (*Ceratotherium simum*) (Turner et al., 2002) and cheetah (*Acinonyx jubatus*) (Wells et al., 2004), which all showed elevated FGCM values after transfer to a new location. Release programmes can reasonably assume the animals will have a significant biological response caused by the transfer and associated procedures, independent of their release into the wild. Animals released before they have recovered from transfer will already be in a state of chronic stress, making them vulnerable to the potential negative side effects associated with a long-term heightened GC response. Thus, release practitioners wishing to reduce the cumulative effect of stress during release or translocation should take measures to help the animal recover from the stress of the transfer prior to exposing them to the additional stress of release.

5.7.2 Prediction 2: The FGCM response associated with the transfer will decrease over a period of weeks.

We found evidence to support this prediction as FGCM values significantly decreased over the first four weeks post transfer. FGCM values of the first group, which had a relatively undisturbed environment post-transfer, were relatively consistent between the animals, remaining relatively elevated through week three then showing the greatest decrease in FGCM values during the fourth week in the enclosure. These results contrast with those for wild Grevy's zebra, which did not acclimatise much to a pre-release enclosure over 40 days (Franceschini et al., 2008). The differences between the two species may be true species differences or may relate to differences between translocation of wild animals and the release of captive raised animals. While captive-raised animals may be habituated to cages, feeding routines and humans, wild animals may not. It is also important to account for individual responses to transfer in groups of captive animals (Vick et al., 2012). Repeated exposure to stressors can result in decreased concentrations of corticoids and responsiveness to stressful stimuli over time (Mormède et al., 2007). The decrease in FGCM values over the four weeks may be due to the animals experiencing reduced stress or a reduced stress response. From a behavioural stand-point, the mandrills were initially agitated post transfer and their reaction to novel environmental stimuli reduced over a period of weeks. This stress showed in teeth-grinding by the dominant male and frequent alarm calls by all animals. The reduction in stress-related behaviours over time suggests that the reduction in FGCMs is due to decreased stress, rather than a reduced stress response. Moreover, in release Groups 2 and 3, where more environmental and social stressors were present FGCM values continued to vary across time, indicating that the FGCM response was still functioning in those animals over a similar time period. These findings highlight the importance of recording

behavioural and physiological measures of animal welfare as a part of the translocation and release process in each study species.

5.7.3 Prediction 3: Release will lead to an increase in FGCM values.

We did not find evidence to support the prediction that release would lead to an increase in FGCM values. This may be due to variation in duration and exposure to novel stimulus while in the pre-release enclosure and variation in confounding factors such as enclosure construction, introduction of novel individuals, and the timing of final health checks. The two most notable increases in FGCM values were in the dominant male and a subordinate adolescent male. Their responses were similar in magnitude to the effect of transfer from the sanctuary to the pre-release enclosure. Post-release, the dominant male, who could not easily climb trees, could no longer mate-guard the females, who frequently copulated with the younger males in the trees. The dominant male displayed, grunted and threatened the copulating monkeys but they no longer responded to him if he could not reach them. The subordinate adolescent male, who also spent most of the time on the ground, frequently exhibited submissive behaviours to the dominant male post-release. All four of the animals with decreased values post-release were low ranking and had spent much of their time in the enclosure avoiding the more dominant animals. They may have showed a decrease in FGCM values post-release because they could feed and rest further from other members of the group.

While both wild and habituated animals would experience acute stress from darting and transfer, the wild animals may be more likely to experience chronic stress caused by a captive environment at the release site. Pre-release enclosures may be more useful for reducing the FGCM values of captive animals than wild animals because captive animals have already had an opportunity to habituate to the presence of humans and an enclosure. However, there may be habituation benefits of pre-release enclosures for wild animals other than stress reduction. The enclosure may help the animals

familiarise with the immediate surroundings and work though disruptions in the dominance hierarchy caused by the transfer. The translocated group of Grevy's zebra found their captive environment stressful and their FGCMs decreased post-release over a four-week period post-release (Franceschini et al., 2008). In a group of mantled howler monkeys (*Alouatta palliata*), FGCMs increased during translocation but decreased 1-4 weeks post-release (Aguilar-Cucurachi et al., 2010). As we found in the mandrills, individual zebras responded different to release and this may have been caused by social factors.

5.7.4 Prediction 4: The FGCM response associated with the release will decrease over a period of weeks

FGCM levels did not decrease significantly over the four weeks following the release. This is not surprising, because we did not find evidence of an increase in FGCM values on release. Our findings suggest that the animals habituated to the environment and did not find the transition into the surrounding environment stressful. The presence of human followers and provisioning may have also helped with this transition. Individual responses also varied. This variation could be related to some animals being less stressed in the forest where they were not exposed to the stressors related to captivity. The animals were no longer confined to a restricted space with limited enrichment options and they had greater variability in how they used the environment. Increases in FGCM values may have also been driven by additional physical activity and unrelated to environmental stressors. It may be useful to account for activity budget in studies of FGCMs when there are going to be large changes in activity patterns. Five of the animals showed a decrease in FGCM values between weeks 1 and 2 and five of the animals showed an increase post-release between weeks 1 and 2. Seven of the animals showed decreases in FGCM values between weeks 3 and 4 whereas only one of the animals for whom we had data showed an increase in FGCM values at that time. Finally, the variation in weekly means may also

be the result of exposure to novel objects and establishing or re-establishing relationships within the group. Younger and low-ranking animals were more likely to have decreases in FGCM values in the week following the release.

5.7.5 Prediction 5: The magnitude of the glucocorticoid response at release will be less than at transfer to the pre-release enclosure.

Five animals showed slightly higher values and six showed a slightly lower FGCM values during their first week of release when compared to their first week post transfer. The results varied between sexes and across all three release groups. The dominant male and female showed similar slight increases in FGCM values post-release as did the highly subordinate adolescent male who also showed a notable increase in FGCM values between the pre-release enclosure and post-release. Five of the six animals that showed a decrease in FGCM values were younger low-ranking animals. This decrease may be a result of the additional space and a decreased proximity to larger more dominant individuals post-release. A notable exception to this trend was the adolescent male who showed a decrease in FGCM values post-release. This mandrill spent less than a month in the pre-release enclosure and was sedated during the final week in the enclosure to be fitted with the GPS collar. These findings show that it is important to understand individual variation in responses to the release process.

5.7.6 Prediction 6: FGCM values will be lower post-release than in the sanctuary.

We found conflicting results for this prediction. The uncorrected results showed FGCM values were significantly higher post-release. However, after correcting for drying time we found no significant difference between the sanctuary and post-release values. In the wild the animals exhibited a range of locomotive and social behaviours that were not possible in their captive environment. This additional activity may have led to increased FGCM values post-release independent of emotional stress. Observing the animals before

and after release, the unlimited space allowed them to spend more time resting and foraging without aggression from a dominant individual, suggesting that they might be less stressed post-release.

5.8 Conclusions and recommendations

Stress is an unavoidable aspect of translocation and appropriate measures should be taken to minimise the stress translocated animals experience (Dickens et al., 2010). We found evidence that a pre-release enclosure reduced physiological stress that may impair an animal's chance of survival. We recommend animals should remain in a stable environment with a stable group structure in the pre-release enclosure for 1-3 months. Based on our findings we make the following recommendations for release projects and studies using FGCM analysis in release projects:

5.8.1 Planning construction and populating the enclosure

Cashflow issues, safety considerations and adjustments to workflow demanded that we construct portions of the enclosure after some of the animals were in the pre-release enclosure compound. We added an additional section to the enclosure to give the animals more space. We also added two safety corridors after the release of Group 1. These additions were necessary to allow the staff protected access to all of the enclosures from the river to deliver food and from the food preparation area for enclosure cleaning and food delivery. This was not ideal and enclosure construction should be complete prior to the arrival of animals when possible. Enclosure construction is stressful in felids (Chosy et al. 2014) and may account for the differences in the FGCM response in the enclosure between release groups. It would have also been ideal to limit the number of humans the animals were exposed too. Additionally, darting the animals to fit them with collars and for final health checks also added stress and introduced variability into the FGCM values during the time in the pre-release enclosure. Under ideal conditions the

environment would have been entirely stable, and the animals would be fitted with their collars during their pre-transfer health check.

Post-release the animals remained near the enclosure. In the mornings and evenings, they were led away from the release site twice per day with supplemental food. The animals consumed the supplemental food then foraged on wild foods at the feeding site. To ensure the safety of the animals from soldier ants and humans we maintained two staff at the pre-release enclosure at all times. One of the staff was a registered nurse who was responsible for heating and processing of the faecal samples and any minor wound care that was needed. At the release site, sleeping and food preparation took place in a section of the pre-release enclosure that was protected from the animals. The remaining staff were located at the base camp down river from the enclosure. At the conclusion of the FGCM study we discontinued the collection for faecal samples but continued following the animals to maintain the researcher presence in the area and assure the animal's long-term wellbeing.

5.8.2 Sample Processing

When heating samples in a solar oven or Dutch oven a cross breeze may be helpful to increase the rate of evaporation. A small fan powered by rechargeable batteries may be sufficient, but the method should be validated prior to implementation. When electricity is available an electric source of heat or ideally a professional dryer would reduce drying time and thus variability in drying times. Sample collection, validation and sample analysis are all time-consuming and costly. Time and financial budgeting of non-invasive hormone monitoring should be considered and included in the early stages of project planning. Faecal sample collection can be inconsistent depending on the group size, access to samples and the likelihood of some samples being unidentifiable or contaminated. This can present a challenge when trying to measure the effect of specific events on specific individuals.

It is also important to build an area to process the faecal samples that is protected from the released animals and weather.

5.8.3 Group dynamics

When planning to conduct successive releases in the same location it is important to plan for the safe transfer of food past released animals in the enclosure design. After released animals have grown accustomed to animals in the enclosure, they were reluctant to leave the release site. When they left, the animals in the enclosure showed signs of agitation and repeatedly contact called.

In primate releases, the time spent in transfer cages and pre-release enclosures at the release site varies greatly and is largely based on the opinions of those conducting the release (Guy et al. 2013) and financial and logistical constraints. The IUCN guidelines include holding animals in the transfer cage as compliant with a soft release (Soorae & Baker, 2002). Short stays or no stays in enclosures may be adequate for some species but based on previous releases and our results it does not appear to be adequate in mandrills. Species-specific recommendations derived from physiological measures for the minimum duration in a pre-release enclosure would be useful to help inform the decision to conduct a soft or hard release, whether animals are held in an onsite enclosure and if so for how long to reduce the potential negative consequences associated with the cumulative effects of stress.

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Chapter 6: Discussion

Life on earth is at a critical turning point and though we are not entirely responsible for the current state of things, the choices we make now will determine how many species go extinct in the near future. Central West Africa is one of many regions where the conservation decisions made now at the local and national levels will soon lead to positive or negative outcomes for the stressed human and wildlife populations living there. The bushmeat trade is coming to a head in countries like Republic of Congo where the human urban populations consume the most bushmeat (Mbete et al., 2011) and the country is trending towards urbanisation (UNFPA, 2016). For these reasons we took how we conducted this mandrill release very seriously and our efforts had positive outcomes. The project had 100% survival and three wild born offspring, fostering the beginning of a small stable resident group of mandrills in the release area. Not all success indicators are directly related to the animals themselves (IUCN/SSC, 2013). This project created alternative sources of income for local community members. It also led to the publication and sharing of findings in this thesis so that future projects can build on these findings and improve upon existing conservation best practices. The aims were to conduct a successful soft release of the mandrills, better understand the functionality and limitations of GPS and Argos collars, and test if non-invasive biological measures could be used to inform the release process. This chapter summarises the methods, key findings and recommendations derived from the research outlined in Chapters 3, 4 and 5.

Mandrills share habitats and behaviours with wildlife species around the world from the perspective of GPS collars. They move from forested areas to non-forested areas, have a 3D relationship with their environment, and place the collars in many

different positions and circumstances that have been shown to affect the amount of fixes GPS collars receive and the accuracy of those fixes. The basic methods in the translocation process are applicable to most species of wildlife species (IUCN/SSC, 2013). With this in mind, we developed tests that would be broadly applicable in wildlife conservation while also informing the methods of the mandrill release.

In Chapter 3 I introduced GPS radio collars and their use in wildlife field studies, follow by discussion of the environmental factors that affect fix success rates and the implications of the bias this effect can introduce in GPS collar data. We measured the effect the presence of a simulated animal, collar position, forest density and a collar height within a forest structure had on fix success. We found the presence of the simulated animal did not have a significant effect on fix success rates but did have a significant effect on time to fix. This may indicate that under conditions such as dense canopy cover the effect would be more pronounced. The effect of the simulated animal on GPS collar performance should therefore be investigated further. Test using simulated animals have used a range of substrates but not tested the different effect of those substrates. The manufacturer assumed that the simulated animal improved collar performance. However, we found that they negatively affect performance. Fitting the collar to a simulated animal as described in the methods section of Chapter 3 and then performing quality assurance testing under field conditions may provide critical insight into how collars will perform under field conditions.

The main finding of Chapter 3 was confirmation that collar height significantly affected GPS collar fix rates and time to fix. This has a host of implications that we tested further in Chapter 4. Performing the tree height tests was logistically challenging and risky for both the researcher and the collars. It may not be useful or practical to perform a host of tests as we did here, but the four habitat test proved a safe, easy way to uncover

malfunctioning collars. The collar malfunctions did not become clearly apparent until they were under the conditions of the tree height tests and the four habitat test.

We did not analyse the collar test data until after we retrieved the collars from the mandrills, so we did not discover the collar malfunction before releasing the mandrills. This highlights the importance of testing the collars that will be used under the conditions they will be used in, and analysing the data prior to deployment. Three of 10 of the collars we tested were not functioning prior to deployment. Two animals carried around bulky collars with limited utility for the duration of the study. The implications of deploying broken collars on released animals are serious and avoidable. One of the animals wearing a malfunctioning collar left the release group and finding and retrieving that animal was very difficult. When we did find the animal its body condition had deteriorated so much that we removed it from the release program for rehabilitation back at Tchimpounga. Fortunately, the collar was an ARGOS collar and it functioned just enough to retrieve the animal with a great effort. If the collar had been a VHF-only collar or store-on-board GPS collar the animal would have very probably died. Functioning collars which transmit the animals coordinates via satellite should be strongly considered in species such as mandrills, with a published history of day journey lengths that exceed the range of a VHF transmitter and adult and adolescent males promptly leaving the release area post-release.

In Chapter 4, I introduce the implications of height-induced bias for wildlife studies. Semi-terrestrial and arboreal animals have a three-dimensional relationship with vegetation and objects that can block their collar's access to satellites. Thus, the relationship between forest density and fix success varies with the animal's height at the time of the fix attempt. I examined data from the tree height tests and confirm that height also has an effect on the precision of GPS collar data. I then reviewed the collar data collected during the mandrill release and explored the relationship between collar data and the animals body mass and the amount of time it spent on the ground. Animals with

smaller body mass were more likely to have successful fixes and the distribution of their points more accurately represented their forest use than animals with larger body mass.

The main finding of Chapter 4 was that a collar's height in the tree affected the error in the 3D fixes. 3D fixes acquired at the top of the tree had a radial spread of ~25 m whereas those acquired at 0.5 m had a radial spread of ~250 m. Therefore any GIS calculation based on forest density from a 2D perspective is flawed because it does not account for the collar's relative height within that forest. This may cause day journey estimates for terrestrial forest dwelling animals to be overstated. Interestingly, in the analysis of the height in a ravine test in Chapter four, the two platforms located in the tree had similar point spreads to the collars at 0.5 m. This suggests that fully arboreal wildlife species would have less height-related error in their collar data than semi-terrestrial species, although they use various heights in the canopy. A limitation of this study is the tree height tests were conducted in only two trees. A larger study with multiple trees in varied environments would be useful to better understand the influences of height on collar data.

In Chapter 5 we demonstrated that field-friendly FGCM methods can be used to measure biological responses of mandrills to the stages of release into the wild. Measuring the mandrills' FGCM values at the sanctuary, in the pre-release enclosure, and post-release provided insight into the utility of using pre-release enclosures to reduce the cumulative effects of stress in release projects. Moreover, it provided a biological foundation for estimating how long the mandrills should be held at the release site prior to release. We found transfer to the enclosure caused a significant increase in FGCM values that decreased over a period of weeks in the pre-release enclosure. One aim of the pre-release enclosure is to reduce the cumulative effects of stress the animals experience at the time of release. Thus, our findings suggest mandrills benefit from being held in a pre-release enclosure for at least four weeks prior to release. Longer stays may be useful for

reasons not directly related to the biological stress response, such as stabilising social dynamics. Projects should also assess behavioural and environmental factors at the time of the release when deciding if 4 weeks is sufficient for their release group.

There was variation in the FGCM responses to release into the wild across the animals. This variation could relate to the individual experiences post-release. The dominant male was no longer able to enforce rule as he had in the enclosures. Post-release he spent much of his time visibly stressed while watching monkeys copulate in the trees. Conversely, subordinate animals had more options for privacy and access to prime resources than they had in the enclosure and were visibly more relaxed than they had been in the enclosure. Pre-release enclosures at the release site should thus be made as large and complex as possible to best simulate the social and environmental conditions the animals will experience post-release.

The practical experience the animals gained while receiving support at the release area was useful. Supporting the animals post-release while they explored the release area helped them learn how to interact with their environment and understand where local resources were distributed. The soft release provided support as the animals have differing responses to the release. In the case of mandrills all current evidence indicates they require a soft release with supplemental support to be successful in the wild.

6.1.1 Release strategy

The release strategy of these mandrills was heavily influenced by experience acquired at H.E.L.P. Congo. Some of the methods that worked with chimpanzees also worked with mandrills and some of them did not. Based on experience releasing chimpanzees the mandrills were released in multiple smaller batches. Separating the stable group into three smaller batches permanently disrupted the social dynamics. When the group was brought back together there was conflict amongst the animals requiring

surgery for three animals and two other animals to be removed from the release program. Keeping the group together through the release process and then releasing them at the same time may be a better strategy. Post-release supplementation

and medical attention was necessary for several of the animals.

In the prior mandrill releases where the animals did not have extended stays in a pre-release enclosure, they fled from the release site within hours or days. After being held in the pre-release enclosure all but one animal in our study stayed with the group. In the CIRMF mandrill releases the mandrills left the release site after a period of hours. None of the mandrills in this project fled the release site immediately post-release. One subadult male who had spent only 21 days in the pre-release enclosure prior to release left the group after several days, probably because he received elevated levels of aggression from other members of the group after the group was split and then reconstituted.

An extended stay in the pre-release enclosure may also be useful in reducing the likelihood of animals leaving the release area. In this project the animals who had been in the enclosure for 197 days did not leave the release area. In contrast, in the CIRMF mandrill release project the animals spent 12-34 days in the pre-release enclosure and left the release area even when supplemental food was available. The departure may have also been due to the enclosure being located in a savanna. Leaving the release area could make providing supplemental food difficult or impossible, forcing a hard release. Hard release is not justified in this species because the animals are likely to die or suffer greatly without support; thus, extended stays in the pre-release enclosure might be needed to keep the animals in the intended release area where provisions are available.

In the CIRMF release, animals had died or were thin and needed supplementary food at 8 weeks post-release. During the JGI release a sub-adult male who left the group was also very thin and needed to be extracted for rehabilitation. In the CIRMF release, sub-adult and adult males were more likely to leave the group post-release. Because provisioning is required post-release and adult and late adolescent males are more likely to disperse, they may be less suitable for release than juvenile and early adolescent males and females. Many of the animals in this project and the CIRMF project required aid to survive. Without aid, many of the animals at the CIRMF project died or disappeared. Animal welfare cannot be used to justify a hard release of this species.

Our enclosures were constructed for a long-term project. For projects that would like to habituate the animals and then deconstruct the camp and leave no footprint, strong chain link, metal sheeting for the roof, a water supply and posts made of wood treated for insect resistances would likely be sufficient for a pre-release enclosure. This would greatly reduce material, transport and labour costs for temporary projects. Our enclosure did not have a corridor leading to the river. After the first group was released transporting food to the animals still in the enclosure was not safe. The chain link corridor was a relatively cheap and easy way to assure staff safety when transporting food between the river and the enclosure.

6.1.2 Other key lessons

It became clear early on that local and not national rules and regulations would affect our project. Local authorities said in direct and indirect terms that noncompliance would result in risk to the safety of the staff and the lives of the animals being released. At the beginning of the project our vehicles were used to help extract a poacher who had been arrested the for killing elephants in the park. He did not stay in prison long and on his release held the project partially responsible and burnt down a major bridge on the way to the release site after animals were at the release site. The project had to reconstruct

the bridge and for several months we need to carry provisions in. As the PI on the project I was a focus of his aggression and could not stay on site until unfortunate circumstances led to his passing. Helping the eco-guards with the extraction of a poacher seemed like an obvious decision but it put the staff and the project at risk. It may be best for research projects to keep a distance from any activity that might be perceived as law enforcement where possible (Setchell 2019).

The release site was at an abandoned mining camp. To be eco-minded we decided to reuse materials at the site to construct our staff housing. We secured government approval to use the remaining portions of a shipping container and other material abandoned at the site for decades. From the local perspective, those materials were their property and because we were using them they had value. Though we had government approval we did not consider there would be a sudden perceived value of the materials at the site once we started using them. When I returned to the release area later in the week, the local government took me by force to a meeting where they spent several hours asking for compensation for the materials. I was eventually released and told not to return. This situation was resolved by government officials who negotiated on our behalf and came to a resolution that allowed me to return and for the project to continue. We should never assume that any materials or resources do not or will not have value at the local level. After getting government level approval, local approval should also be acquired prior to doing anything in the area. When the situation escalated at the local level the government level approval was vital to getting the situation resolved. Therefore, negotiations should be conducted at both the local and national levels of government (Setchell 2019). It is very important to maintain a calm composure even when in stressful situations because the success of the project is dependent on maintaining those long-term relationships.

6.1.3 Considerations for the selection of release candidates

Individuals in the release group reacted differently to different observers and it was important to explain the personality traits and how to react to the individual's tendencies to new observers. The dominant female in Group 1 in particular was affiliative or highly aggressive depending on which staff members were present and what they were holding in their hand. We were aware of this behaviour from pre-release behavioural observations but our attempts to avoid inciting aggression were not effective post-release and we had to remove her from the project and take her back to Tchimpounga. In the presence of released mandrills, it may be safer for observers to avoid standing near or interacting with other observers. However, even with these precautions some animals may still act aggressively and need to be removed. Three of the females bit observers and attempted to incite other mandrills to act aggressively towards the observers on their behalf. The other mandrills did not react in the instances where the observer did not react to being bittern. No adult male mandrill bit a staff member during the project. It is prudent to wear sturdy trousers and long sleeves when working with mandrills.

Interaction across age groups seemed important to the development of important social behaviours in the release group. The adult male in the release group (Kiki, Table 2.2, p 39) arrived as a young adolescent and integrated with the group but did not know how to copulate. He enforced order in the group and post-release appeared to be the authority who decided where the group was going on day journeys. He defended the group when it came close to chimpanzees, elephants and a pangolin. Although he was useful for group cohesion, he was not useful for producing offspring and had atypical sexual behaviour post-release. He often approached observers aggressively and held their leg while he masturbated on their boots. He would then leave without causing any harm. Nobody was ever harmed because new staff were warned of this behaviour and knew not to react. Due to cultural norms, national staff were not comfortable explaining this

behaviour to people of authority especially when they were female. It is therefore prudent for researchers to take it upon themselves to encourage staff to report any important safety concerns, even if they are awkward to discuss. It is equally important for researchers to explain important personality traits of the released animals and how to react to the behaviour in question to staff.

The amount of time the release subjects spent in captivity and the level of interaction they had with humans prior to arrival was important to their release ability. A male mandrill who was reared as a pet at a hotel was not releasable because he had been trained to search the owner's pockets for treats. This behaviour translated into serious safety concerns for the owner as the monkey entered late adolescence. As a full adult the animal associated humans with food and was aggressive. It is common for primates to become aggressive towards humans as they reach late adolescence (Tregle et al. 2011; Jones-Engel et al. 2005). Therefore, males reared to adulthood by humans may not be suitable for release. A female who arrived at the sanctuary as an adult was also un-releasable because she did not appear to identify as a mandrill. She was comfortable with humans but would cower and scream when placed in enclosures with other monkeys especially the above-mentioned adult male. Over a period of months this behaviour diminished but she showed other abnormal behaviours including excessive grooming of herself and others, greatly reduced mobility during oestrus, and leaving her tongue out for extended periods. Conversely, juveniles who went through quarantine together at the sanctuary developed close bonds which persisted even after mixing with other animals in the enclosure and post-release. The adult female mandrill may have bonded with humans whereas the juveniles are still able to develop a strong bond with conspecifics. Thus, holding juveniles in reasonably small enclosures together for 60-90 days may be useful to develop cohesion amongst individuals who are unrelated. Monkeys raised by humans to late adolescence or adulthood may not be suitable for release.

6.1.4 Conclusion

Releasing wildlife into the wild is challenging because wild populations are typically in decline due to pressures that are increasing and have no easy solutions. The relationships built with the local community members were critical to the success of this release project and are fundamental to any effort to reverse wildlife population declines. The bushmeat trade is driven by human activity. Long-term release projects can provide a non-militant opportunity to build relationships with local populations and earn their support in protecting the release animals and the surrounding areas.

Release projects can also generate the funds needed to responsibly conduct wildlife release projects. The primary conservation benefit of reintroduction and release projects in the developing world seems to come from the nonviolent occupation of land and establishment of long-term relationships with the communities in the surrounding area. Hard release without follow-up is not conservation and there is little reason to believe those animals or their offspring will survive without a persistent presence in the area. In contrast, good animal welfare supports positive conservation outcomes. This is especially true with mandrills. As we saw with this project the animals required a great deal of support to survive their transition back into the wild. The animals were not hunted because we established relationships with and employed local community members. This mandrill release project has now effectively protected the study area from poachers for several years. After we occupied the area for the release project, poachers passed through our study area but rarely hunted in it. We also did not find a single active snare on any of our trails during the study. Years after the conclusion of this research project the Jane Goodall Institute is still maintaining a presence in the area, and that gives me hope.

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Appendix

Appendix a

PASA Mandrill Project- Education Report

EDUCATION PROJECT REPORT

for PASA

October 2014

the Jane Goodall Institute's
AFRICA PROGRAMS

1. INTRODUCTION

Tchimpounga Chimpanzee Rehabilitation Centre is the Jane Goodall Institute's sanctuary in the Republic of Congo. For over a decade, the sanctuary has been receiving and carrying or mandrills as well as chimpanzees. Since 2012, the Institute has been undertaking a program to release wild born mandrills back into the Conkouati-Douli National Park. As part of this release program, we are undertaking a long term conservation education program with the local communities, who have been known to illegally hunt in the National Park. This program is being conducted by our education team alongside our mandrill release team. As part of the monitoring process of the education program, the team will conduct pre and post testing of community members in the villages closest to the mandrill release site.

The first visit that included the pre testing and first program with community members and school aged children was conducted in Mpoumbou (Conkouati National Park)

2. PRE-TESTING

The mission took place in Mpoumbou as a part of the education project. The goal of this mission was to conduct pre-evaluation tests, in order to assess the knowledge level about the laws protecting gorillas, chimpanzees and mandrills in Congo.

The field team was formed by three (3) workers of the JGI education team; Alain Sitou (mission manager), Achille Nsafou and Stéphane Fouenga. According to the planning, the team left the sanctuary of Tchimpounga on the 13th of March at 9 a.m. towards the Conkouati National Park to spend seven (7) days on the field. The program was to visit seven (7) villages: Koutou, Sintou-Nkoila, Kam, Nkoila, Bioko, Mabana and Tronzo (see Map 1).

2.1. Pre-testing fieldwork development:

2.1.1. Logistics:

The interviewers had two (2) sets of images each, one representing a mandrill and other animals (see image 1) and another representing a caged chimpanzee to symbolize they don't belong to captivity. The goal of the first set was to assess if the villagers were able to difference among the targeted species.

Image 1-2: Sets of images to conduct the questionnaires

Map 1: Sensitized villages and mandrill release site locations

Regarding the questionnaires (see Annex 1), each person of the team had to complete 25 in each village. A vehicle was used for transport between towns.

2.1.2. Field work proceedings:

The targeted villages are around the area where the mandrill releases are taking place. The field work started in Mbamba and finished in Koutou.

Previous awareness had been conducted by the chief of the town, thus the villagers knew our goal conducting the questionnaires. We completed the questionnaires by knocking at each door.

Image 3-6: Workers and villagers during the activity

2.1.3. Population reaction:

All towns were reasonably welcoming, since nobody neither rejected nor refused the team, except in Sintou-Nkola where two people refused answering the questionnaires.

The villagers were afraid to talk about the natural resources, due to the presence of ecoguards in the National Park. They reported a serious problem with elephants as they are damaging their fields and the only ways of subsistence are hunting, fishing and agriculture. Nevertheless, hunting is actually forbidden in Conkouati National Park, so the population asks to develop new methods to keep away the elephants and complains that neither the government nor NGO have helped them.

In almost all towns, the children already knew about our job, because the teachers implemented the awareness programs provided by the JGI since November 2013.

During the field work, the villagers of Koutou, Sintou-Nkola, Nkola and Bioko requested projections of the Super Kodo movie or to provide them with DVDs and viewing materials in order to improve wildlife knowledge.

2.1.4. Comments :

Children were not able to difference among chimpanzees and gorillas, and even adults had problems recognizing mandrills.

In general, the fieldwork was successful. The ecoguards of Conkouati National Park are permanently on the field rising awareness against hunting.

2.2. Pre-testing results:

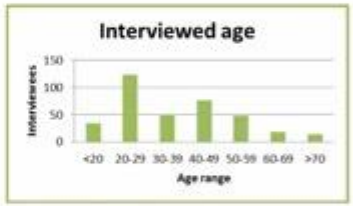
2.2.1. Summary of population sample:

In total, 326 people were interviewed during the pre-evaluation process. Within this population sample, 149 were women and 177 men. The average of interviews conducted per town was 40 and everyone was Congolese, except four people from Democratic Republic of Congo.

Regarding age, the breakdown of interviewed ages shows that every age range is well represented.

LOCATION	INTERVIEWS
Sitou-Nkola	46
Koutou	36
MBamba	41
Mongo-Bissafi	45
Tchionzo	37
Bioko	34
Nkola	47
KM4	40
TOTAL	**326**

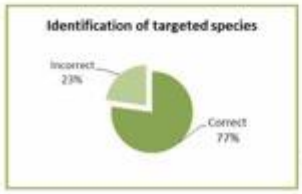
Table 1: Number of interviews per village



Graphic 1: Interviewed age ranges

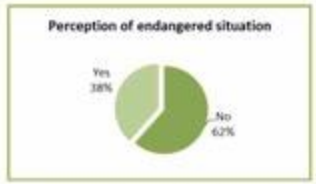
2.2.2. Population knowledge:

Using images of six different primates, interviewers asked to identify baby and adult chimpanzees, gorillas and mandrills. Three quarters of the answers were correct (see Graphic 2), so the majority of the population can distinguish between the targeted species.



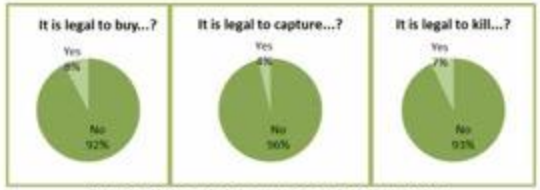
Graphic 2: Identification percentage of targeted species

Moreover, they also checked the population perception on the endangered situation of these species (see Graphic 3). When the interviewers asked if they thought than chimpanzees, gorillas and mandrills are threatened in Congo, approximately 60% of interviewees said "no".



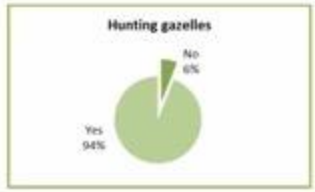
Graphic 3: Perception percentage of endangered situation of targeted species in

Finally, to assess the knowledge of the law they asked whether it was legal to buy, capture or kill a chimpanzee, gorilla or mandrill. Results show that the population knows the law regulating great ape protection (see Graphics 4-6), as the knowledge percentages is higher than 90%.



Graphics 4-6: Knowledge about protection law of endangered species

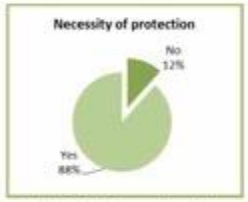
In contrast with the answers given for the endangered species, 94% of the population answered "yes" to the question if it is legal to hunt gazelles, as Graphic 7 shows. The fact that they were capable to distinguish between protected and non-protected species shows the high level of awareness about the law.



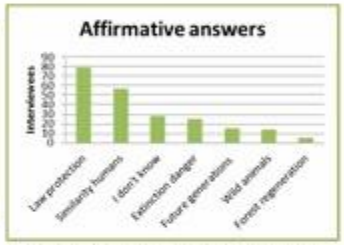
Graphic 7: The population knows that it is legal to hunt gazelles

2.2.3. Population attitude:

In general, from the point of view of the population, the targeted species should to be protected. The results show that 88% of the population questioned agreed (see Graphic 8). The main reasons to believe so were; "The law already protects them" and "They are similar to humans"(see Graphic 9). Therefore, the population thinks that the fact that they are already protected means that they should be protected and, likewise the closeness and similarity between humans and these primates makes them special to people. Other reasons were: "They are in danger of extinction", "We want our children to be able to see them", "They are wild animals" and "They contribute to the forest regeneration".

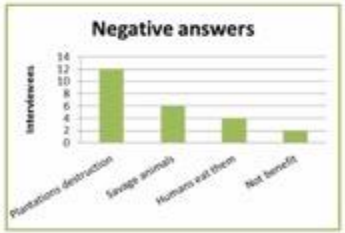


Graphic 8: Population percentage believing the targeted species should to be protected



Graphic 9: Reasons to believe that targeted species should be protected

Concerning negative answers, in the most cases people don't agree with their protection because "They destroy our plantations", so it makes clear serious conflict among villagers and chimpanzees, gorillas, and mandrills. Other reasons were "They are savage animals", "We eat these species" and "We don't benefit from them".

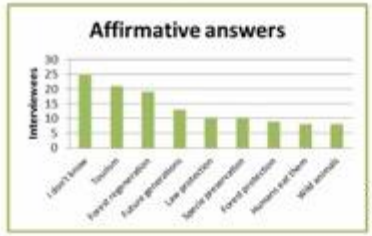


Graphic 10: Reasons to believe that targeted species should not be protected

The interviewers also asked about the importance of these species and people again agreed with it. An 87% of answers were "yes" as showed in Graphic 11, but the reasons specified were very varied (see Graphic 12).

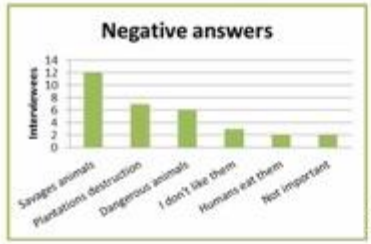
Graphic 11: People percentage believing the targeted species are important

Even if people said "yes" to the question, the most answers were "I don't know", nevertheless other answers stated; "For tourism development", "For the regeneration of the forest" and "For the future generations".



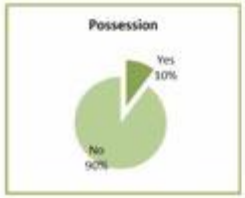
Graphic 12: Reasons to believe the targeted species are important

The negative answers to this question were almost the same than the previous (see Graphic 13). People don't consider they are important because "They are savages animals" and "They destroy our plantations", there was an additional reason; "They are dangerous".

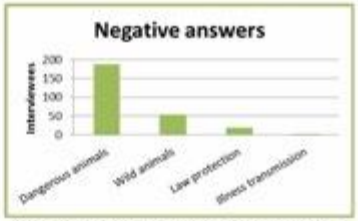


Graphic 13: Reasons given to not believe the targeted species are important

We lastly asked if they thought it was right to have chimpanzees, gorillas and mandrills as pets. 90% of the interviewees answered "no" (see Graphic 14), the three main reasons given were: "they are dangerous" "they are wild animals" "They are protected by the law". In a few cases they moreover gave their concern about illness transmission (see graphic 15).

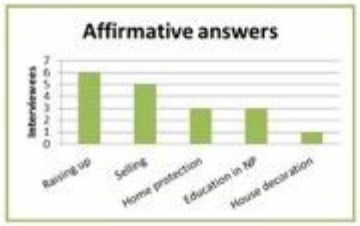


Graphic 14: Thinking about targeted species possession as a pets



Graphic 15: Reasons given to not believe the targeted species can be pets

Despite the percentage of affirmative answers being low, we obtained a large diversity of answers about the benefits of owning a chimpanzee, gorilla or mandrill. The majority of the affirmative answers said they would have one of these species just to nurture them when they are young. Some individuals had no problem to admit they would sell these protected animals.

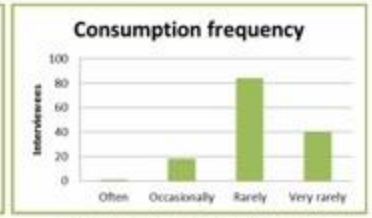


Graphic 15: Reasons given to believe the targeted species can be pets

2.2.4. Population practice:

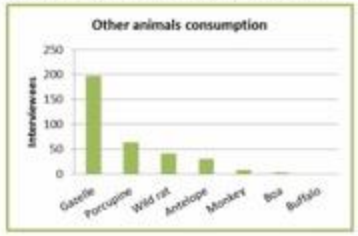
In the present question of the questionnaire we were interested on knowing how familiar individuals were with eating mandrill meat (see Graphic 16). Almost half of the interviewees disclosed they knew somebody that eats mandrills' meat (see Graphic 17). Nevertheless the frequency of consumption is low with most answers stating "rarely" when asked "how often".

Graphic 16: Mandrill meat consumption

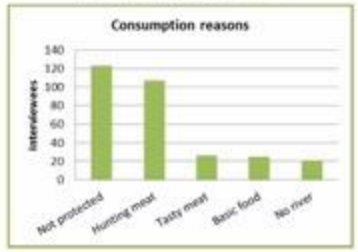


Graphic 17: Mandrill meat consumption frecuency

The population consumes other wild animals, especially gazelles, but also porcupines, wild rats, antelopes and monkeys (see Graphic 18). They hunt these species justifying they are non-protected by the law and the mean reason to hunt them is the population needs to eat, particularly in locations where fishing cannot be practiced (see Graphic 19). The most common hunting tools are traps, shotguns and, in a few cases, nets.

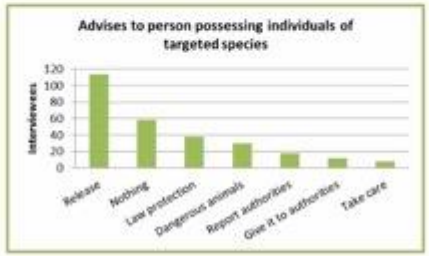


Graphic 18: Consumption of other animals



Graphic 19: Reasons to consume other species

The last question was what the person would do if he/she encounters a person possessing a chimpanzee, gorilla or mandrill. The most frequent answer was "I would ask to release it", sometimes justifying "they are protected species" and "they are dangerous". A lot of people wouldn't do anything. Someone would directly report to the authorities the case, but others would advise the person to give the animal to the authorities or, by contrast, to take care of the animal or just to have it at home or sell it.



Graphic 20: Answers to question "why would you do if you encounter someone possessing a chimpanzee, gorilla or mandrill"

3. EDUCATION PROGRAM :

In the context of scholar community sensitization, two (2) workers of the JGI education team piloted the first education program on the 26th of May in the school of NKola, one of the villages where the pre-tests were conducted.

Image 7: People of the villages with sensitization materials

Prior to this activity, teachers of the MPoumbou area participated in a workshop in which they learnt the strategies to transmit the knowledge about mandrills in order to make the students aware. Therefore, the goals of JGI education team mission were; to verify the improvement in the students' knowledge and sensitize about the mandrills extinction danger in Congo.

3.1. Development of the sensitization activities:

3.1.1. Logistics:

The education team had two (2) sets of sensitization materials, one representing a story of a captured mandrill (see Annex 2) and an awareness poster with the slogan: "I am not a domestic animal" (see Annex 3). They use these materials to ask the students about the activity subject: the status protection of mandrills and the consequences of breaking the law.

3.1.2. Fieldwork proceedings:

JGI workers conducted, first, a class activity in which they asked students about the notions transmitted to teachers in the workshop to verify the transfer of knowledge. Finally, they leaded an activity in the playground (see Image 8-9).

Image 8-9: Class activities and students with sensitization materials

3.1.3. Comments:

The sensitization has recently started and more activities will take place in the other villages, but this first action permitted us to know that even though the students have improved their knowledge, at least two (2) sensitization actions per school and year would be necessary to have a long-term impact.

Image 10-11: Children with the JGI education team members

Annex 1: Pre-testing questionnaire

JGI QUESTIONNAIRE

Date:
Interviewer:
Location:

1. **Gender** ___M ___F
2. **Age:**
3. **Employed?** ___ Yes ___No If yes, what is your job?
4. **Education level** Learning Primary Secondary University
5. **Resident?** ___Yes ___No State the name of the neighborhood or town you live in
6. **Have you got children in primary school?** ___Yes ___No If yes, state the name of school
7. **Nationality:** Congolese Non-Congolese African Non-African nationality

Knowledge

1.Using the pictures of 6 different primates, ask them to choose the Mandrill and check if they can identify the chimpanzee, gorilla and mandrill babies and adults. Correct Incorrect
2.Are mandrills, chimpanzees and gorillas threatened in Congo? ___Yes ___No
3.Answer Yes or No to these questions:
Is it legal to buy mandrills, gorillas or chimpanzees? ___Yes ___No
Is it legal to capture mandrills, gorillas or chimpanzees? ___Yes ___Non
Is it legal to hunt gazelles? ___Yes ___Non
It is legal to kill mandrills, gorillas or chimpanzees? ___Yes ___Non

Attitude

4.Do you think mandrills, gorillas and chimpanzees should be protected? ___Yes ___No
Why?
5.Are they important species? ___Yes ___No
Why?
6.Do you think it is right to have a mandrill, gorilla or chimpanzee as a pet? ___Yes ___No
Why?

Practice

7.DO you know anyone that eats the meat of mandrills? ___Yes ___No
If yes, how often does he/she eat it? Frequently (Approximately 1 time per week)
Occasionally (Approximately 1 time per month)
Rarely (Approximately 1 time each 3 months)
Very rarely (Approximately 1 time per year or less)
8.Do you know someone that eats other animals? ___Yes ___No
If yes, which species?
Why?
How do they get it?
9.What would you do if you meet someone that has a mandrill, gorilla or chimpanzee?

Annex 2: Educational materials

L'histoire d'un petit mandrill au parc de Conkouati

Institut Jane Goodall

Annex 3: Awareness poster

Appendix b

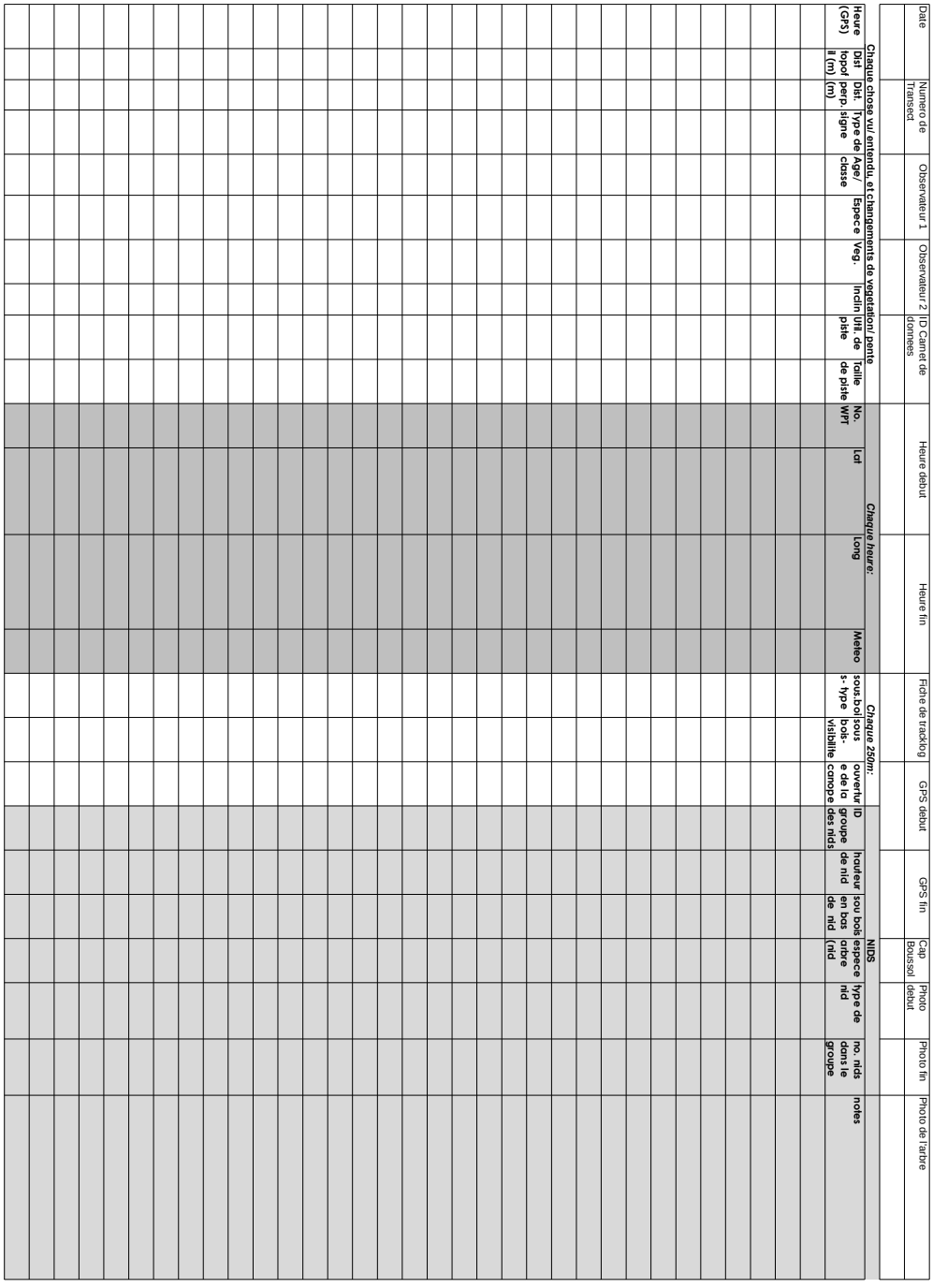
Multidisciplinary team involved in the mandrill release project

Name	Affiliation	Title
Mbani Akalanga	Ministère du Développement Durable, de l'Economie Forestière et de l'Environnement	Governmental Liaison
Rebeca Atencia	the Jane Goodall Institute	Congo Executive Director and Veterinarian
Tamara Bettinger	Disney's Animal Kingdom/ GRACE Gorilla Sanctuary	Animal Operations Director
Carol Collins	the Jane Goodall Institute	Associate Director, Budgets and contracts, Affrica Programmes
Debby Cox	the Jane Goodall Institute	Techincal Advisor
Anna Gibson	the Jane Goodall Institute	Vice President, Development and Marketing
Jane Goodall	the Jane Goodall Institute	Founder
Guy Kilendo	the Jane Goodall Institute	Mandrill Camp Manager
Shana Lavin	Disney's Animal Kingdom Animals, Science and Environment	Research Manager
Nianga Leckosso	Ministère du Développement Durable, de l'Economie Forestière et de l'Environnement	Tchimpounga Conservator
Jean Josue Maboto	the Jane Goodall Institute	Animal Care Supervisor
Errol Mavoungou	the Jane Goodall Institute	Forester
Achille Nsafou	the Jane Goodall Institute	Forester
Tammy Palmer	the Jane Goodall Institute	the Jane Goodall Institute
Lilian Pintea	the Jane Goodall Institute	Vice President, Conservation Science
Gregoire Bonassidi	Ministère du Développement Durable, de l'Economie Forestière et de l'Environnement	Conkouati Conservator
Missilou Boukaka Roland	Ministère du Développement Durable, de l'Economie Forestière et de l'Environnement	Conkouati Conservator
Russell Hill	Durham University/ Primate Predator Project	Professor Durham University /

Joanna Setchell	Durham University/ International Journal of Primatology/ International Primatological Society	Professor Durham University /Editor-in-Chief/Vice-President (Research)
Shawn Sweeney	the Jane Goodall Institute	Senior Director Community Engagement
Herve Tchikaya	the Jane Goodall Institute	Nurse and Head Vetrinary Assistant
Fernando Turmo		Congo Coordinator of Communications and Imagery
Hilde Vanleeuwe	Wildlife Conservation Services	Conkouati Project Director
Catharine Wheaton	Disney's Animal Kingdom Animals, Science and Environment	Research Manager Reproductive Manager Disney Animal Kingdom, Animals, Science
Miles Woodruff	the Jane Goodall Institute/Durham University	Mandrill Release Manager and Principal Investigator /PhD Student Durham University

Appendix c

Adaptation of WCS Observation Sheets for Line Transects



Appendix d

WCS Survey codes for conducting a field survey

Chaque 250m:		
Groupement végétal du Sous-	**Canopée**	**Sous bois**
Herbes=**H**	0-25%=**0**	Très ouvert (>15m)=**TO**
Arbustif=**A**	26-50%=**1**	Ouvert (10-15m)=**O**
Lianes=**L**	51-75%=**2**	Fermé (5-10m)=**F**
Steppes=**S**	>75%=**3**	Très fermé (<5m)=**TF**
Chaque fois que la vegetation change:		***Chaque 250m:***
Vegetation (Transects)	**Code**	**Pente**
Bais (clairière marécageuse)	B	Plat=0
Bosquet (petit forêt dans une savane)	BO	Faible=1
Cuirasse - Foret	CF	Moderee=2
Cuirasse - Savanne	CS	Raide=3
Forêt de Bambous	FB	plus clinometre
Forêt de Colonisation	FC	
Forêt de Lianes	FL	
Forêt de Lianes avec Rotins	FLR	
Forêt de Marantacée	FM	
Forêt Inondée Saisonnièrement	FI	
Forêt Mixte Sous-Bois Ferme	FMSF	
Forêt Mixte Sous-Bois Ferme Liane	FMSFL	
Forêt Mixte Sous-Bois Ferme Marantacée	FMSFM	
Forêt Mixte Sous-Bois Ouvert	FMSO	
Foret Monodominante	Fmono	
Forêt Secondaire Jeune	FSJ	
Forêt Secondaire Vieille	FSV	
Galerie forestière	GF	
Inselberg	INS	
Jachère	JAC	
Marécage	MC	
Marécage de Lianes	MCL	
Plantation	PLT	
Raphiale	RAP	
Rivière	RIV	
Rocher	ROC	
Route abandonnée	ROAB	
Route active	ROAC	
Saline	SAL	
Savane arbustif	SA	
Savane boisée	SB	
Savane herbeuse	SH	
Trouée (Chablis)	TR	
Chaque heure:		
Meteo	**Code**	
Ensoleillé	E	
Très ensoleillé	TE	
Légèrement nuageux	LN	
Nuageux	N	
Pluvieux	P	
Chaque heure:		
Point GPS		
Chaque crotte d'elephant		
Age de crotte	**Code**	
Fraîche	F	
Récente	R	
Vielle	V	
Très vielle	TV	
Fossilisée	FO	
CLASSE DE CROTTE	**Code**	
Tout amas intact	A/B	
50% - 100% des boules intact	C1	
< 50% des boules reste intacte	C2	
Aucune boule reste intacte.	D	
plat- fibres.Pas de matiere fecale	E	

Traces des animaux	**Code**
Alimentation	A
Boue sur l'arbre	B
Crotte	C
Decortissage	D
Empreinte	E
Nid	N
Observation Directe	O
Piste	P
Passage	PA
Activité saline	S
Vocalisation	VO
Traces humaines	**Code**
Carcasse	Carcasse
Arbre coupe/ abattu	AC
campement	CA
Carriere (quarry)	CAR
coups de fusil entendu	CF
coupe de machete	CM
douille de cartouche	DC
écorcement d'arbre	EA
extraction de latex	EL
extraction de miel	EM
extraction du vin	EV
feu	FE
fumoir	FU
bruit de moteur	M
observation directe	O
piste	P
peche	PE
piège	PG
recolte de fruit	RF
signe de passage	SP
utilisation artisanales	UA
village abandonne	VA
Type du nid	**Code**
Arbre	A
Zero	Z
Minimum	M
Herbacee	H
Mixte	Mx
Ligneuse	L
Ligneuse detachee	LD
Palmier	P
Vegetation (Recces)	**Code**
Bai	B
Forêt de Colonisation	FC
foret mature sur terre firme	FM
foret monodominante	Fmono
Marecage	MC
Utilisation	PLT JAC
Saline	SAL
Savane	SAV
Crottes des ongulees*	
Petites	U1
Moyennes	U2
Grands	U3

Nom Scientifique	Nom français	Code
Allenopithecus nigroviridis	Singe de marais	AN
Aonyx congica	Loutre aux joues blanc de Congo	AC
Atherurus africanus	Atherure	AA
Atilax paludinosus	Mangouste des marais	AP
Bdeogale nigripes	Mangouste a pattes noires	BN
Canis adjustus	Chacal à flancs rayes	CA
Canis aureus	Chacal commun	CH
Caracal aurata	Chat doré	FA
Cephalophus callipygus	Céphalophe de Peters	CP
Cephalophus dorsalis	Céphalophe bai	CD
Cephalophus leucogaster	Céphalophe à ventre blanc	CL
Philantomba monticola	Céphalophe bleu	CM
Cephalophus nigrifons	Céphalophe à front noir	CN
Cephalophus ogilbyi	Céphalophe de Ogilby	CO
Cephalophus rufilatus	Céphalophe à flancs roux	RU
Cephalophus spp.	Céphalophes rouges	CR
Cephalophus sylvicultor	Céphalophe à dos jaune	CS
Cephalophus wenysi	Cephalophe de Weyn	WE
Cercocebus agilis	Cercocebe agile	CG
Cercocebus torquatus	Cercocebe a calotte rouge	CQ
Cercopithecus aethiops	Grivet	CT
Cercopithecus ascanius	Singe d'Ascane	AS
Cercopithecus cephus	Moustac	CC
Cercopithecus dryas	Salongo monkey	DR
Cercopithecus erythrotis	Red-eared monkey	CE
Cercopithecus hamlyni	Cercopithèque D'Hamlyn	CY
Cercopithecus l'hoesti	Cercopithecus de l'hoesti	LH
Cercopithecus mitis	Cercopitheque a Diademe	MI
Cercopithecus mona	Mone	MO
Cercopithecus mona wolfi	Cercopitheque de Wolf	CW
Cercopithecus neglectus	Singe de Brazza	BZ
Cercopithecus nictitans	Hocheur	NI
Cercopithecus pogonias	Singe courounée	PG
Cercopithecus preussi	Singe de Preuss	PR
Civettictis civetta	Civette	CV
Colobus angolensis	Colobe noir et blanc d'angola	CAN
Colobus guereza	Colobe guereza	GZ
Colobus satanas	Colobe noir	SA
Crocuta crocuta	Hyène	HY
Crossarchus ansorgei et alexandri	Mangue d'Ansorge et d' Alexander	CAL
Crossarchus platycephalus	Mangue de crane plat	FC
Dendrohyrax dorsalis	Daman d'arbres	DD
Ecureils	Ecureils	US
Erythrocebus patas	Patas	EP
Genetta spp.	Genet spp.	UG
Genetta victoriae	Genette geante	GV
Gorilla gorilla	Gorille	GG
Grands singes	Grands singes	GS
Herpestes naso	Mangouste a long museau	HN
Hippopotamus amphibius	Hippopotame	HI
Homo sapiens	Humain	HS
Hyemoschus aquaticus	Chevrotain aquatique	HA
Hylochoerus meinertzhageni	Hylochère	HM
Hystrix cristata	Porc-epic	HC
Kobus ellipsiprymnus	Kobe défassa	KE
Kobus kob	Kobe de Buffon	KK
Leptailurus serval	Serval	FS
Lepus saxatilis	Scrub hare	LS
Lophocebus albigena	Cercocebe à joues grises	LA
Lophocebus aterrimus	Cercocebe noir	LAT
Loutres	Loutres	UO
Loxodonta africana cyclotis	Eléphant	E
Lutra maculicollis	Loutre a cou tachete	LM
Lycaon pictus	Lycaon	LP

Nom Scientifique	Nom français	Code
Mandrillus sphinx	Mandrill	MS
Mangouste	Mangouste	UM
Manis gigantea	Pangolin géant	MG
Mellivora capensis	Ratel	MC
Nandinia binotata	African palm civet/ Nandinie	PC
Neotragus batesi	Antilope de Bates	NB
Okapia johnstoni	Okapi	OJ
Orycteropus afer	Oryctérope	OA
Osbornictis piscivora	Genette aquatique	OP
Ourebia ourebi	Ourébi	OO
Pahtaginus tricuspis	Pangolin à écailles tricuspides	P3
Pan troglodytes	Chimpanzé	PT
Panthera leo	Lion	PL
Panthera pardus	Léopard	PP
Papio anubis	Babouin doguera	PA
Perodicticus potto	Potto	PE
Phacochoerus africanus	Phacochère	PH
Potamochoerus porcus	Potamochère	PO
Potamogale velox	Potamogale	PV
Procavia sp.	Daman de rocher	RH
Procolobus pennantii ssp.	Colobe rouge	PB
Sylvicapra grimmia	Céphalophe de Grimm	SG
Syncerus caffer	Buffle	SC
Thryonomys swinderianus	Cane rat	TH
Tragelaphus euryceros	Bongo	BO
Tragelaphus scriptus	Guib harnaché	TS
Tragelaphus spekei	Sitatunga	ST
Uromanis tetradactyla	Pangolin à longue queue	MT

Appendix e

Survey tree species at release site

	Family	Species	Jan	Feb	Mar-Apr	May
Vine	Leg-mim	*Mimosa comosa*				
Tree	Annonaceae	*Cleistopholis patens*			Fl	Fr
Tree	Ebenaceae	*Diospyros*				
Tree	Myristicaceae	*Coelocaryon preussii*		Infl	Infl	Fr
Tree	Burseraceae	*Santiria trimera*			Infl	Fl
Tree	Myristicaceae	*Staudtia gabonensis*		Infl	Infl	Fr
Tree	Olacaceae	*Coula edulis*				Fl
Tree	Verbenaceae	*Vitex doniana*	Fl	Fl	Fl, Fr	Fr
Tree	Sapindaceae	*Soreindea sp*				
Tree	Annonaceae	*Polyalthia suaveolens*			Fl	Fr
Tree	Leg-caesalp	*Dialium lopens*		Infl	Infl	Fl, Fr
Tree	Annonaceae	*Xylopia sp*	Fr	Fr	Fr	Fr
Tree	Annonaceae	*Pachypodentium*	Fr	Fr	Infl	
Tree	Euphorbiaceae	*Uapaca sp*			Infl	Fr
Tree	Myristicaceae	*Pycnanthus angolensis*	Fl	Fl	Fl, Fr	Fr
Tree	Burseraceae	*Aucoumea klaineana*				
Tree	Leg-mim	*Pentaclethra macrophylla*	Fr	Fr	Fl	Fl
Fern	Balanophoraceae	*Thonningia sanguinea*	Fr	Fr	Fr	
Tree	Leg-mim	*Parkia bicolor*				
Tree	Anacardiacea	*Trychoscypha acuminata*				
Tree	Leg-caesalp	*Anthonotha macrophylla*		Infl	Fl	
Tree	Leg-mim	*Afzelia bipendensis*				
Tree	Leg-caesalp	*Erythrophleum ivorense*				
Vine	Celastraceae	*Salacia sp*				
Tree	Loganiaceae	*Warneckea sp*	Fl	Fl	Fl	
Vine	Gnetaceae	*Gnetum africanum*				
Shrub	Commelinaceae	*Palisota sp*				
Vine	Apocynaceae	*Landolphia sp*		Fr	Fr	
Tree	Annonaceae	*Xylopia sp*				
Tree	Annonaceae	*Uvariastrum sp*				
Tree	Leg-caesalp	*Dialium lopens*				Fl
Tree	Rubiaceae	*Porterandia sp*				
Tree	Annonaceae	*Enantia clorantha*				
Vine	Arecaceae	*Raphia sp*			Fr	
Shrub	Zingiberaceae	*Aframomum sp*	Fr	Fr		
Tree	Sterculiaceae	*Cola sp*				Fl
Tree	Annonaceae	*Hexalobus*		Infl	Infl	Fr
Tree	Annonaceae	*Uvariastrum*				
Tree	Annonaceae	*Cleistopholis patens*			Fl	Fr
Tree	Cecropiaceae	*Myrianthus arboreus*	Fr	Fr	Fr	Fr
Tree	Annonaceae	*Xylopia aethiopica*				
Tree	Euphorbiaceae	*Maprounea membranacea*				
Tree	Guttiferae	*Pentadesma*	Fr	Fr	Fl	Fl, Fr
Tree	Leg-pap	*Pterocarpus soyauxii*				
Tree	Olacaceae	*Ongokea gore*				
Tree	Moraceae	*Ficus etrangla*				Fl
Tree	Tiliaceae	*Desplatsia*	Fr	Fr		Fl
Tree	Irvingiaceae	*Klainedoxa gabonensis*				
Tree	Annonaceae	*Monodora myristica*				Fr
Tree	Humiriaceae	*Sacoglottis gabonensis*				Fr
Tree	Annonaceae	*Pachypodanthium staudtii*		Infl	Fl	Fl

Tree	Arecaceae	*Elaeis guinensis*	Fr	Fr	Fr	Fr
vine	Leg-pap	*Millettia sp*				
Tree	Tiliaceae	*Grewia coriacea*	Fr	Fr	Fr	
Tree	Annonaceae	*Xylopia aethiopica*				
Tree	Pandaceae	*Panda oleosa*				
vine	Leg-mim	*Mimosa comosa*				
Tree	Irvingiaceae	*Irvingia robur*	Fr	Fr	Fr	Fr
Tree	Guttiferae	*Symphonia globulifera*	Fr	Fr	Fr	Fr
Tree	Cecropiaceae	*Myrianthus arboreus*	Fr	Fr		
Tree	Burseraceae	*Dacryodes*				
Tree	Burseraceae	*Canaruim shweinfurthii*				Fl
Tree	Guttiferae	*Allanblanckia*	Fr	Fr	Fr	Fr
Tree		*Poga oleosa*	Fl	Fr	Fr	Fr
Tree	Irvingiaceae	*Irvingia grandifolia*		Infl	Fr	
Tree	Cecropiaceae	*Musanga cecropiodes*		Fr	Fr	Fr
Shrub	Costaceae	*Costus*				
Tree	Irvingiaceae	*Klainedoxa gabonensis*		Infl	Fl	
Tree	Sapotaceae	*Synsepalum*		Infl	Fl	
Tree	Euphorbiaceae	*Bridelia*				Fl
Tree	Anacardiacea	*Pseudospondias*				
Tree	Rubiaceae	*Psychotria*		Fr		
Tree	Annonaceae	*Monodora*		infl	Fl	
Tree	Rubiaceae	*Nauclea p*				Fl
Tree	Humiriaceae	*Hexalobus*		Infl	Fl	
Tree	Ebenaceae	*Dyospyros mannii*				
Tree	Guttiferae	*Pentadesma*		Infl	Fl	
Tree	Leg-caesalp	*Dialium lopens*		Infl	Fl	
Tree	Leg-mim	*Piptadeniastrum africanum*		Infl	Fl	Fr
Tree	Euphorbiaceae	*Macaranga*			Infl	Fl
Shrub	Dracaenaceae	*Dracaena*				
Tree	Humiriaceae	*Saccoglotis*		Infl	Fl	
Tree	Rubiaceae	*Pausinystalia yoyimbe*				
Tree	Rubiaceae	*Porterandia*		Infl	Fl	
Tree	Salicaceae	*Caloncoba*			Fl	Fr
Tree	Sapotaceae	*Baillonela toxisperma*			Fl, Fr	Fl, Fr
Tree	Sterculiceae	*Sterculia*				Fr
Tree	Rubiaceae	*Museanda*				Fr
Shrub	Costaceae	*Costus*				
Tree	Arecaceae	*Raphia*				
Tree	Irvingiaceae	*Klainedoxa gabonensis*		Infl	Fl	
Tree	Sapotaceae	*Synsepalum*		Infl	Fl	
Tree	Euphorbiaceae	*Bridelia*				
Tree	Anacardiacea	*Pseudospondias*				
Tree	Rubiaceae	*Psychotria*		Fr		
Tree	Annonaceae	*Monodora*		infl	Fl	
Tree	Rubiaceae	*Nauclea p*				
Tree	Humiriaceae	*Hexalobus*		Infl	Fl	
Tree	Tiliaceae	*Duboscia macrocarpa*		Infl	Fl	
Tree	Ebenaceae	*Dyospyros mannii*				
Tree	Leg-mim	*Piptadeniastrum africanum*		Infl	Fl	
Tree	Euphorbiaceae	*Macaranga*				
Shrub	Dracaenaceae	*Dracaena*				
Tree	Humiriaceae	*Saccoglotis*		Infl	Fl	

Appendix f

Health Management Plan Sedation Procedure

All mandrills undergo a minimum of 3 months quarantine from the time of confiscation to the time of integration with other confiscated individuals. Only after successful integration and the group settled, would release be considered. The Jane Goodall Institute as a member of PASA is obligated and follows the guidelines of PASA veterinary manual with regards to quarantine and long term captive care of any primate. Additionally, as part of PASA protocols, all PASA sanctuary members must follow IUCN guidelines to reintroduction of primates back into the wild.

No individual would be released if there are behavioural or veterinary concerns with regards to the suitability of the individuals. All primates arriving at Tchimpounga, great ape or monkey undergo a 3-month quarantine programme. During this quarantine period, 3 TB tests are carried out with 1 month interval between the tests; blood is taken and analysed, as is faeces, urine and salvia all tested. Only when an individual is deemed clear of any communicable diseases after three months will it be integrated with the resident population.

Just prior to an individual being deemed suitable for release the veterinary treatments and examinations will be carried out:

Anti-parasitic programme includes:

Drug	Dose	Action	Spectrum
Praziquantel	15mg/kg/day	Taken once and repeated after 20 days just prior to release	cestodes
Albendazole	8mg/kg for 3days	Taken once prior just prior to release	Nematodes , oesophagostomus
Mebendazole	25 mg/kg for 3 days	Two times a day within 2 weeks prior to release	Nematodes: hookworm and whipworm
Ivermectin	0,2mg /kg /day	Given once then repeated in 15 days	Nematodes, sarcoptes
Metronidazole	30mg/kg day	Once a month for three months	Protozoa's (giardia , amoebas)
Tinidazol	60 mg/jour over three days	1 time only	B coli

We performed a protocol of testing for diseases primarily recommended for the IUCN reintroduction guidelines, under the control of infectious diseases communicable to preserve the native fauna.

Bacteriology and Virology	Sample Taken	Examination Carried Out
Tuberculoses	Intradermal	Tb test/ serology
Hepatitis B	Serum	Serology
Hepatitis A	Serum	Serology
Salmonella	Fecel	Culture
Campilobacter	Fecel	Culture
Shigella	Fecel	Culture
Streptococus pneumonia	Secretios respiratoire	Culture/ LM
Parasitological		
Nematodes	Fecel	LM/culture
Cestodes	Fecel	LM
Protozooses	Fecel	LM
Malaria	Blood	LM (blood smear,droplets)
Hematology		
Numeration	Blood Smear	LM
HT	Blood	Using Capillary
HB	Blood	Sahli Method
Dermatology		
Sarcoptes	Cutaneous scraping	LM
Dermatitis	Cutaneous scraping	LM

Biological samples of all individuals are also stored for purposes of carrying out a bio bank:
- Serum (frozen in cryotube)
- Plasma (frozen in cryotube)

- Blood and paper kept giligagel (for viral research)
- Hair (sample genetics)

Husbandry: All mandrills are kept at the sanctuary for no less than three months before entering the release programme. Most individuals spend much more time at the sanctuary. All undergo a three month quarantine in isolation of all other individuals. This is done off sight, at another location that is 4 kms from the sanctuary. After three TB tests, free parasite load testing and clean blood work, any new arrival is then slowly integrated into the existing mandrill group. The group currently live in a facility that has 5 interconnecting cages that allow separation of communal living, depending at want state the integration process is going.

Mandrill enclosures, as per all other facilities at the sanctuary are cleaned daily. Water is provided via water nipples (self drinking devices) and food is provided 3 times per day.

During the time in captivity at the sanctuary, the mandrills will be assimilated with wild food plants such as:

- Aframomum (Tondolo)
- Landolphia (Malombo)
- Cola gabonensis (Bissiese)
- Tchicophila acuminata (Tsouteke)
- Niotum africanum (Foumbu) ....

This is to ensure they remember and are willing to eat wild food plants once back in the forest. In the first few weeks of release, depending on the mandrills needs, we will provide supplementary food until the field team are assured the mandrills are finding enough wild food to support themselves. As per the first release, we expect this process to be quick, as we will release in the wet season when food availability is high.

Staff screening and health

All staff of JGI are given annual health checks, including TB, HIV testing,blood and fecal analysis is done annually. Fecal samples can and are done ad hoc if individuals are exhibiting signs of illness or are complaining of illness. All staff are trained not to come to work if they have any upper respiratory infections.

Sedation Procedure:

All mandrills will be sedated with the following to be transferred from their enclosures to the transport boxes on the day of the transfer. Once the mandrills are sedated, each will be marked with a purple dye in addition to the radio collars for monitoring. Once safely in the box, the anesthetic will be reversed, so they are awake during the transportation process. Sedation will either be done by hand injection where possible, if not blow pipes will be used. Most individuals will present for grooming and so, most can be hand injected without need to stress individuals with the use of the blow pipe.

Anesthetic agent and dosage is:

Alfa 2 agonist medetomidine 0'05 mg / kg

Cicloxamide Ketamine 5 mg / kg

Antidote Atipamezole; given in same volume as for medetomidine

Appendix g

Mandrill Ethogram

The Mandrill ethogram used in the pre release testing will be based off of Mellen 1981, Setchell 1999. Measures of self directed behaviours will be taken from Castles 1999 and Aggression levels will be divided into three levels of aggression ranging from non-physical to physical outlined in Otivac 2007. Vocalizations are from Kudo 1987.

Behaviour	Description
Body Position	
Sit, Lay, Climb, Jump, Run,	
States of activity	
Sitting, Climbing Eating, Running, Walking, Playing, Fighting, Sexual, Foraging, Digging, Scanning	
Aggressive behaviours	
Level 1	
Head bob	Actor plants feet and jearks head forward towards individual being threatened. Mouth is closed and nuchal crest is raised.
Ground Slap	Actor slaps one or both hands in a fast movement on the ground.
Level 2	
Lunge	Actor lunges in the direction of another animals but does not move more than a meter
Cage Slap	Actor slaps the fence surrounding the enclosure or grips and shakes the cage. Usually accompanied by repeated grunting
Threat Rush	Actor rushes towards another animal, stops or changes direction before fully chasing or attacking.
Chase	Actor runs rapidly after recipient which who is fleeing.
Dirt throw	Actor leaps vertically in the air while kicking dirt behind them.
Dirt Slap	Actor slaps dirt with for arms casing dirt to fly perpendicularly away from the subject
Grimace	Actor's mouth was open to varying degrees and the lips retracted horizontally and was closed and the nuchal crest was raised. This could be repeated several times. A grunting vocalization often accompanies a head bob.
Level 3	
Hit or Grab	Actor hits or grabs the recipient.
Bite	Actor bites the recipient.
Attack	Actor uses a combination of hitting , biting, chasing and grabbing
Affiliative and social behaviour	
Groom	Actor parted the recipient's hair with one or both hands. The thumb and index finger were used to pick at the skin and transfer particles to the mouth.
Grin	In the adult male mandrill, the mouth is closed and the corners of the mouth are drawn back exposing the canines and premolars. The lips are drawn together or almost together over the incisors in the shape of a horizontal figure-eight. Crest is raised, ears are flattened and head is slowly shaken from side to side. In adult females and infants, the mouth does not form as distinctive a figure eight as in the adult male. The lips form more of an oval shape, exposing the incisors, canines and premolars
Head-shake	Actor shook its head one or more times sideways. Typically accompanied 'Grin'.

Grooming solicitation posture	Individual stands quadrupedally with all four limbs fully extended. Head is held slightly up, facing away from the potential groomer, with the hind quarters toward the groomer. Tail is flattened back over the spine. A second grooming solicitation posture involves the soliciting individual in a sitting posture with head tilted to one side exposing an area of the neck or armpit toward the groomer's face. Eye contact between the two individuals is brief or non-existent.
Approach	Actor moved towards the recipient, passing him, or standing near him.
Affiliative and social behaviour	
Avoid	Actor moved away from an approaching animal.
Mount	Same as for 'Full mount' but actor and recipient were male–male, female–female, infant–infant or infant–adult.
Sternal gland marking	Animal rubs sternal area in an anterior-posterior motion on an object; chin is usually raised
Self-Directed Behaviour	
Self-scratch	Movement of the hand or foot during which the fingertips are drawn across the fur or skin.
Self-groom	Picking through and/or slowly brushing aside fur with one or both hands.
Self-touch	Other forms of body touching with the hand including wiping eyes, inspecting feet and placing hand to mouth.
Body shake	Shaking movement of entire body (similar to that of a wet dog).
Yawn	Brief gaping movement of the mouth. Not recorded as an SDB if accompanied by aggressive signals such as eye flash or canine whetting.
Submissive behaviours	
Flee	Mandrill ran away from another animal
Presentation	Same as Sexual presentation but actor and recipient were male–male, female–female.
Sexual behaviours	
Male sexual behaviour	
Attempted mount	Male attempted to mount a female or male, who either accepted or refused the mount.
Full mount	Male successfully mounted female and ejaculated.
Masturbation	The erect penis was rolled back and forth between the hands or stroked longitudinally. Ejaculation could occur.
Follow female	Male walked after a female at a short distance, often looking at the sexual skin.Typically indicated sexual interest and formed part of pre–copulatory behaviour.
Inspect	Male looked closely at a female's perineum, often lip-smacking or grinning. Males also touched the female's vagina and licked or sniffed their hands, or sniffed the female's genitalia.
Mate guarding	Prolonged and persistent following and maintenance of proximity to a female by amale.
Smacking	Smacking noise made with mouth. Accompanied by head shaking and grinning, typically immediately followed by sexual behaviour
Female sexual behaviour	
Presenting	Individual either stands with limbs bent or crouches with ventral surface touching the substrate. The rear is oriented toward the individual presented to. The individual presenting repeatedly looks over its shoulder at the individual presented to.
Refuse	Refusal of a male's mounting attempt by a female, by sitting down, showing aggression or moving away rapidly.
Masturbation	Clitoris rubbed vigerously with hand or stick.

Accept	Female accepted a mount from a male.
Vocalizations	
Long distance call 2 phase grunt	Made by the adult male. Low groaning 2 syllable vocal sound. Sound unit of .5 sec. duration is repeated with an interval of 2 sec.
Crowing	Begins in the form of vibration with discreet units of .04-,06 sec. and is followed by a continuous sound with harmonic structure. Persists for 1.8 seconds or longer
Short distance Yak	Repetition of a sharp pulse like sound of.15-.25 sec. Persists for .05-3 sec.
Grunt	Short and intense expiration of breath repeated once or twice
K-alarm	2 syllable sharp and short sound emitted in one unit with the second syllable much louder than the first
K-sound	Sharp and loud with various nuance [Kyakya] [kwakwa]
Scream	Noisy sound [gyaa]. [gii]. First par is slightly tonal the other is with large energy distribution. Persists for .25 -several sec.
Griney	2 syllable sharp and short sound emitted in one unit with the second syllable much louder than the first
2 phase moan/sigh	Made by alpha male first part of the two syllable sound goes up and second goes down. Low energy.
1 phase moan/sigh	Same as the second syllable of the 2phase moan/sigh

Appendix h

Observation sheet with Self-directed behaviour

Appendix I

Staff observation sheet

Observation des Mandrilles — Observateur — Date — the Jane Goodall Institute / Duke University

Heure	Behavior	Associat	Contact	Autre/distance/activité	Alimentation	éviter	Haut	Commentaire
	Positionner/Activité	Submissive	Aggressive	Sexual	Affiliative	nom spécifique			
0								
2								
4								
6								
8								
10								
12								
14								
16								
18								
20								

Nombre de mandrills collar number

Toutes les 20 minutes

Toutes les mandrills présents Nome.Behavior.Associat Contact.distance.Haut

Vegetation-sous-bois/%

Vegetation-canopée/%

Point GPS

Meteo

Heure	Behavior	Associat	Contact	Autre/distance/activité	Alimentation	éviter	Haut	Commentaire
	Positionner/Activité	Submissive	Aggressive	Sexual	Affiliative	nom spécifique			
0								
2								
4								
6								
8								
10								
12								
14								
16								
18								
20								

Nombre de mandrills collar number

Vegetation-sous-bois/%	Point GPS
Canopée/%	Meteo

Toutes les 20 minutes Toutes les mandrills présents
Nome.Behavior.Associat Contact.distance.Haut

Code	Nom
KM34-Mpacka	
OB18-Obia	
GEO22-George	
DOM13-Dominique	
MAD20-Madol	
TCH-Kiki	
GAG-Gagaga	
VDL-Vieux Delo	
GAY-Gayard	
MOB-Mboté	

Positionner/Activité: assise, déposer, montée, marcher, saut, courir, manger, sexe, masturber, creusement, se gratter, D'outils, Marcher à 2 pattes, pansage, jouer, reste, agressif, fourrage

Submissive: 1) Soumettre, bobi de la tête, fente, 2) Chasse, cage, taper, 3) Frapper, mordre, seize

Aggressive: 1) Présent, 2) Toucher, inspecter, examiner, 3) Mount/fullmount, tentative de montage, montage

Sexual

Affiliative: 1) 1 mètre, autre; 2) Sourire, autre; 3) Pansage, autre; 4) 1-5 mètre, autre

Alimentation: 1 tige, 1 feuille, fleur, fruit, 2 graines, insect, 3 avage, 4 ...

Hauteur: 0=sol; 1=<5m; 2=5-15m; 3=15-30m; 4=>30m

Activité...=se gratter, bâillement, secouer, se toucher, se pansage,

Appendix j

Durham University Ethics Form

PERSONAL AND CONFIDENTIAL

DURHAM UNIVERSITY
LIFE SCIENCES ETHICAL REVIEW PROCESS COMMITTEE
TWO YEAR REVIEW FORM – UNLICENSED WORK

Review reports will be requested after 2 years and after 4 years where unlicensed work is continuing. These reports must be written in language which a lay person can readily understand, and should not generally exceed four A4 sides in length. Column one is to replicate your original outline application form; column 2, any changes made since; and column 3, explanations for those changes.

Please use the following format and headings for your report.

	ORIGINAL OUTLINE APPLICATION FORM	TWO YEAR REVIEW REPORT	EXPLANATIONS FOR ANY CHANGES SINCE THE ORIGINAL OUTLINE APPLICATION FORM
1	Title of Project A 18-month study based on the release of eight wild-born *mandrillus sphinx* into the Conkouati-Douli National Park; Republic of Congo	Title of Project A 18-month study based on the release of eight wild-born *mandrillus sphinx* into the Conkouati-Douli National Park; Republic of Congo	Title of Project
2	Proposed dates of commencement and completion October 2012-October 2015	Actual dates of commencement and completion October 2012-October 2016	Actual dates of commencement and completion The transfer of the animals to the release site was delayed a year because of funding and logistical issues. The field work will finish shortly after the collars drop off of the animals on June 9th of 2015.
3	Name of Applicant Miles Cooper Woodruff; M.C.WOODRUFF@DURHAM.AC.UK 1(925)297-6696	Name of Applicant Miles Cooper Woodruff M.C.WOODRUFF@DURHAM.AC.UK 1(925)297-6696	Name of Applicant Miles Cooper Woodruff
4	Name/s of Assistant/"Deputy"/Co-worker/s Dr. Jo Setchell, joanna.setchell@durham.ac.uk; Dr. Russell Hill, r.a.hill@durham.ac.uk	Name/s of Assistant/"Deputy"/Co-worker/s Dr. Jo Setchell, joanna.setchell@durham.ac.uk; Dr. Russell Hill, r.a.hill@durham.ac.uk	Name/s of Assistant/"Deputy"/Co-worker/s
5	Name/s of any collaborator/s Dr. Rebeca Atencia Jane Goodall Institute Congo Director, rebecatencia@hotmail.com	Name/s of any collaborator/s Dr. Rebeca Atencia Jane Goodall Institute Congo Director, rebecatencia@hotmail.com Dr. Tamara Bettinger tamara.L.Bettinger@disney.com Disney Animal Kingdom	Name/s of any collaborator/s Disney Animal Kingdom came on as a partner with the noninvasive hormone work.
6	Purpose of the work, and advantages expected to be gained from the work The primary purpose of my research is to masure the conservation impacts and success of a Mandrillus sphinx reintroduction project over an 18 month period. I will also document the little-studied ranging patterns and free ranging behaviors of the mandrills. I will compare the release methods used during the release with the methods suggested in the IUCN guidelines to assess their direct practical applications. I will document how the presence of researchers and eco-gaurds impact illegal human activities within the study area. I will compare the reliability of the GPS radio collars to hand held GPS units to assess their usefulness in primate reintroduction. The project will inform future primate releases and will further the understanding of mandrill behavior in the wild. Additionally the project will give practical feedback to IUCN on how the guidelines are bing used in release. Documenting the impact researchers have on illegal human activities will be useful information for future conservation efforts.	Purpose of the research - is that purpose being met? Yes we are in alignment with the original purpose and have added the additional biological component with the non-invasive fecal hormone study.	Purpose of the research - is that purpose being met? The hormone study will inform the IUCN guidelines' recommendation of using a pre-release inclosure and conducting a soft release. The non-invasive fecal hormone study supports the previous work by providing a bio-marker for the stress the animal is experiencing during each stage of the reintroduction and allow us to measure how long the animals stress levels remained elevated after the transfer and release.
	----------	Are the expected advantages as originally proposed? Yes we they are.	Are the expected advantages as originally proposed? We are learning about mandrill behavior in the wild and changing the hunting behavior in the area. We also completed the GPS collar study and had significant results that will inform GPS collar usage.

PERSONAL AND CONFIDENTIAL

7	Summary of procedures and anticipated impact on the animals The mandrills will undergo a soft release and be followed for 18 months using VHF collars. Prior to the release they will be sedated, fitted with radio collars and transferred to a pre-release enclosure at the release site. In this enclosure they will undergo behavioral observations to measure stress and social affiliations. These observations will include scan and focal sampling. The animals are wild born but captive raised and are habituated to the presence of human observers. After two weeks of acclimation and observation the cage will be opened. The mandrills will receive supplemental feeding for a minimum of three months and supplemental feeding will continue as needed. The mandrills will be tracked using VHF radio transmission collars for 9-18 months. The collars will automatically release at the end of the study. Animals who are sick or injured will be recaptured and receive treatment. If the release subjects interact with wild mandrills researchers coninue to observe the released subjects but will leave the area if the wild population becomes visibly stressed.	Actual impact on the animals	Actual impact on the animals Three of the original release subjects removed from the project for various reaso adult female mandrill was removed becau was biting the staff. An adolescent ma removed because the country director fel necessary based on its color changin animal had not been released from the the point of its removal. The third dispersed from the group into an area th unregulated hunting. All of the animals a and healthy at this point. We have contin supplemental feeding as a method monkeys to leave the release site and exp surrounding forest. We were not able to fi the animals with collars because the would have been more than 5% of the weight. We have not had any contact w mandrills.
8	Location of the work in the University Conkouati-Douli National Park; Republic of Congo	Location of the work in the University Conkouati-Douli National Park; Republic of Congo & Tchimpounga Chimpanzee Reserve; Republic of Congo	Location of the work in the University A portion of the collar study was conducte Tchimpounga Chimpanzee Reserve. The study was testing the function of the collar did not use animals.
9	Numbers and species of animals involved Ten mandrills sphinx	Numbers and species of animals involved to today's date There are 19 mandrills in the Congo project.	Numbers and species of animals involved today's date The increase in the number of subjects is primarily due to confiscations of illegally h animals. The confiscated animals are then over to the sanctuary for care. We also ha monkey born in the wild and one monkey captivity.

	----------	Numbers and species planned for use during the remainder of the project We expect the number to remain at 18 animals.	Numbers and species planned for use remainder of the project We are not expecting any new anima at this time but it is a possibility.
10	Are any of the animals of a rare or threatened species? Yes, mandrills are on the IUCN Red List of threatened species	Are any of the animals of a rare or threatened species? Yes, mandrills are a threatened species.	Are any of the animals of a rare or thre species?
11	Can the work be undertaken without any living animals at all? [Replacement] No. Because it is an observational study based on the released of living primates, it will require living primates.	Can the work be undertaken without any living animals at all? [Replacement] No. Because it is an observational study based on the released of living primates, it will require living primates	Can the work be undertaken without a animals at all? [Replacement]
12	Where animals have to be used, is the number of animals involved the absolute minimum necessary to obtain valid results where Replacement is not possible? [Reduction] Yes, the observations will be limited to a single group of animals. The sample size is small and replacements will not be available.	Where animals have to be used, is the number of animals involved the absolute minimum necessary to obtain valid results where Replacement is not possible? [Reduction] Yes the animals are still at a minimum. We have added animals to the study with the inclusion of the non invasive fecal work we are doing with Disney.	Where animals have to be used, is the animals involved the absolute minimu necessary to obtain valid results where Replacement is not possible? [Reducti
13	Have all the procedures been Refined to minimise the adverse effects [Refinement]? Yes The animals are habituated to the presence of human observers and the proposed observational study should not negatively impact their welfare. If observations seem to be causing the animals stress observations will stop and observers wil retreat. Tracking will continue using VHF collar signals from a distance of 100-200m	Have all the procedures been Refined to minimise the adverse effects [Refinement]? We removed a female from the group because she sought out observers and aggressed them. No one was seriously injured but we thought things might escalate. The female is healthy and will continue to live at Tchimpounga.	Have all the procedures been Refined minimise the adverse effects [Refinem
14	Funding sources and collaborators the Jane Goodall Institute is funding the release of the mandrills.	Funding sources and collaborators the Jane Goodall Institute Disney and private donors	Funding sources and collaborators the Jane Goodall Institute Disney and donors

PERSONAL AND CONFIDENTIAL

15	Other matters - statistical advice taken, anything to be drawn to the Committee's attention The sample sizes and statistical issues have been discussed with my supervisors.	Other matters - statistical advice taken, anything to be drawn to the Committee's attention The sample sizes and statistical issues have been discussed with my supervisors.	Other matters - statistical advice taken, a to be drawn to the Committee's attention
	Alterations/Changes *(with details of LSERP Committee approval dates)* *No we are not planning to bring any samples into the country*	Alterations/Changes *(with details of LSERP Committee approval dates)* We did bring in fecal, hair and fingernail samples.	Alterations/Changes *(with details of LSE Committee approval dates)* For the non-invasive hormone study we hair and fingernail samples collected dur routine health checks as well as the feca extracts collected non invasively and pro in the field. We had CITIES export perm exportation of the samples as well as the letter of explanation for import into the U
16	Intended method of dissemination of findings The results will be written up as a postgraduate dissertation.	Dissemination of findings to date In addition to writing up the results for the postgraduate dissertation we are preparing the results of the collar and hormone study for publication.	Dissemination of findings to date

Signature .....

Date 11/12/2014

Appendix k

Approval for the establishment of the mandrill base camp

DURABLE

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Projet d'Appui à la Gestion
Parc National Conkouati-Douli

B.P. 498, Pointe Noire
Tél : 068631764/ 055497477 ; 055440034
Département du Kouilou,
République du Congo

WILDLIFE CONSERVATI SOCIETY

N° 023 /MEFDD/ DGEF/PAG-PNCD

Conkouati, le 12 Mars 2013.

Le Conservateur du Parc
National Conkouati-Douli.
Conkouati

bjet : Installation au camp Faucon
éférence : Votre lettre du 08 Mars 2013.

A

Madame la Directrice Exécutive
de l'Institut Jane Goodall Congo
Tchimpounga

adame la Directrice,

ır lettre citée en référence ci-dessus, je donne mon accord pour installer le camp
r l'ancien site Faucon pour le relâcher et le suivi des mandrills.

vous prie d'agréer, Madame la Directrice, l'expression de ma parfaite
nsidération.

npliations :
EF:1
EFK:1
gade MK :1
M/Kayes :1
T :1
hive : 1/5

Appendix I

Approval for the mandrill release

MINISTERE DU L'ECONOMIE FORESTIERE
ET DU DEVELOPPEMENT
DURABLE
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REPUBLIQUE DU CONGO
Unité * Travail * Progrès
-=-=-=-=-

Projet d'Appui à la Gestion
Parc National Conkouati-Douli

B.P. 498, Pointe Noire
Tél : 068631764/ 055497477 ; 055440034
Département du Kouilou,
République du Congo

WILDLIFE CONSERVATION SOCIETY

N° 079 /MEFDD/ DGEF/PAG-PNCD Conkouati, le 29 Octobre 2013

Le Conservateur du Parc
National Conkouati-Douli.
Conkouati

Objet : Réparation du pont
Référence : Votre lettre du 28 Octobre 2013.

A

Madame la Directrice Exécutive
de l'Institut Jane Goodall Congo
Tchimpounga

Madame la Directrice,

Après notre conversation téléphonique du 26 Octobre suite à la destruction du pont sur la rivière Tissa, isolant le site du relâcher des mandrills du village Km4, suite à ma visite dudit pont et pour faciliter le ravitaillement du site, par lettre citée en référence ci-dessus, je donne mon accord pour la réparation du pont de la rivière Tissa.
Je vous prie d'agréer, Madame la Directrice, l'expression de ma parfaite considération.

Ampliations :
DGEF:1
DDEFK:1
Brigade MK :1
S/P M/Kayes :1
RNT :1
Archive : 1/5

Roland MISSIE OU BOUKAKA
Conservateur Chef de S[illegible]

Appendix m

Cites permit for exporting dried faecal, hair and nail samples.

RÉPUBLIQUE DU CONGO

CONVENTION SUR LE COMMERCE INTERNATIONAL DES ESPECES DE FAUNE ET DE FLORE SAUVAGES MENACEES D'EXTINCTION

PERMIS / CERTIFICAT N° 038 — Original

☒ EXPORTATION ☐ REEXPORTATION ☐ IMPORTATION ☐ AUTRE

2. Valable jusqu'au 18/03/15

3. Importateur (nom et adresse)
NICOLE SHARPE, C/O MC COWAN LAB
DEPARTMENT OF POPULATION, HEALTH AND
REPRODUCTION, VETERINARY MEDICINE 3B
UNIVERSITY OF CALIFORNIA, DAVIS

4. Exportateur/réexportateur (nom et adresse, pays)
RESERVE NATURELLE DE TCHIMPOUNGA
B.P. 1206
REP. DU CONGO

3a. Pays d'importation: USA

5. Conditions particulières
ESPECES INTEGRALEMENT PROTEGEES (ANNEXE I)
ANALYSES EN LABORATOIRE

Pour les animaux vivants, ce permis ou certificat n'est valable que si les conditions de transport sont conformes aux Lignes directrices pour le transport des animaux vivants ou, en cas de transport aérien, à la Réglementation IATA du transport des animaux vivants

5a. But de la transaction (voir au dos): S

5b. Timbre de sécurité n°: CC 1125666

6. Nom, adresse, sceau/cachet national et pays de l'organe de gestion
Direction Générale de l'Economie Forestière
Direction de la Faune et des Aires Protégées
B.P. 98, Brazzaville - République du Congo

	7. Nom scientifique (genre et espèce) et nom commun de l'animal ou de la plante	8. Description des spécimens, marques ou n° d'identification (âge/sexe si vivant)	9.	10. Annexe et source (voir au dos)	11. Quantité (et unité)	11a. Total exporté/Outre
A	7.a CHIMPANZE (Pan troglodytes)	ECHANTILLONS DES SELLES SECHES		I W	300	
	12. Pays d'origine * / Permis n° / Date: CONGO / 038 / 18/09/14			12a. Pays de provenance / Certificat n° / Date		12b. N° de l'établissement ** ou date de l'acquisition **
B	7.a MANDRILL (Papio sphinx)	ECHANTILLONS DES SELLES SECHES		I W	1500	
	12. Pays d'origine * / Permis n° / Date: CONGO / 038 / 18/09/14			12a. Pays de provenance / Certificat n° / Date		12b. N° de l'établissement ** ou date de l'acquisition **
C	7.a MANDRILL (Papio sphinx)	ECHANTILLONS D'ONGLES ET POILS		I W	40 (Sachets plastiques)	
	12. Pays d'origine * / Permis n° / Date: CONGO / 038 / 18/09/14			12a. Pays de provenance / Certificat n° / Date		12b. N° de l'établissement ** ou date de l'acquisition **
D	7.a //////////	8. //////////	9. //////////	10. //////////	11. //////////	11a.
	12. Pays d'origine * / Permis n° / Date			12a. Pays de provenance / Certificat n° / Date		12b. N° de l'établissement ** ou date de l'acquisition **

* Pays dans lequel les spécimens ont été prélevés dans la nature, sont nés et ont été élevés en captivité ou reproduits artificiellement (seulement en cas de réexportation)
** Uniquement pour les spécimens d'espèces inscrites à l'Annexe I nés et élevés en captivité ou reproduits artificiellement à des fins commerciales
*** Pour les spécimens pré-Convention

13. Ce permis/certificat est délivré par: LE DIRECTEUR GENERAL DE L'ECONOMIE FORESTIERE

BRAZZAVILLE — Lieu
18/09/14 — Date
signature et cachet officiel

14. Approbation de l'exportation: 15. Connaissement/lettre de transport aérien n°

Joachim KONDI

Bloc	Quantité
A	
B	
C	
D	

Port d'exportation — Date — Signature — Timbre officiel et quantité

PERMIS/CERTIFICAT CITES N° 038

Appendix n

Overview of EIA procedures used in the assay validations covered fully in

(Lavin et al., submitted)

Preparation of 69a (1:900,000) and 69a AB (1:17,000) assays for four plates each. To prepare the 69a label (1:900,000) we added 150ul 100% methanol to a 69a label tube shook well then let it stand for 20 minutes. On the day of use we combined 70ul label/methanol and 42m; 1x assay buffer to make a 1:900,000 solution then shook it and let it stand for 20 minutes. To prepare the 69a AB (1:17,000) we added 600ul ddH20 to a 69a antibody tube, shook well then let it stand for 20 minutes. On the day of use we combined 295ul antibody/water and 42ml 1x assay buffer to make a 1:17,000 solution then shook it and let it stand for 20 minutes. We stored unused label in the freezer.

Assay methods

We first pipetted the standards or diluted samples into a clear microtiter plate coated with Protein A. The 69a-biotin conjugate was then added to the standards and samples in each of the wells followed by the addition of the 69a antibody to each well to initiate binding. We then left the plates to incubate for 24 hours before washing them and added streptavidin-peroxidase conjugated to bind the 69a-biotin conjugate. We then allowed the pates to incubate for a further 45 minutes, washed them again, then added the, room temperature, TBM substrate to initiate the reaction with the bound 69a-perozidase conjugate. After a further 45minute incubation, we stopped the reaction by washing the plate quickly drying it upside down on a paper towel and adding TBM substrate to each well. We used a microplate reader capable of measuring 450-650 nm to measure the colour intensity of the wells. We calculated the concentration of the 69a in the well using SoftMax Prosoftware (pg/50ul). The 69a concentration in the well is calculated by correcting for the dilution of the sample and mass of the faecal sample (g) and the extraction volume (ml) and correction for the creatinine (ug/mass hormone measured /ml) or specific gravity.

Appendix A Materials, material source and the concentration/volume of the buffer or chemical used to conduct the assay

Buffer/Chemical used	Material Source	Concentration	Volume used per well	Volume used per plate*
DAK 1x Assay Buffer	Prepared in house	1x	50µl	<1ml
69a standards & controls	Prepared in hose (Stock 2 = 500,000 pg/50ul)	Prepared in house 5.12-7812.5 pg/50µl	50µl	150µl
	Rupert Palme (tube = 25,000 pg; for 10 plates: add 0.2ml to tube and transfer to 2.3ml buffer for 500 pg/50ul top standard)	Rupert Palme 2.048-500 pg/50ul (serial dilutions of 1:2.5)		
69a AB	Rupert Palme	1:17,000	100µl	>10ml
69a label	Rupert Palme	1:900,000	100µl	>10ml
DAK 1x Wash Solution	Prepared in house	1x	6x250µl	~150 ml

Strepavidin-POD-conjugate	In house prep (2ul/48ml)	1:24K	250µl	~25ml
TMB substrate	Moss	Neat (2.5mmol/L)	250µl	~25ml
HCl stop solution	Fisher	Neat, 1N (1 Molar)	50µl	~5ml
Plates and Consumables				
Protein A coated plate (8x strips or full plate; 250ul coating/well)	In house prep (0.002mg/ml of Sigma P-7837 or P-3838)			
Plate cover	Plastic			
Consumables: glass vials for AB & label, tips, disposable troughs, strip tubes/test tubes, stir bars (label only)				

* 96 Wells per plate