USING FEEDING ECOLOGY TO INFLUENCE CAPTIVE SLOW LORIS (*NYCTICEBUS* SPP.) NUTRITION AND HUSBANDRY

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ABSTRACT

Despite the advancement of science within the animal nutrition field, specifically production and domestic animals, exotic animal nutrition is very little studied. Some species are so understudied or shrouded in routine and anecdotes that both zoos and rescue centres manage them the same way, the wrong way. The slow lorises *Nycticebus* spp. are one of these species. I aimed to investigate the diet of wild Javan slow lorises, in order to create an appropriate captive diet for them. My objectives were to assess the current state of captive slow loris diets, calculate the nutrient intake rates and energy expenditure in wild individuals, assess the importance of natural food items within their diet, and finally, to trial a new diet and assess its long term impacts on health. From June 2014 to June 2015 I collected behavioural and feeding data on 17 radio-collared wild Javan slow lorises near Cipaganti, Indonesia. Food samples were collected and analysed for proximate and fibre analyses. Our diet trials were conducted in a rescue centre where we introduced gum into their diets and recorded food passage time. We collecte faecal samples of wild and captive individuals and analysed them for chitinolytic activity. We developed a new diet and compared nutrient intake, digestion and passage rate of the old and new diets. Wild diet was gum, insects and plant parts with seasonal variations in intake. Average intake was high in protein and fibre, low in sugars. They are able to vary their behavior to adjust energy expenditure. Captive animals increased passage rates when fed gum and potentially can digest chitin. Our new diet of gum, insects and vegetables had similar physiological effects than wild diets: slower and more efficient digestion and more appropriate nutrient intake. It was conducive to optimum weight and health.

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I can pinpoint the moment when my career was born. When Dr Amy Plowman offered me an internship in animal nutrition following my masters when her dedicated student dropped out. Things have a funny way of happening and working out for me. Always giving me the benefit of the doubt and supporting me in whatever situation I was in all while remaining perfectly British. I will always remain deeply loyal to you. You have my eternal gratitude.

I never would have looked into slow lorises this intently if it wasn't for Matthew Webb. His exact sentence was "You should look into slow lorises; they have loads of issues". Following his wise advice, I started to look into slow loris nutrition. For sending me on this path Matt, I am very thankful. This led to a short paper with a sample size of two which happened to catch the eye of my supervisor to be, Prof Anna Nekaris. She saw the potential for an entire PhD which I accepted. You have been a constant source of patience and advice throughout this journey. Coupled with the counsel and effort from Dr Giuseppe Donati and Dr Ellen Dierenfeld, I felt like I was in safe hands. I can never repay the time and energy you have all spent on me, I am humbled. You have showed me how important it is to be selfless and to nurture the next generation of researchers who can make a difference and solve the issues of tomorrow, even if this problem is properly feeding small nocturnal primates. I hope you do not regret the time spent on me.

My fieldwork was the most significant time of my life. Never have I, nor will I be forever changed by a period of my life like this, that I would never want to forget, yet never want to repeat. I had both the best and worst moments of my life. I learned a lot about myself, found a different version of myself I didn't know existed and I met both the best people I will ever meet but I also met the worst. To my trackers, I literally owe you my life as I would surely have perished without you. Thank you Dendi Rustandi Pondiana, Adin Sopian, Dede Ahmad Aconk and Yiyi Muhamed. Saya sangat menghargaii pekerjaan kalian. Terimah Kasih. Thank you

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And last but not least, thank you to the slow lorises. I have seen many devastating scenes at animal markets and rescue centres, where the only release for these poor animals would be death. We are used to hearing about the strength of an elephant and the speed of the cheetah and the prowess of the lion. No one speaks about the will to live of a slow loris. No one knows how strong they are, how much they can suffer through and what they can come back from. No one sees their struggles hidden behind their clown-like faces. I saw you little ones, and I hope I can help some of you have a second

chance. This was all for you.

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ABBREVIATIONS

ADF = Acid Detergent Fibre

AZA = Association of Zoos and Aquaria

BLR = Binary Logistic Regression

BMR = Basal Metabolic Rate

CWRC = Cikananga Wildlife Rescue Centre

EAZA = European Association of Zoos and Aquaria

EEP = European Endangered species Plan

FMR = Field Metabolic Rate

FNG = Framework of Nutritional Geometry

GIT = Gastro-Intestinal Tract

GLMM = General Linear Mixed Model

MRT = Mean Retention Time

NDF = Neutral Detergent Fibre

NPE = Non-Protein Energy

PCA = Principle Components Analysis

PE = Protein Energy

PSM = Plant Secondary Metabolites

SSP = Species Survivial Plan

TAG = Taxon Advisory Group

TEE = Total Energy Expenditure

TT = Transit Time

TNC = Total Non-structural Carbohydrates

WSC = Water Soluble Carbohydrates

PREFACE

This thesis was constructed as a walk through the necessary steps for creating and validating nutrient recommendations for captive animals beginning from wild observations. For this reason, the thesis must be read as a whole and some data obtained in some chapters are only used in later chapters.

Chapter I – This chapter gives a broad literature review about exudativorous mammals and how they are currently fed in captivity and highlighting linked health issues that they may be facing.

Chapter II – This chapter details the field site, study species and the methods repetitively used within this thesis.

Chapter III – This chapter initiates the rational behind why this research is warranted, providing data that slow lorises (*Nycticebus* spp.) in captivity suffer from dental issues which are linked with their diet.

Chapter IV – This chapter collects the bulk of the data and information which sets the path of this thesis. The diet and nutrient intake information in this chapter is used as a guide to create the trial diets used in later chapters. Data from this chapter are often referred to but most importantly, the average nutrient intake of wild slow lorises is not presented until Chapter VII where this data is useful.

Chapter V – This chapter uses metabolic equations to estimate the energy of free ranging males and females but discusses it in a captive context.

Chapter VI – This chapter looks at the importance of gum as being part of the captive diets. Diet information from Chapter IV is referred to.

Chapter VII – This chapter looks at the importance of having insects as part of the captive diets.

Chapter VIII – This chapter combines data from chapter IV, VI and VII to trial and validate a new captive diet. Every chapter was conducted specifically to inform how this chapter was conducted, therefore, this is where the nutrient recommendations are listed, although this data originated from Chapter IV.

The following thesis is written in the first person using the plural ‘we’ rather than ‘I’. I received the help of various research assistants throughout this journey and similarly advice from supervisors and colleagues during analysis and writing up helped to shape this thesis. For this reason, it doesn’t sit right to claim this entirely as my own.

CHAPTER I

"EXPLOITING A READILY AVAILABLE BUT HARD TO DIGEST RESOURCE: A REVIEW OF EXUDATIVORY IN MAMMALIA AND ITS IMPACT IN CAPTIVE CARE"

1.1 INTRODUCTION

The use of gum as a resource by mammals, especially in terms of nutrient uptake, remains largely unstudied despite field studies increasingly revealing the vital nature of this resource to mammals as an obligate or fallback food. In this review, we examine the published literature on the use of this food, how mammals adapted to consume it, and the nutrition it contains. Although reviews of exudativorous primates have been previously published (Burrows and Nash, 2010; Nash 1986; Smith 2010), the current version opens the scope to encompass mammals in general, and includes literature additions since 2010. Additionally, we will use this information to discuss the current nutritional management of gummivorous species in a zoological setting, and discuss the importance of gum as a component of diets.

1.1.1 The Nature of Gum

The very nature of what gum is can be confusing based on definitions available in the literature. All gums are exudates, but not all exudates are gums. The term exudate includes gums, resins and latexes. We use the definition of gum from Nussinovitch (2009): a fluid that is produced by some plant through gummosis, following the creation of an injury, that hardens upon exposure to air. Contrary to some usage, sap is not an exudate as it is simply leaking phloem liquid. Sap and gum also both have very different chemical compositions with sap being mostly soluble carbohydrates and water while gum is mostly non-digestible soluble polysaccharides (Ushida et al. 2006). In order to exploit this food item fully, a mammal must support a synergistic microbial population capable of fermenting the beta-linked polysaccharides compriseing = gum into short chain fatty acids for assimilation (Ushida et al. 2006). Except as a source of difficult-to-obtain energy, gums are not a particularly worthwhile source of macronutrients. Micronutrients, however, may present a different situation. Minerals such as calcium, magnesium, potassium and iron can be found in appreciable quantities in gums relative to other

food sources (Hladik 1979). For example, by ingesting only 30 g of gum, a chimpanzee (*Pan troglodytes*) can meet its daily needs for calcium, manganese, magnesium and potassium (Ushida et al. 2006). Exudates can transit undigested through the small intestine of mammals (Power and Oftedal 1996); if the species ingesting gum does not have fermentation/digestive capacity within the hindgut, potential micronutrients may not be available for assimilation. Gums also often contain secondary plant secondary metabolites (PSM) such as phenolics and tannins, which may limit the amount of gum a particular mammal can ingest (Heymann and Smith 1999 but also see Wrangham and Waterman 1981).

1.1.2 Gum Eating by Mammals

Even with the documented low nutritional quality of exudates, several mammalian species are commonly acknowledged as using gum. The majority of mammal taxa commonly used as examples are heavily biased in Primata and the petaurid marsupial groups. Some taxa use it as an obligate food source (*Nycticebus spp.:* Cabana and Nekaris 2015b, Starr and Nekaris 2013, Wiens et al 2006, Das et al. 2014; *Cebuella:* Coimbra-Filho and Mittermier 1977; *Petaurus breviceps:* Smith 1982), some seasonally as a fallback food (*Saimiri:* Stone 2007; *Saguinus spp:* Egler 1992), and some only opportunistically (*Pan:* Ushida et al. 2006). We expect each of these groupings to possess different morphological and physiological feeding adaptations that may allow some mammals to exploit gum food sources more efficiently than others (Bearder and Martin 1980). Year-long food availability, competition, seasonal effects on plant phenology, and varying insect abundances should also impact how beneficial or necessary gum is to a particular species and/or population (Garber 1984).

1.2.3 Gum eaters in captivity

The lack of research investigating the importance of gum within the diets of mammals is reflected in the captive management of these species. Captive

husbandry manuals rarely stress the importance of gum as part of a proposed diet. Diet recommendations either totally omit gum, or suggest it only as an enrichment ingredient or feeding-behaviour enhancement technique. For those species which use gum as an obligate food item year round (possibly with some seasonal increases), lack of dietary gum may lead to health issues and in turn impact the breeding and survivability of captive populations (e.g. Cabana and Nekaris 2015). Conversely, reduced gum intake for opportunistic gummivores should result in minimal impact. There is no evidence yet as to how seasonal gum feeders are affected by a lack of gum ingestion.

In this paper, we first review every mammalian taxon reported in the literature to ingest gum, categorized by importance of gum in their feeding ecology either as an obligate, facultative or opportunistic diet ingredient. Secondly, we collate the nutritional information of wild gum samples known to be ingested by these taxa, and lastly interpret why some species depend more on exudates as a food source than others. These results were used to question current husbandry practices for captive gum eating mammals, with particular emphasis relative to the proportion of gum in their wild diets, to evaluate potential resulting physiologic/psychological effects.

1.2 RESULTS

We collated information on 94 species of mammals that eat gum in the wild. Of these, 11 are marsupials, 78 are primates, and one each is a rodent, perissodactyl or procyonid. All mammals are from equatorial or subtropical climates. For the purpose of this review, we distinguish between the exudativorous obligate -feeding marmosets and the facultative-feeding tamarins within the Callitrichidae. Listed in Table 1.1 are species that have been observed feeding on gum as an obligate food (n=27 spp), Table 1.2 describes facultative feeders (n=34 spp) and Table 1.3 contains opportunistic feeders (n = 33 spp).

Table 1.1 Mammal species which have been identified to be obligate gum feeders, where gum is a major part of their feeding ecology.

Common Name	Scientific name	% of foraging	References
	Marsupials		
Striped Possum	*Dactylopsila trivirgata*	26	Rawlins and Handasyde 2002
Leadbeater's possum	*Gymnobelideus leadbeateri*	29-77	Irlbeck and Hume 2003, Smith 1984
Yellow-Bellied Glider	*Petaurus australis*	59-91	Henry 1985, Quin et al 1996
Sugar Glider	*Petaurus breviceps*	29-55	Smith 1982, Quin 1995, Henry 1985
Squirrel glider	*Petaurus norfolcensis*	59	Irbeck and Hume 2003, Quin 1995, Sharpe and Goldingay 1998, Menkhorst et al. 1988
	Primates		
	Strepsirhines		
Madame Berth's Mouse Lemur	*Microcebus berthae*	49	Hammhahn and Kappeler 2010
Reddish-Gray Mouse Lemur	*Microcebus griseorufus*	78	Genin 2008
Golden-brown Mouse Lemur	*Microcebus ravelobensis*	50	Radespiel et al. 2006
Hairy-eared dwarf lemurs	*Allocebus trichotis*	19†	Biebouw 2009
Masoala Fork-marked lemur	*Phaner furcifer*	65-85	Schulke 2003
Pale Fork-marked Lemur	*Phaner pallescens*		Charles-Dominique 1977
Bengal slow loris	*Nycticebus bengalensis*	76.5-85.3	Das et al. 2014
Sunda slow loris	*Nycticebus coucang*	43	Wiens et al. 2006
Javan Slow Loris	*Nycticebus javanicus*	54	Rode-Margonno et al. 2014
Pygmy Slow Loris	*Nycticebus pygmaeus*	51	Starr and Nekaris 2013
Southern Needle-clawed Bushbaby	*Euoticus elegantulus*	75	Charles-Dominique 1974
Lesser bushbaby	*Galago moholi*	Major	Bearder and Martin 1980
Thick-tailed Greater Galago	*Otolemur crassicaudatus*	5-90	Bearder and Martin 1980, Crompton 1983, 1984, Harcourt 1986, Clark 1978
	Platyrhines		
Pygmy Marmoset	*Cebuella pygmaea*	67	Soini 1982, Moynian 1976, Castro and Soini 1977
Silvery Marmoset	*Mico argentatus*	59	Veracini 1997
Common marmoset	*Callithrix jacchus*	30-70	Thompson et al 2013, Alonso and Langguth 1989, Stevenson and Rylands 1988, Cunha et al 2006, Alonso 1984, Castro 2003
Black-tufted Marmoset	*Callithrix penicillata*	25-70	Muskin 1984, Rylands 1984, de Fonesca and Lacher 1984
Geoffroy's Marmoset	*Callithrix geoffroyi*	14-69.7	Passamani and Rylands 2000, Passamani 1998, Garber 1984, Dawson 1979
Buffy-tufted marmoset	*Callithrix aurita*	12.9-50.5	Muskin 1984, Rylands 1984, Correa et al. 2000, Correa 1995, Martins and Setz 2000, Coutinho and Correa 1995, Ferrari et al. 1996
Buffy-headed marmoset	*Callithrix flaviceps*	65.7-87	Correa et al. 2000, Ferrari 1991
	Catarhines		
Patas monkey	*Erythrocebus patas*	0.1-36.9	Isbell 1998
Grivet Monkey	*Chlorocebus aethiops*	Moderate	Isbell 1998

† % of total activity

Table 2. Mammal species identified as exploiting gums as a facultative food. Although gum may be in the diet all year long, it will seasonally become more important when another more preferred resource is less available.

Common Name	Scientific name	% of foraging	References
	Marsupials		
Tasmanian bettong	*Bettongia gaimardi*	Minor	Irbeck and Hume 2003, Taylor 1992
	Primates		
	Strepsirhine		
Geoffroy's Dwarf Lemur	*Cheirogaleus major*	1	Lahann 2007
Grey Mouse Lemur	*Microcebus murinus*	4-69	Dammhahn and Kappeler 2008, Radespiel et al. 2006, Lahann 2007, Joly and Zimmermann 2007
Giant Mouse Lemur	*Mirza coquereli*	0-20	Hladik and Hladik 1969, Pages 1980
Potto	*Perodicticus potto*	20-21†	Oates 1984, Charles-Dominique 1977
Senegal Bushbaby	*Galago senegalensis*	15-60	Harcourt 1986
Kenyan galago	*Galago senegalensis braccatus*	Major	Nash and Whitten 1989
Prince Demidoff's Bushbaby	*Galagoides demidovii*	0-10	Charles-Dominique 1977, 1974
	Platyrhine		
Snethlage's Marmoset	*Mico emiliae*	Minor	Lopes and Ferrari 1994
Hershkovitz's Marmoset	*Mico intermedius*	15.5	Rylands 1982
Santarem Marmoset	*Mico humeralifer*	5†	Rylands 1984, Stevenson and Rylands 1988
Black-tailed Marmoset	*Mico melanurus*	Minor	Rylands 1984
Wied's marmosets	*Callithrix kuhlii*	7-28.4†	Raboy and Dietz 2004, Rylands 1989, Raboy et al. 2008
Goeldi's monkey	*Callimico goeldii*	1-14	Porter 2001, Porter et al 2007
Pied Tamarin	*Saguinus bicolor*	0-17	Egler 1992
Emperor Tamarin	Saguinus imperator	Minor	Terborgh 1983
Brown-Mantled Tamarin	*Saguinus fuscicolis*	12-14.4	Peres 1993, Haymann and Smith 1999, Porter 2001
Illiger's Saddle-back Tamarin	*Saguinus fuscicollis illigeri*	3.4-42	Terborgh 1983, Soini 1987, Garber 1988
White-Lipped Tamarin	*Saguinus labiatus*	8	Porter 2001
Golden-handed Tamarin	*Saguinus midas*	Minor	Smith 2010
Moustached Tamarin	*Saguinus mystax*	10.4	Peres 1993, Haymann and Smith 1999, Castro and Soini 1977
Black-handed Tamarin	*Saguinus niger*	3.1	Oliveira and Ferrari 2000
Black-Mantled Tamarin	*Saguinus nigricollis*	Minor	Izawa 1978
Cotton-Topped Tamarin	*Saguinus oedipus*	5-14.4	Neyman 1977, Power and Oftedal 1996, Hladik and Hladik 1969, Garber 1980, Garber 1984
Black-faced Lion Tamarin	*Leontopithecus caissara*	Minor	
Golden-rumped Lion Tamarin	*Leontopithecus chrysopygus*	12.5-15.2‡	Albernaz 1997, Passos 1999, Valldares-Padua 1993
Golden lion headed tamarin	*Leontopithecus chrysomelas*	5-55	Raboy and Dietz 2004, Rylands 1989, 1993
	Catarhine		
Squirrel Monkey	*Saimiri sciureus*	11.5	Stone 2007
Yellow-Breasted Capuchin	*Sapajus Xanthosternos*	2-9	Canale et al. in press
Red-tailed Monkey	*Cercopithecus ascanius*	Minor	Chapman et al 2002
Blue Monkey	*Cercopithecus mitis*	1.9-2.8	Cords 1986
Vervet monkey	*Chlorocebus pygerythrus*	30	Wrangham and Waterman 1981
Yellow Baboon	*Papio cynocephalus*	8-15	Altmann et al 1977, Post 1982
Human	*Homo sapiens*	Minor	Sugiyama and Koman 1992

† % of total activity
‡ Estimated % of diet

Table 1.3 Mammalian species identified as only opportunistically ingesting gum and not being a necessarily important part of the mammal's feeding ecology.

Common Name	Scientific name	% of total foraging	References
	Marsupial		
Rufous rat-kangaroo	*Aepyprymnus rufescens*	Minor	Irlbeck and Hume 2003
Brush-tailed bettong	*Bettongia penicillata*	Minor	Irbeck and Hume 2003
Black-striped wallaby	*Macropus dorsalis*	Minor	Irbeck and Hume 2003
White-eared Opossum	*Didelphis albiventris*	Minor	Alessio et al 2005
mahogany glider	*Petaurus gracilis*	Minor	Irbeck and Hume 2003, Jackson 2001
	Primates		
	Strepsirhine		
Fat-tailed Dwarf Lemur	*Cheirogaleus medius*	2	Lahann 2007, Martin 1972
Brown mouse lemur	*Microcebus rufus*	2	Atsalis 1999
Aye Aye	*Daubentonia madagascariensis*	Minor	Petter 1977
Ring-tailed Lemur	*Lemur catta*	Minor	Sussman et al 2003
Brown Lemur	*Eulemur fulvus*	Minor	Smith 2010
Black Lemur	*Eulemur macaco*	0-2	Simmen et al 2007
Black-and-white-Ruffed Lemur	*Varecia variegata*	Minor	Ratsimbazafy et al 2002
Mysore Slender Loris	*Loris lydekkerianus*	3	Nekaris and Rasmussen 2003
	Platyrhine		
Golden Lion Tamarin	*Leontopithecus rosalia*	1.6	Dietz et al 1997
Tufted Capuchin	*Sapajus apella*	Minor	Peres 1993, 1994a, b
Northern Night Monkey	*Aotus trivirgatus*	Minor	Hladik and Hladik 1969
Peruvian Red Uakaris	*Cacajao calvus*	Minor	Bowler and Bodmer 2011
White-footed Saki Monkey	*Pithecia albicans*	Minor	Peres 1993, 1994a, 1994b
Red-handed Howler Monkey	*Alouatta belzebul*	Minor	Bonvicino 1989
Mantled Howler Monkey	*Alouatta palliata*	Minor	Hladik and Hladik 1969
Guianan Red Howler Monkey	*Alouatta seniculus*	Minor	Izawa 1975
Red-faced Spider Monkey	*Ateles p paniscus*	1	Van Roosmalen 1985
Woolly monkey	*Lagothrix lagotricha*	6.9	Peres 1994b
	Catarhine		
Putty-nosed Monkey	*Cercopithecus nictitans*	Minor	Gautier-Hion et al 1980
Tana River Crested Mangabey	*Cercocebus galeritus*	0-6	Gautier-Hion et al. 1980, Homewood 1978
Hanuman langur	*Semnopithecus entellus*	1	Newton 1992
Capped Langur	*Trachypithecus pileatus*	Minor	Solanki et al 2008
Chimpanzee	*Pan troglodytes*	Minor	Ushida et al 2006
	Rodentia		
Black Agouti	*Dasyprocia fuliginosa*	Minor	Peres 1993, 1994a, b
Silky Desert Mouse	*Pseudomys apodemoides*	Minor	Cockburn 1981
Bush Squirrel	*Paraxerus cepapi*	Minor	Viljoen 1977
	Procyonidae		
Ring-tailed Coati	*Nasua Nasua*	Minor	Peres 1993, 1994a, b
	Perissodactylae		
Lowland Tapir	*Tapirus terrestris*	Minor	Peres 1993a , 1994a, b

Table 1.4 A compilation of published chemical compositions of gums that have been observed being ingested by mammals in the wild, where all nutrients are presented as % concentration on a dry matter basis.

Species eaten	CP	Sugar*	NDF	Galactose	Arabinose	Fructose	Mannose	Xylose	Rhamnose	Glucose	Tannins	Ash	Na	K	Ca	Mg	P	Reference
Anacardium excelsum	1.7												0.02	0.31	0.55	0.02	0.08	1
A. occidentale	2.8			61	14		2	2	7	8		1.3	0.03	0.32	0.41	0.06		2
Lannea coromandelica	1.38			69.5	11	2.5						3.5						3
Spondias mombin				43	30		7		3									4
Commiphora arofy	4.4																	5
Terminalia mantalis	2.4																	5
T. manteliopsis	1.4																	5
Terminalia sp.	1.12			20	51		9	4	5			1.8						6
Anogeissus latifolia	4.18	76.36		21.97	37.07		1.93	1.28	1.09	0.78		1.14						7
Acacia dealbata	1.6			63	20				10			1.3						8
A. drepanolobium	3.29		4.99								0-0.49	2.23	0.08-0.14	0.11-0.16	0.6-0.78	0.07-0.09	negl	9
A. irrorata	9.8			44	30				9			2.7						10
A. karroo	1.025			50	31				6			3.35	0.02	0.99	0.97-1.036	107	0.011	11
A. mearnsii	7.0	95.0		46	43				7	trace								12
A. nitolica	1.265			34.1	44.5				2.7			2.24						13
A. obliquinervia	4.4																	14
A. seyal																		14
A. tortilis	5										49.475	2						15
A. xanthoplhoea	1.79		3.73								0.27	3.225		0.13	0.72	0.07	negl	15
A. auriculiformis	5.75			59	8				5			5.3						16
A. terminalis	3.4			82	12				1			1.1						17
Albizia mainaea	11.3																	17
A. procera	7.34			30.17	44.92	0.02			0.22			4.1	0.02	0.05	0.03	0.23		18
Albizia sp.	3.18										0.42	5.5						19
A. zygia											0.1-1		0.09	0.24	1.19	0.04		20
Allantsilodendron alluaudianum	21																	5
Delonix decaryi	3.9																	5

Species eaten	CP	Sugar*	NDF	Galactose	Arabinose	Fructose	Mannose	Xylose	Rhamnose	Glucose	Tannins	Ash	Na	K	Ca	Mg	P	Reference
Enterolobium sp.	1.18			46	19				12		0.2	3.95	0.03	0.46	0.88	0.1		21
Parkia bicolor	5.8			74	9							3						22
P. nitida	13.9												0.08	0.12	0.26-0.36	0.1	negl	23
P. pendula	2.575			30	62							1.5			1.07		0.02	22
Rhopalocarpus similis	4.6																	5

negl=negligible
NDF=neutral detergent fibre
Sugar fractions are % of total sugar, not of total dry matter
*Actual sugar amounts here may not theoretically be all sugar, and may instead be the value for soluble structural carbohydrates.

References: 1 Garber 1984, 2 de Pinto et al. 1995, 3 Anderson and Hendrie 1970, 4 de Pinto et al. 2000, 5 Genin et al. 2010, 6 Anderson and Bell 1974, 7 Kang et al. 2011, 8 Anderson et al. 1973, 9 Mhinzi et al. 2008, 10 Anderson et al. 1984, 11 Hladik 1979, 12 Grein et al. 2013, 13 Kapoor and Farooqi 1991, 14 Lindenmayer et al. 1994, 15 Wrangham and Waterman 1981, 16 Anderson and Gill 1975, 17 Anderson et al. 1971, 18 Pachuau et al. 2012, 19 Mhinzi 2002, 20 Ushida et al. 2006, 21 Clamens et al. 2000, 22 Anderso and de Pinto 1985, 23 Anderson et al. 1990

1.3 DISCUSSION

1.3.1 Gum Feeders and their Adaptations

Mammals which feed on gum as an obligate food source (26 species) are represented by primates (*Nycticebus, Cebuella, Phaner, Cheirogaleus, Callithrix)* and Australian possums (*Petaurus*); all but *Cebuella* and *Callithrix* spp. are nocturnal. Being an obligate feeder requires a unique set of evolutionary adaptations, not only for surviving on this low quality diet (metabolic), but also for harvesting and processing it (anatomical, behavioural). For this reason, mammals of this group can also be considered exudativores/gummivores or gum specialists.

Obligate feeders must have the capability of inducing the production of gum, such as a well-developed dentition adapted to damage a tree or liana severely enough for it to produce gum, a process called gouging. All exudativores have evolved specialised dentition to gouge and harvest gum (Burrows et al. 2016; Coimbra-Filho and Mittermeier 1977; Ravosa et al. 2010). The upper first premolars are used as an anchor for the teeth on the upper mandible to scrape the cambium away, stimulating gummosis (Nussinovitch 2009). The possums and gliders also have this adaptation (Smith 1982). The lower canines of exudativorous primates are incisiform and form a toothcomb with the incisors such as seen in *Nycticebus spp. (*Nekaris 2014). The same specialisations are clear in marmosets and have arisen through convergent evolution (Burrows et al. 2016).

In terms of extremities, we also see some elaborate adaptations for reducing the energetic strain congruent with the arboreal lifestyle of obligate gum feeders. Marmoset species and *Phaner* all have keeled nails, which allow them to negotiate climbing and clinging to tree trunks for long periods, reducing the energy necessary during gouging (Hladik 1979). *Nycticebus* do not have keeled nails, instead a special set of blood vessels in their limbs called *retia-mirabilia* (Nekaris 2014). This allows them to contract their hands and feet into vice-like grips, yet only use small proportions of energy.

Lastly, gum is composed mainly of soluble structural carbohydrates/polysaccharides that require a host of digestive adaptations. Fermentation, rather than intrinsic enzymatic digestive processes, is necessary to fully digest gum (Anderson and Bell 1974; Power 2010). All obligate gum feeders possess some gut fermentation capabilities.

This process is accomplished either by possessing an expanded caecum and large intestine or a complex hindgut (Dierenfeld et al. 2006). Within these chambers, some established microbes possess the capability of cleaving the β-bonds (Hladik 1979). Although many arboreal possums ingest gum, the only species that are obligate feeders*, P. breviceps and P. leadbeateri*, have the largest caeca in relation to body size among marsupials (Smith 1984). Gliders also display a relatively long food mean retention time, of about 29 hours, which allows time for gum to ferment (Dawson 1979). All of these exudativores share similar traits that enable efficient harvest, processing and digestion of gum as an obligate food source. The patas monkey (*Erythrocebus patas)* is not described as an exudativore in the literature, yet it uses gum as an obligate food source (Isbell 1998). Although lacking other adaptations, their hindgut must be able to ferment a portion of the ingested gum for patas monkeys to obtain sufficient energy.

The species that are the most exudativorous may also have a modified metabolism that can assist in coping with this diet ingredient. Using *N. coucang* as a model, Muller (1979) showed that they have a basal metabolic rate (BMR) which is 60% that of a similar sized primate. The *Cheirogaleus, Petaurus* (in addition to marsupial lower BMR), and some *Galago* spp. can enter daily torpor in order to reduce overall metabolic costs (Schmid et al. 2000 but see Mzilikazi et al. 2006). Whether this evolved concomitantly with their feeding ecology or not, it has allowed exudativores to be able to thrive on gums and insects.

Seasonal facultative gum feeders identified in Table 1.2 (35 species) may possibly have some, or none of the necessary adaptations for exudativory, with an example being the many *Saguinus* spp. Tamarins do not possess the specialised dentition necessary to gouge tree bark (Soini 1987). Rather, they harvest gum from pre-occurring tree wounds (Soini 1987). The mean retention times (MRT) of tamarins are heavily dependent upon the structure of food ingested (Power and Oftedal 1996). When large indigestible particles are present, such as seeds, the MRT deviates from normal and becomes shorter in order to void this nutritionally inaccessible item, leaving space for more digestible foods. When gum was added to experimental diets, tamarin MRT values did not significantly differ, unlike the marmoset MRT which increased by 40 to 60 minutes, most likely to maximise fermentation opportunities (Power and Oftedal 1996). While facultative feeders may digest and assimilate some nutrient content of gum, they exploit other seasonal food sources such as fruit, where fermentation is a helpful, yet less essential adaptation (Heymann and Smith 1999). Forest guenons are described as frugivores, yet contain a complex hindgut with active fermentation (Cords 1986, Chapman et al. 2002). When the fibrous fruits are not in season, this adaptation would be necessary to extract nutrients and energy from gum (Homewood 1978).

The opportunistic gum feeders are almost as numerous as the obligate feeders (31 species). This group appears to have no targeted adaptations for dealing with this foodstuff. Some species may possess the necessary fermentation chambers to digest the carbohydrates within gum, such as black-striped wallabies (*Macropus dorsalis*) and langurs (*Semnopithecus entellus* and *Trachypithecus pileatus),* which possess foregut microbial fermentation (Newton 1992, Irbeck and Hume 2003). Remaining opportunistic gum feeders must rely on varying levels of hindgut fermentation. Any adaptations an opportunistic feeder may possess to harness or process gum is the product of some other selection pressures. Clearly gums are not considered a major dietary component for these species, which is why

for the remainder of the discussion; we shall focus on obligate and facultative feeders.

1.3.2 Gum Composition

We identified that the 92 mammal taxa in this study consume gums from 144 species of plants, belonging to 78 genera in 35 families. Chemical compositions were found for gums from 32 plant species (Table 1.4). From a plant's perspective, a primary function of exudates is to seal off wounds from the outside environment to prevent entry of pathogens, as well as to minimize desiccation (Nussinovitch 2009). A high PSM load should theoretically deter predators. In a study of fallback feeding behaviours of vervet monkeys (*Chlorocebus pygerythrus*) on two different *Acacia* species, Wrangham and Waterman (1981) documented that the monkeys preferentially ingested *A. xanthophloea* rather than *A. tortilis* gums, attributing selection to its lower PSM concentrations (0.23-0.31% versus 27.99-70.96% DM basis). Exudativorous species may have further physiologic mechanisms for coping with high dietary PSM loads, including detoxification of compounds through gut microbes or specialised saliva (Rode-Margonno and Nekaris 2015). Conversely, seasonal and /or opportunistic feeders may behaviorally ingest gums with less concentrated PSM. Animals that feed on exudates seem to be very sensitive to different PSM compounds and sometimes seem to select a gum for its particular PSM (Wrangham and Waterman 1981).

Gum exudates have been generally described as being virtually devoid of lipids, low in protein, and relatively high in trace minerals (Garber 1984, Nash and Whitten 1989, Power 2010); the summary data in Table 1.4 support this description. Indeed, crude protein of tree gums can range widely from 1.10-11.3% (DM basis), although high values are exceptional and most gums tend to have a protein concentration nearer to the low end of this range. Pod gum protein concentrations (*Parkia spp.*) are generally higher, from 2.2 to 13.9% (DM basis). Both trunk and pod gums are abundant in minerals and have a high calcium to

phosphorous ratio (Ca:P). It has been shown repeatedly that a positive dietary Ca:P ratio is necessary for proper nutrition in all life stages, and especially important during gestation, lactation and juvenile growth (Dierenfeld et al. 2006). Gums also contain minerals that are considered limiting in a tropical context such as sodium, copper and iron (Rode-Margono et al. 2014).

Gums comprise complex linkages of monosaccharides and typically have no sweet taste (Power and Oftedal 1996). Numerous sugars have been identified from gums (see Table 1.4); however, these compounds can be misleading in understanding the nutrition of exudativores since chemically they comprise the end product of laboratory hydrolysis rather than primary substrates available for digestion (Hall 2007). The declared sugar content of gums depends on plant species and perhaps analytical methodology. The complex linkages of sugars in gums are very different in digestibility compared to simple sugars found in saps, nectars and domestic fruit, which are generally completely digested. Thus, fermentable fibres in gum are not interchangeable with sugars. Gum fermentation typically does not result in the same carbohydrate end products, and fermentation can be inhibited by a drop in pH (and accompanying change in gut microbiome) that may occur when high soluble sugar (i.e. domestic fruit) diets are fed, which may lead to negative health consequences (Topping et al. 1988). Development and maintenance of appropriate microfloral populations in the digestive tract of the all gum feeders, but especially the obligate feeders, are critical for animals' ability to obtain energetic benefits from gums.

For obligate feeders, the nutrients found in gums are integrated into their daily metabolism and are used in conjunction with other diet components to reach nutrient targets. Simply looking at the nutrient concentrations found in their selected food types can be an indication of the role gum plays in a species' diet. *Nycticebus, Phaner* and some *Cheirogaleus* have natural diets comprising insects, nectar, perhaps sap, and other plant parts (Schulke 2003, Genin 2008, Starr and Nekaris 2013). Fruit is not a main component of their diets, nor is it for possums

and gliders (Smith 1980). This removes a major seasonal variability from their feeding ecology because, except for some blossoms and perhaps overall abundance of some insects, most of their diet is available year round. Marmosets have more food diversity than other obligate feeders, and do exploit fruit seasonally (Garber 1984). As explained by Gaulin (1979), the Jarman/Bell theory allows small mammals to subsist on insects and gums because they do not have large total nutrient requirements. They theoretically should be able to meet nutrient targets by balancing gum, insects and plant matter intake. This is not feasible for larger mammals with greater nutritional needs (Gaulin 1979).

Erythrocebus patas has a relatively low fruit diet and ingests mostly insects and gum (Isbell et al. 2013). Insects are known to be a concentrated source of animal-based nutrients, high in protein, often fat, some vitamins and some minerals as well as dietary fibre in the form of chitin (Finke 2015). They also have typically inadequate calcium to phosphorous ratio which renders an insect-only diet inappropriate for long periods of maintenance, and anytime during growth or reproduction (Garber 1984, Finke 2015). The trends observed in this review (Table 1.4) corroborate Garber's (1984) hypothesis that insects and gums appear to provide nutritional complementarity contributing to a balanced diet. With insects and gum available throughout most of the year, this feeding strategy is relatively stable and requires little dietary divergence. Isbell et al. (2013) showed how *E. patas* are able to reach their nutrient targets with a diet of insects, gum and some plant matter, as well as gestate and lactate on this diet. This feeding strategy is limited by habitat to locations where gum trees and insects are abundant, and competition for the gum resource isn't too severe. Isbell (1998) found that *E. patas* is indeed an exception to the Jarman/Bell rule, being a medium-sized primate that subsists on insects, gum and little other plant matter.

One main characteristic that unifies the facultative feeders is their highly seasonal diet due to their preference for fruit (29 of the 33 species listed in Table 1.2). Not possessing one of the aforementioned adaptations may rendered the

energy gained from gum not worth the energy spent harvesting it when fruit is present. The Australian marsupials in this category each have their unique niches that include honey, ants, nectar and fruits (Jackson 2001, Rawlins and Handasyde 2002). Severe nutritional stress periods are common when fruits are seasonal and appear in patches. Correa et al. (2000) discusses the inverse relationship that exists between the consumption of fruits and exudates. *Leontopithecus chrysopygus* increase their gum consumption as the fruiting period ends (Albernaz 1997). Different species may target fruits at different phenological stages - small or large unripe or ripe -- or even by fruits' chemical composition (Porter 2001). The fruits selected by *Cheirogaleus major* were high in fibre, low in fat and protein, with moderate sugar content (Lahann 2007). This proportion of nutrients is reflective of most fruits consumed by wild animals, which are generally low in calcium and have a low Ca:P ratio. Both gum and fruit are low in protein, high in fibre (albeit different fibre types: soluble versus insoluble), and can contain PSM (Power and Oftedal 1996). Fruit can be abundant seasonally, as well as distributed in patches, which allows for a much higher energetic yield during fruiting season(s). When gum becomes more important in the diet, the overall energy intake will be lower since the carbohydrates are more difficult to extract, which may lead to a lean season with an overall decrease in body mass (Stone 2007). The lean season also occurs simultaneously with the breeding season of some species, correlated with a diet higher in minerals, particularly essential nutrients during gestation and lactation (Garber 1984). *Saguinus bicolor*, for example, selects gum between June to December, with its birthing season between May and November (Egler 1992). For such species, the intake of gum during this period is essential to provide suitable nutrients to offspring, as well as to slow fat catabolism.

1.3.3 Exudativores in Captivity

Understanding the nature of gum consumption by mammals has great implications for maintaining these species in captivity. Indeed, 71% of the species listed in Tables 1.1, 1.2 and 1.3 are found in captive settings, either as part of a

managed breeding program in accredited zoological institutions worldwide, or in rescue centres being rehabilitated and reintroduced. Duplication of wild diets is often the first step when formulating a captive diet, as well as using domestic or laboratory animal models to determine specific nutrient requirements (Cabana and Nekaris 2015). Many accrediting zoo bodies now produce their own husbandry/best practice guidelines for holders, or endorse studies or guides put together by outside organizations. Exudativores, *Nycticebus* in particular, have typically been fed diets that contain little to no actual exudates (Fitch-Snyder et al. 2001). American Zoo and Aquarium Association (AZA) husbandry manual recommendations mention how gum can be used as enrichment for lorises, but do not actually include them in their diet formulation recommendations.

Since they include obligate as well as facultative gum feeders, husbandry recommendations for the Callitrichidae and Lorisidae should routinely include gum components in their diets. Cabana and Nekaris (2015) provided evidence that diets high in fruit and low in gum contribute to the dental diseases in *Nycticebus*. The free ranging diet of *N. javanicus* significantly differs between rehabilitated and released individuals versus wild individuals (Rode-Margonno et al. 2014). Callitrichids, as well, should receive gum two to three times per week, either as enrichment (Ruivo 2010) or as a dietary essential. According to the IUCN redlist in May 2015, these primate groups contain a high proportion of threatened species (56% for marmosets and 50% for tamarins), placing importance on their respective breeding programs. Marmosets and tamarins have been plagued in captivity with a wasting syndrome since the 1970s, and it is still present in captive populations today (Gozalo et al. 2008). Although no causal link has yet been established, all underlying hypotheses (apart from stress) appear to be of a nutritional basis. Similar to slow lorises, many captive callitrichid diets are high in fruit and generally low in exudates (Nash 1986; Ruivo 2010). Although no case is identical, Jarcho et al. (2013) report a common instigator linked with intestinal inflammation which causes malabsorption, and leads to the observed clinical symptoms. Studies

now focus on the underlying cause of the initial inflammation, with a lack of fibre being one of the current hypotheses (Pham and Barr 1996). The species most affected are the most gummivorous of the tamarins: *S. bicolor* and *S. oedipus,* as well as *C. jacchus, C. geoffroyi* and *Calimico* goeldii (Gozalo et al. 2008). The lack of gum in diets has been suggested as one of the factors linked with marmoset wasting syndrome (Nash 1986).

The Australian exudativorous marsupials may also not receive adequate amounts of gum in their captive diets. The species most commonly kept in captivity include *Dactylopsila trivirgata, Peturus australis, P. breviceps, P. gracilis* and *P. norfolensis,* none of which have gum listed in common diet recommendations of high protein mixes, nectar replacers and fruits *(*Dierenfeld et al. 2006*).* Similarly, to *Nycticebus,* the most studied species of gliders *Petaurus breviceps*, also suffers from several health ailments in captivity (Dierenfeld et al. 2006), including tetany and osteodystrophy, both of which can be related to inappropriate, imbalanced high fruit diets. As described in Table 1.4, gum is a source of calcium, which is also found in the natural diet of sugar gliders. A reduction of fruit, and increase in gum, could positively impact the health of these captive animals, specifically reducing calcium based illnesses (assuming vitamin D intake was sufficient). Reported dental issues, diarrhoea and kidney issues may also benefit from the gum's lower sugar, higher fibre and low protein levels, respectively. Additionally, gut health and overall microbiome may be improved (Dierenfeld et al. 2006, Solden et al. 2015). Other species that would provide interesting case studies, are either not maintained in captivity (e.g. *Phaner*) or do not yet have a husbandry manual. Due to the adaptable behaviours seen in the feeding ecology of fall-back and opportunistic gum feeders, primary health issues related to lack of gum in captive diets are less anticipated (or reported) in these species.

1.4 CONCLUSION

Much remains to be learned about exudate consumption in mammals. We suspect that upon further study, many other taxa will be categorized as opportunistic consumers. Clearly gum consumption has evolved in multiple mammal lineages, with a variety of morphological adaptations to cope with the exploitation, processing and digestion of this readily available, yet difficult to digest food source. The evident evolutionary adaptations to this resource mean than an understanding and certain mimicry of their natural feeding ecologies may be critical to their proper management in captivity. Future research should focus on the physiological effects that gum ingestion poses on different digestive systems. This would enable us to assess other potential evolutionary driving influences related to exudativory.

STRUCTURE OF THE THESIS

We have shown in CHAPTER I how current literature and anecdotal animal husbandry practices are ill equipped to inform and instruct on how to provide for exudativorous primates. In order to properly inform on dietary husbandry and lead to changes within the zoological world, some questions must first be answered. The slow loris is an excellent model of choice due to its hardy and resistant nature in captivity. They are long lived and unfortunately for them, can cope with a large amount of inadequate husbandry and welfare before going into a critical state. This will allow us to retroactively assess the effects of the diets on them.

Q1. Do slow lorises in zoological institutions actually suffer of poor health and/or welfare because of their current nutritional management?

If they do, a better diet must be devised. We must start from scratch and identify how different are the captive diets compared to wild diets of slow lorises not necessarily in terms of food items, but in nutrients.

Q2. What is the nutrient intake of wild slow lorises, and do males and females differ in their selection? Does their ratio of protein energy to non-protein energy differ?

Q3. Do males and females expend a similar amount of energy? How does their energy expenditure affect their nutrient selection?

Comparing actual components may also be of value. With the absence of gum and perhaps of insects in captivity being foreshadowed, we must collect empirical evidence on just how important gum may be for captive slow lorises.

Q4. What effects does gum have on the physiology of the slow lorises?

Q5. Can slow lorises use insect chitin as an energy/nutrient source?

Once these questions are answered can we then proceed to trialing a new, evidence based naturalistic slow loris diet, able to address the slow lorises physiology, morphology and behaviour. The framework for this new diet would be the nutrients ingested from wild slow lorises.

Q6. Can free-ranging slow loris nutrient intakes be used as a framework for an appropriate captive diet which recreates the same physiological reactions as wild slow lorises?

In order to answer these questions, this thesis is structured like articles, so the reader can understand the structure and the steps taken to inform the next chapter and cumulate in the last diet trial chapter.

CHAPTER II

METHODS FOR WILD AND CAPTIVE NUTRITIONAL ECOLOGY RESEARCH

2.1 Brief Overview

This thesis focuses on the captive care element for slow lorises, aimed at helping zoos and rescue centres. In order to complete this, there are two elements, captive and wild, to the field work. Both elements are used in all chapters as this research project was built in such a manner that both elements work symbiotically in an integrative manner. Thus, the general methods include:

- An introduction to the conservation project, The Little Fireface Project and the field site where free ranging animals were studied and methods used with wild Javan slow lorises (section 2.2)
- An introduction to the captive site and methods used with captive individuals (section 2.3)

2.2 Wild Field Work

2.2.1 The Javan Slow Loris

Our chosen model species is the Javan slow loris *(Nycticebys javanicus*), a primate which belongs to the infraorder Strepsirhini. Although they may share the moist nose, grooming claw on the second digit of their hind limbs and a toothcomb with other Strepsirhines (Nekaris and Bearder, 2011), the purpose of using them as a model are for exudativorous primates, and not of a taxonomical purpose. The Javan slow loris will serve as a model species for the seven other slow loris species currently recognised from Munds et al. (2013) (Table 2.1).

Table 2.1 List of the eight currently recognised slow loris species within Southeast Asia.

Species	Common Name	Geographic Range
Nycticebus pygmaeus	Pygmy slow loris	Cambodia, Laos, Vietnam
Nycticebus menagensis	Philippine slow loris	Brunei, Indonesia, Malaysia, Philippines
Nycticebus kayan	Kayan slow loris	Indonesia (Kalimantan), Malaysia (Bornea)
Nycticebus bancanus	Sody's slow loris	Indonesia (Bangka, Belitung)
Nycticebus borneanus	Bornean slow loris	Indonesia (Kalimantan)
Nycticebus coucang	Greater slow loris	Indonesia (Sumatra, peninsular Malaysia, Thailand, Singapore)
Nycticebus javanicus	Javan slow loris	Indonesia (Java)
Nycticebus bengalensis	Bengal slow loris	Cambodia, Laos, Thailand, Vietnam, India, Burma, Bangladesh, Bhutan, China

Geographic range and species descriptions from Munds et al. (2013) and Nekaris (2014).

Slow lorises are nocturnal arboreal primates that cannot leap and instead cross canopy gaps by using a movement termed cantilevering (Nekaris and Bearder, 2011). Their hands and feed are adapted to suit their lifestyle, with modified blood vessels in their limbs, allowing strong grasping force throughout a

prolonged period of immobility (blood vessels are known as retia mirabilia) (Hill 1953). This also has a conservative effect on ther basal metabolism which is estimated to be 40-60 % lower than a primate of similar body mass (Muller 1979). To suit their nocturnal lifestyle, they possess a *tapetum lucidum*, an extra membrane within the retina that reflects light back, allowing for excellent night vision (Fleagle 2013). Their coat is also thick and well insulated, and has a distinct dorsal stripe and face mask (Nekaris and Jaffe, 2007).

The main threat to the conservation of the slow lorises is the pet trade (Nekaris and Jaffe, 2007). Due to their cute physical appearance, they are caught from the wild and sold in animal markets within large Indonesian cities (Shepherd et al. 2004; Nekaris and Nijman, 2007). Most of the trade is described as being domestic, however some are illegally smuggled to Russia, Eastern Europe, Japan and the Middle East. When confiscations do occur, the animals are sent to one of the main rescue centres within Indonesia, either belonging to an NGO or the government (Moore 2012).

Rescue centres on Java are inundated by slow lorises, some of which have had their teeth removed and must live in the centre until they die, or until a suitable release site and funding is found. Matters are further complicated by slow lorises being marked as ‘evidence’ against the smugglers and often must remain in the rescue centres until the trial occurs.

2.3 Field Work

2.3.2 The Little Fireface Project and Field Site

I was based at the Little Fireface Project which was founded by Prof Anna Nekaris in 2012. Prof Nekaris began researching Asian lorises in 1993 and later began this research and conservation project. The project tasks itself with researching the ecology of slow lorises (*Nycticebus*), educating local people about slow lorises, nature and their role within it and lastly to empower them take pride and action in actively protecting slow loris habitat and rescuing animals from the illegal wildlife trade (Nekaris, 2016). Topics of research at The Little Fireface

Project include ecology, nutrition, translocation, illegal wildlife trade, captive behaviour, physiology – specifically venom, conservation and occupancy/ecological modelling. The main field site for the project, and where we were based, was in the small village of Cipaganti (*Desa Cipaganti*), Cisurupan (*Kecamatan Cisurupan*), regency Garut (*Kabupaten Garut*), province West Java (*Provinsi Jawa Barat*) in Indonesia, at S7°6'6 - 7°7'0& E 107°46'0 - 107°46'5 between June 2014 and May 2015. This village was at the foothills of the active volcano Mount Papandayan. The village was situated roughly at 1200 m asl with the slow loris study population being found between 1200 and 1600m asl (according to GPS data). This area was an agro-forest mosaic of about 50 ha, with rows and thickets of trees and vegetation between agricultural plots harbouring the slow lorises. Overall there was good connectivity due to the bamboo thickets, however due to the intensive usage of the plant rows, thickets were routinely cut down in their entirety. Popular crops included tea, chili, potato, carrots, cabbage, ginger, coffee etc. The rows of plants and thickets are not representative of the original forest at that location, with most trees being planted for timber (*Gigantochloa* spp., *Eucalyptus* spp., *Acacia* spp.) or fruit (jackfruit- *Arctocarpus heterophyllus*, persimmon - *Diospyros kaki*, mango – *Mangifera indica*, avocado – *Persea americana*). Althoug anthropogenically modified, the rows of trees gave us good visibility, much more than in previous *Nycticebus* research where results were based on a few hours of observations nightly e.g. Starr and Nekaris 2013; Wiens et al. 2006.

As described by Rode (2014), the climate at the field site varied in temperature and rainfall throughout the year (Figure 2.1). Temperatures ranged between 12.4 and 20.7 ° Celsius (with a maximum of 2° Celsius variance depending on altitude). Although not meeting the criteria of a "Season", the field site has a distinct wet and less wet period. During the less wet period, precipitations were below 50mm per month with a slightly colder average temperature. The wet period brought on rains of more than 200 mm and up to 800 mm. We will be using the terms austral summer for the wet period and austral winter for the less wet period.

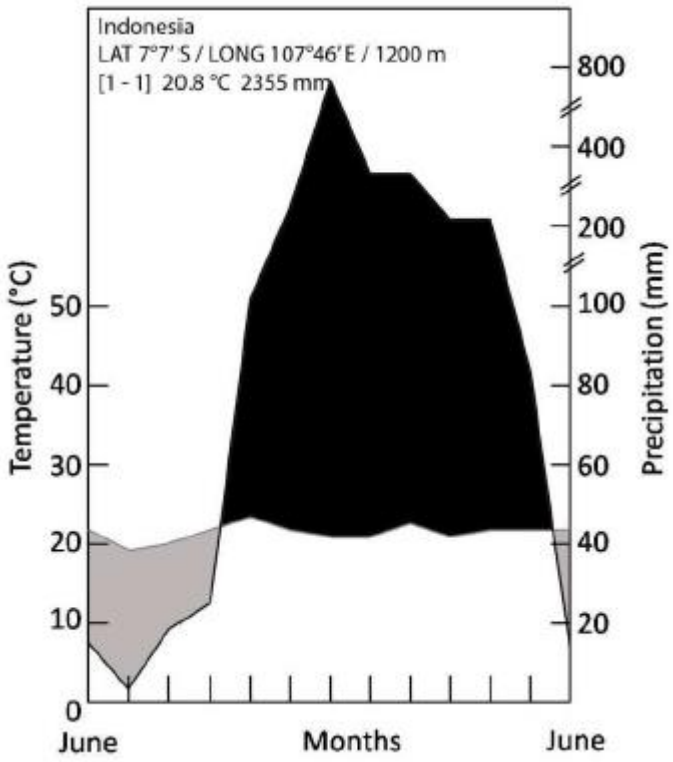


Figure 2.1 The average monthly rainfall and temperature variations at the Cipaganti fieldsite, showing two clear rainfall periods (from Rode-Margono 2014).

2.3.3 Slow Loris Observations

I followed 17 free ranging slow lorises between June 2014 and June 2015 in our field site, but ended up only with usable data from only 15 individuals as unfortunately one individual died and another individual did not have enough follows in the Austral summer for comparison. Each individual maintained its own territory, so we knew the general vicinity of expected location. In order to follow

the slow lorises, we caught each individual and equipped it with a radio collar (BioTrack, UK). These collars weigh 17g on average (approximately 1.9% of body weight). We tracked the slow lorises using a six-element Yagi antenna and SIKA receiver (BioTrack, UK) and observed them using next generation LED headlamps with a red filter (CluLite, UK). In order to calculate activity periods, we equipped four individuals (male n=2, female n=2) with ActiWatch Mini Loggers (CamnTech, Germany) on their radio collars for four months, allowing us to multiply quantified intake rates by the appropriate activity times. Epoch lengths were set at every minute at 100% intensity.

We followed radio-collared slow lorises in two observation periods during the night, from 18:00h to 0:00h and 00:00h to 06:00 h, each covering one different animal per period. Every individual slow loris was observed on a random schedule to prevent bias, however all observations were counterbalanced throughout the wet and Austral winters. We caught each slow loris at least every six months for a health check that included weighing using a hanging clip scale (Pesola). We used values for 13 adult or sub-adult slow lorises with weight information (female n=42, male n= 29) obtained between 2012 and 2015. Females may have been gestating when weighed, which would result in higher BW. This is not an issue with regards to analysis as these variations may impact the nutrient intake of gestating females, and understanding this strategy is essential to comprehend their natural feeding ecology. We used instantaneous behavior sampling (Altmann, 1974) with a 15-minute interval for the behaviours listed in Table 2.2 as well as all occurrence feeding behavior sampling (Nekaris, 2003). We collected data on a total of 256 days over the course of 12 months (1470 hours, 5.8 hours/night average), totaling 7191 instantaneous points of data. I conducted inter-observer reliability tests between all long-term research assistants every month to ensure all results were above 90%. If they were below this amount then I would remove the data collected by the research assistant for the last month, which only occurred in one situation with a volunteer who left us early.

Table 2.2 Ethogram used in the behavioural observations for the Javan slow lorises

Behaviour	Definition
Alert	Remain stationary like in "rest" but active observation of environment or observer
Feed	Actual consumption of a food item
Investigate	Movement associated with looking for food (often includes visual and olfactory searching) or any other stimulus (scent marking etc.)
Freeze	Interrupt locomotion to maintain motionless, rigid posture in standing or sitting position for at least three seconds, extremely slow movement not associated with foraging
Groom	Autogroom, lick or use tooth comb on own fur
Rest	Remain stationary, often with body hunched, eyes open
Sleep	Remein stationary, head between the knees, eyes closed
Social	Definiton of social behaviours:
	Agression - Fight, bite including attempts, threat, chasing; often accompanied agonistic vocalisations
	Allogroom - Lick or comb with toothcomb other loris' face or fur - usually while clasping him or her
	Play - Behaviours serving no immediate, definable purpose, including friendly attempted bite or manual attack and clasping, dangle by feet, wriggle body with arms over head. No vocalizationsas when fighting.
	Other Social - Social activity while being in contact or close proximity (<5 m), like mating, social follow, sniffing, social explore
Travel	Continuous, directed movement from one location to another
Out of Sight	Behaviour of animal not discernible
Other	Other behavior not included above

At each instantaneous sample point when the slow loris was observed feeding, we recorded the category of food being consumed and plant species. During all occurrence sampling, we recorded the category of food being ingested, plant species, and the measurement of intake (Knotts, 2002). We recorded gum

feeding only when a slow loris was visibly ingesting tree gum and not simply gouging the tree cambium; we recorded duration in seconds. We defined nectar feeding as a slow loris using its tongue to lap up nectar without consuming the flower; we recorded the number of flowers visited. If one flower was revisited, it was not counted again for this evening. We defined fruit feeding as a slow loris eating the non-flower reproductive plant part. We noted if the seed(s) was/were ingested or not, and recorded the leftover weight after consumption. After the slow loris moved away, we approached the left over fruit and collected the leftovers and placed them in a sealed plastic bag. It was impossible to identify the insects being eaten. We thus could only record the size of the insect in relation to the slow loris' hand(s). We thus divided insect feeding into three size categories small (much smaller than the palm of one hand, caught with mouth or one hand), medium (caught in air or on substrate with one hand with the insect being roughly the same size as the slow loris' hand) and large (caught with two hands, insect being larger than one of the loris' hands) with number caught consumed being recorded. For leaf eating, we noted whether a leaf was mature or immature and how many were consumed. We defined flower feeding as the consumption of flower reproductive parts, with the amount of flowers ingested. We calculated the average amount of nectar within one *Calliandra calothyrsus* by sampling 100 inflorescences, totaling 451 flowers and measuring the volume of nectar within each flower using a microcapillary tube (Sigma-Aldrich, Jakarta). The average quantity of nectar obtained was 22.55 (SD ±1.82) μL. The average fresh weight of each food item quantified was: 2.5 g (SD ±0.06) for flowers, 1.2 g (SD ±0.03) for young leaves, 83 g (SD ±4.20) for persimmons (*Diospyros kaki*), 990 g (SD ±130.55) for jackfruit (*Artocarpus heterophyllus*), 0.010, 0.60 and 1.1g for small medium and large insects respectively.

2.3.3 *Phenology and Insect Availability*

We organized five 10 by 10 m phenology plots in random locations using a GIS map at five different altitudes inhabited by slow lorises: 1200, 1300, 1350, 1500 and 1600 m above sea level that contained 21, 14, 54, 13, 23 adult trees and bamboo species, respectively using modified methods from Chapman et al. (1999). A total of 16 different tree and bamboo species occurred within our plots, which is representative of the agro-forest environment. We did not count domestic crop plants in the phenology plots. We numbered and labelled trees and each month scored those with a diameter at breast height (DBH) $\geq$ 5 cm for the amounts of young leaves, mature leaves, flowers, unripe fruits, and ripe fruits. The scoring system used included: 0 = none present, 1 = 0-50% of capacity reached, and 2= $\geq$ 50% of the capacity reached.

For insect availability, we calculated catch density using a malaise trap and three sticky traps twice a week for the duration of the study (Benton et al. 2002; Rode-Margono et al. 2015). Both traps were used in three different locations that rotated weekly. The malaise trap was only placed in areas between trees used by the slow lorises and sticky traps were placed on trunks and branches of trees often frequented by slow lorises. We divided the weight (g) caught for each month by the total weight caught over 12 months and used as gross indicators of availability for each category. Catch rate was so low that it was not possible to attain a large enough mass for the three different size categories of insects recorded in this study (small, medium and large). Therefore, the samples of all three insects had to be combined in proportions reflecting their yearly intake, so one analyzed sample reflected the weighted yearly intake.

2.*3.4 Sample Collection and Nutritional Analyses*

We collected any food item for chemical analyses that we observed being ingested by any slow loris. We collected at least 500 g (fresh weight) of the food item from the trees actually fed on. We only analyzed the plant part ingested by the slow lorises. If more than one tree of the same species was fed on for the same food item, we took samples from many different trees and pooled the results in order to

create a representative sample. We weighed samples and placed them in trays wrapped in mosquito netting and dried them in indirect sunlight for two days (24 hours' worth of sunlight) with temperatures reaching up to 32°C within the tray. We then reweighed dried samples and placed them in plastic sealed bags with silica gel packets, and then placed them in another plastic bag of silica gel. We placed all samples in a cooler in a dry equipment room until transported for laboratory analysis.

We conducted nutrient analyses in the Nutrition Laboratory of Lembaga Ilmu Pengetahuan Indonesia (Indonesian Institute of Sciences, also known as LIPI) in Bogor, West Java, Indonesia. Nutritional analyses followed Norconk and Conklin-Brittan (2004). Crude protein (CP) was estimated by the Kjeldahl method for total nitrogen, multiplying results by 6.25 (Norconk and Conklin-Brittain 2004; Pierce et al. 1958), total ash was determined by incinerating the sample (0.5 g) at 550°C overnight in a muffle furnace, crude fat was determined by ether extract for four days (Williams 1984), free soluble sugars via phenol/sulfuric acid colorimetric assay, calibrated for sucrose (Strickland and Parsons, 1972), and total non-structural carbohydrates (TNC). Fibers were analyzed as neutral detergent fiber (NDF) and acid detergent fiber (ADF) using the Van Soest method (Van Soest, 1996). It was not possible to collect enough nectar for standard chemical analyses, therefore, 85 *u*l microcapillary tubes were used to measure the average volume of nectar in each flower (Morrant et al. 2009), and a portable hand-held refractometer was used to estimate soluble sugar contents as per Bolten et al. (1979). We only observed the nectar of *Calliandra calothyrsus* being ingested; therefore, we sampled 100 inflorescences, totaling 451 flowers. Energy values of each food item were calculated as per Irwin et al. (2012) in equation 2.1, by summing up the caloric energy values for each macronutrient:

(Equation 2.1) E= (CP*4) + (CF*9) + (TNC*4) + (NDF*1.6)

Where E is total energy in Kcal, CP is percentage of crude protein in dry matter (DM), CF is crude fat in DM and TNC is total non-structural carbohydrates in DM. Total non-structural carbohydrate amounts were calculated by Equation 2.2 where A is ash. Our gross estimate for TNC does not include the fiber fractions of neutral detergent fiber (NDF that is an estimate for lignin, cellulose and hemi-cellulose (Van Soest, 1994). The digestive system of *Nycticebus* is morphologically adapted to ferment some amount of plant fiber matter, although not yet quantified (Stevens and Hume 2004). We assume the Javan slow loris is able to digest approximately 40% of NDF intake, resulting in an assimilation of 1.6 kcal/g of NDF based on the hindgut-fermenting model of the chimpanzee (*Pan troglodytes*) (Conklin-Brittain et al. 2006). We take a conservative approach to our energetic modeling as this is a starting point for understanding slow loris feeding ecology (Sayers et al. 2010).

(Equation 2.2) TNC=100-A-CP-CF-NDF

2.3.5 Intake Rate Calculations

We used the equation 2.3 to calculate food intake rate F (gram/hour), for individual *i*, for food item *f* and for season *s*, modified from Rothman et al. (2008). We summed the collected measurement of intake data for individual *i* of food *f* during season *s* and multiplied it by the intake rate (I) of food *f* to transfer the intake into grams. Recorded values for I are: 0.0212 g/sec for gum, 0.002255 g/flower for nectar, 50 g/fruit for persimmon, 475 g/fruit for jackfruit, 0.010, 0.60 and 1.1 g/insect for small, medium and large insects respectively, 2.5 g/flower for eucalyptus flowers (*Eucalyptus* spp.) and 1.2 g/leaf for young bamboo leaves (*Gigantochloa cf. ater*). The total sum was divided by the amount of hours (H) individual *i* was observed during season *s*.

(Equation 2.3) $F_{ifs} = \frac{\sum_f W_{ifs} I_f}{H_{is}}$

We averaged values obtained by equation 1 to yield average daily values for the wet and Austral winter for the entire study population as well as for each sex separately. Values reported include seasonal daily averages only, similar to Irwin et al. (2015) due to the widely variable counterbalancing of observations for each individual.

We determined average seasonal nutrient intake amounts (*N*) for nutrient *n* ingested by individual *i* during season *s* using equation 4. We multiplied the sum of the food intake rates (eq 4.1) for food *f* for individual *i* and season *s* by the fresh matter content (*M*) of nutrient *n* for food *f* were added up and multiplied by the activity period *A*. We averaged every individual average daily intake for each nutrient to determine the average daily seasonal intake of each nutrient. This was used to calculate protein energy (PE) by multiplying the crude protein amount by 4 kcal/g, and calculating the non-protein energy (NPE) by multiplying TNC by 4 kcal/g DM, NDF by 1.6 kcal/g DM, and crude fat by 9 kcal/g DM and summing.

(Equation 2.4) $N_{nis} = (\sum_n F_f M_{nf})A$

2.4 Captive Research

2.4.1 Captive Field Site

Following my time in Cipaganti, I worked with captive slow lorises at Cikananga Wildlife Rescue Centre (CWRC - S7°03'27.04" and E106° 54'36.63"), near Sukabumi, West Java, Indonesia for two months. I had access to 12 Javan slow lorises (*N. javanicus*), 12 Sunda or greater slow lorises (*N. coucang*) and four Philippine slow lorises (*N. menagensis*).

This centre is split into two different portions, one receiving the seized animals of the illegal wildlife trade (orangutans, gibbons, slow lorises, snakes, crocodiles, birds etc.) and the other breeding critically endangered birds and suids for *in situ* and *ex situ* conservation programs. Both have their separate funding sources with the breeding centre being in much stronger financial state than the rescue centre part. Having a steady supply of funds to procur adequate food items

for the animals is a constant challenge and providing gum to the slow lorises is not a priority when compared to simply giving them anything for them to eat. The constant issue is to get the animals to survive, not necessarily to thrive.

2.4.2 Gum Intake Rate

We harvested one kg of wild gum from Cipaganti and transported it fresh to CWRC. We only collected enough gum to trial on ten slow lorises (weight range 862 --1020 g). Each was given 10 g of the wild gum and, using a stopwatch, recorded the amount of time required to eat this 10 g. If consumption stopped, or the slow loris became disinterested, we stopped the stopwatch until ingestion resumed. We repeated this experiment on two separate occasions one week apart with each slow loris observed on the same night. We provided the gum in one wooden log per individual, each cut to 20 cm long, with a deep longitudinal groove, 5 cm deep, where the gum was placed. We divided the amounts ingested by the amount of time it took to ingest that amount for each individual each night. On average, they consumed gum at the rate of 0.021 g/s (SD ±0.006) (n=10).

2.4.3 Food Passage Rate

We used the methods described in Lambert (2002) to determine transit time (TT) and the mean retention time (MRT). Originally, we used non-toxic plastic beads as the marker, however the slow lorises were able to use their sublingual to filter and spit out the beads that we tried hiding in bananas, guava and gum. We then decided to use glitter as described in Fuller et al. (2011). To validate the glitter technique within slow lorises, we fed it inside guavas and compared the TT and MRT values of the guava seeds versus glitter within the same individuals. Results were identical and the trials proceded using glitter inside a banana as a marker. After the slow lorises awoke, we checked every enclosure hourly and collected every faeces we could locate. If we found glitter in the faeces, we recorded the name of the individual and the time at which the marker was found. One trial consisted of dosing 10 g of gum with 5.0 g (1 teaspoon) of glitter and

observing the markers. We used red and blue glitter and alternated colours between trials. We waited one day between trials to ensure no lag time confused the results. We conducted a total of four trials for each individual for each diet.

The time between ingestion of the marker and its first appearance is the TT, and the MRT is used as the best estimate of food movement through the GIT. This value is calculated by dividing the length of time from ingestion to each occurrence of the marker, divided by the total number of separate faeces with markers present for that trial (Lambert 2002). We averaged each of the four trials per animal to calculate individual MRT values.

2.4.3 Ethics Approval

As part of our captive field work, we wrote and submitted a questionnaire to all zoos within North American and Europe holding *N. pygmaeus* (due to them being most abundant within zoological institutions). Our questionnaire used received full approval from the Oxford Brookes University Research Ethics Committee (Registration number 150900). The questionnaire is available in APPENDIX I and the approved ethics form is available in APPENDIX II.

CHAPTER III

"Do slow lorises in zoological institutions actually suffer of poor health and/or welfare issues because of their current nutritional management?"

or

CAN'T GET NO SATISFACTION: DIETS HIGH IN FRUITS AND LOW IN GUM EXUDATES PROMOTE THE OCCURENCE AND DEVELOPMENT OF DENTAL DISEASE IN PYGMY SLOW LORIS (*NYCTICEBUS PYGMAEUS*)

3.1 INTRODUCTION

Questionnaires have become an important method of gathering information in the zoo community. Such methods have been used to suggest beneficial husbandry methods (Fuller et al. 2013, Rose and Roffe 2013, Wright et al. 2011), identify different research or enrichment methods used and their effectiveness (Fuller et al. 2011, Huber and Lewis 2011), and to survey the health or behavioral issues within a species and success rates of treatments (Lewis et al. 2010, Montaudouin and Le Pape 2005). Surveys have also been used in conjunction with veterinary or post-mortem reports in hopes of identifying predictors or possible causes of specific health ailments as shown by Fuller et al. (2014). Fuller et al. (2014) focussed on lorisid primates within Association of Zoo and Aquariums (AZA) institutions and concluded that more than half of the reports that were studied showed evidence of renal pathology. One fifth of the samples showed signs of cardiovascular, gastrointestinal, endocrine and metabolic or immunologic diseases. One main hypothesis theorised by the authors in an attempt to explain why so many lorisids in captivity show signs of illness, was nutrition, speculation which was first brought up by Debyser (1995).

3.1.1 Slow Lorises in Captivity

Asian slow lorises are found in zoos and rescue centres worldwide with *Nycticebus pygmaeus*, the pygmy slow loris, boasting the largest population in captivity (ZIMS 2015). These Vulnerable primates (Streicher et al. 2008) are largely fed a diet high in fruit and concentrates (Fitch-Snyder et al. 2001). Starr and Nekaris (2011) report feeding observations of free-ranging lorises to be 40% insects, 30% nectar and 30% exudates and sap; the ingestion of fruit was rare and opportunistic. A plant exudate has been defined in different ways to include or exclude different substances produced by plants. The definition used in this paper is from Nussinovitch (2009) which describes an exudate as a fluid which oozes out of wounds in injured trees and hardens upon exposure to air. Namely this includes

gums, resins, latex and chicle but excludes sap. No slow loris has yet been observed ingesting resins, presumably due to the high load of terpenoids and other plant secondary metabolites (Nekaris 2014).

3.1.2 Captive Diets

While feeding fruits may seem to provide a diet that is richer in nutrients and energy than exudates, Cabana and Plowman (2014) showed that a naturalistic diet can be palatable and additionally promoted the occurrence of natural behaviors. Breeding is monitored by the Species Survival Plan (SSP) and by the European Endangered Species Program (EEP) from North American and European zoos, respectively. Individual *N. pygmaeus* are recommended to breed or not and to be moved to other institutions to ensure genetic diversity. Yet breeding is successful only for a few key collections and health ailments are common (Debyser 1995, Fitch-Snyder et al. 2001, Fuller et al. 2014). All studies state nutrition as a possible causative agent of low breeding success and important illnesses, however no attempts at providing empirical evidence were attempted.

Fruits are known to be prevalent within the diets slow lorises, both in zoos and in rescue centres, with gum exudates being used more as enrichment and not a dietary staple (Fitch-Snyder et al. 2001). Health issues have been reported to be widespread, with dental issues being the most prominent. In this chapter we aim at identifying the current trends in captive populations of *N. pygmaeus* worldwide, extracting information about the extensiveness of health issues and indentifying a causative agent within the nutrition of *N. pygmaeus*.

3.2 MATERIALS AND METHODS

3.2.1 Questionnaires

After permission and endorsements from the AZA and EAZA Prosimian Taxon Advisory Groups (TAG) were obtained, questionnaires described in CHAPTER II (section 2.3.6) were sent via e-mail to every zoo with at least one *N.*

pygmaeus which is included in the AZA SSP and EAZA EEP collections. Questionnaires were also sent to zoos and rescue centres in Vietnam, Indonesia and Thailand. The questionnaires were filled in by keepers, curators and veterinarians; however, we asked that our main person of contact at each institution liaise with the relevant authorities for the different parts of the questionnaire. In this questionnaire we asked about the identification of each individual *N. pygmaeus*, as well as its daily husbandry pertaining to diet, and current diet; if the current diet was less than 3 months old, we asked participants to provide data on the previous diet. Husbandry questions were limited to how often food was presented per day, on which surface or container was used to present the food and whether or not a seasonal change in diet occured. In addition, we requested details of all health problems diagnosed during the feeding of the current diet, namely dental, digestive, skeletal, renal and liver diseases as well as pelage/fur conditions. The questionnaire also allowed and encouraged the inclusion of extra information about health issues we did not consider or mention.

3.2.2 Nutrient Analysis

Diet menus listed on our returned questionnaires were analyzed for nutrition concentrations using the Zootrition v2.6 (St. Louis Zoo) software. We used the USDA nutritional data for ingredients listed in AZA diets. Ingredients formerly analyzed from Paignton Zoo, UK and entered into our Zootrition database were used for all European zoos. Nutrient information of food items analysed by us, within Indonesia at the Indonesian Institute of Science Nutrition Laboratory (LIPI), were used for Ingredients used in Asian institutions.

3.2.3 Statistical Analyses

We ran a principle components analysis (PCA) using every nutrient value for each diet that is accounted for by at least 85% of the ingredients by dry mass, in order to identify nutrients which are responsible for the highest variance of nutrient

contents (Jolliffe, 2002). The results of the PCA were then used as possible predictors in a binary logistic regression (BLR) using the presence or absence of an illness as the outcome (Hosmer and Lemeshow, 2000). We also used a chi-square test for association to specifically investigate the effects of fruit within the diet. We assigned each collection a grade of 1, 2 or 3 to reflect none to little, medium and high amounts of fruit respectively. These classifications were determined by removing or adding one half standard deviation to the overall mean of fruit proportion. All statistical analyses were conducted using SPSS version 22.0 (IBM Software). Reported health issues were linked to a diet and not to an individual slow loris, therefore, results will be reported as per collection for descriptive questions and as per diet for analysis results.

3.3 RESULTS

3.3.1 Questionnaire Replies

We sent a total of 55 questionnaires (19 to AZA collections, 28 to EAZA collections and 8 to Asian collections). A total of 39 (71%) were returned (18 from AZA, 13 from EAZA and 8 from Asian collections) representing 160 individuals (31.3% from AZA, 21.3% from EAZA and 47.4% from Asia). Some collections had multiple diets in place for specimens with specific cases such as obesity so we analyzed a total of 47 diets worldwide. Table 3.1 shows the different food categories and how many collections include them in their diets as well as their average proportion by weight in all analyzed diets. Nutrition information for the diets are found in Table 3.2. Dental health issues were the most prominent problem reported in 20 (51.3%) of the collections (Figure 3.1). Most of the diets were fed in bowls or plates fixed on branches (71.8%) followed by bowls/plates on a shelf (20.5%). Very few institutions placed the food directly on the shelf (7.7%). The lorises were most often fed only once/day (59.0% of institutions), followed by twice/day (25.6%) and only 15.3% of institutions divided the daily diet into three feeds. Seasonality was virtually absent with no collections altering the diet to

reflect the natural life history of *N. pygmaeus.* Three (7.8%) institutions did alter the diet seasonally to counter seasonal weight gain and loss.

Table 3.1 Collections that use at least one food category item in any of their diets for *Nycticebus pygmaeus* and the average proportion of each category in all diets based on fresh weight, indicating that fruit is prevalent in diets worldwide and exudates are lacking.

Food Item Category	Number of Collections # (%)	Average Proportion by Fresh Weight % (SD+/-)
Concentrate	26 (66.7)	17.6 (1.5)
Fruit	33 (84.6)	40.9 (24.1)
Vegetable	29 (74.4)	25.8 (22.7)
Animal Product	18 (46.2)	6.5 (8.1)
Dairy Product	7 (17.9)	0.1 (2.7)
Invertebrate	31 (79.5)	7.3 (6.6)
Gum exudate	14 (35.9)	2.9 (5.2)
Grain or Grain-based product	3 (7.7)	0.6 (2.5)
Nectar	5 (12.8)	0.2 (0.6)
Other	6 (15.4)	0.3 (0.9)

Total collections n= 39
Total diets n= 47

Concentrates include pellets or canned food, Animal products include raw or cooked meat, eggs, chicks and newborn mice (pinkies); dairy products include yogurt, cheese and pudding; grains include rice, bread and pasta; other includes honey, peanut butter, seeds and nuts.

3.3.2 Statistical Results

For purposes of the PCA, the nutrients used as variables were from each individual diet and not averaged by collection. All nutrient concentrations on a dry matter basis as well as proportions of each food item category on a fresh weight

basis for each diet were used in the PCA, totalling 31 different factors. Simple structure was obtained with 11 of these factors (Calcium, Ca:P ratio, Ash, Magnesium, Vitamin D, Crude Protein, Gum, Potassium, Acid Detergent Fibre (ADF), Nectar and Energy) which loaded onto 4 components. Results of the rotated component matrix are visible in Table 3.3. The variables retained explain 71.01% of the total variance. We found multi-collinearity between some of our variables. A strong correlation in this case is defined as an *r* value greater than or equal to 0.700. Ash and Calcium ($r = 0.718$), Calcium and Ca:P ratio ($r = 0.849$) all correlated strongly so both Ash and Ca:P ratio were removed from the BLR.

Table 3.2 Average, standard deviation and min-max values of the nutrient concentration of diets of captive *Nycticebus pygmaeus* on a dry matter basis

Nutrient	Units	Mean	SD	Min.	Max.
Ash	%	4.98	1.03	2.70	7.29
C. F.	%	8.58	3.42	2.55	18.30
C. P.	%	19.64	5.19	6.70	30.91
Ca	%	0.55	0.27	0.04	1.04
P	%	0.45	0.15	0.08	0.74
Fe	mg/kg	100.81	77.83	1.02	342.95
Mg	%	0.13	0.04	0.05	0.24
Cu	mg/kg	11.54	6.38	1.90	26.71
K	%	1.04	0.33	0.25	1.91
Se	mg/kg	0.59	1.47	0.02	8.01
Na	%	0.21	0.10	0.05	0.43
Zn	mg/kg	47.39	34.79	0.78	126.92
Vit C	mg/kg	683.09	516.41	66.11	2291
Vit D	IU D/g	5.46	4.85	0.58	21.25
Vit E	mg/kg	105	77.79	17.55	306.81
NDF	%	9.98	5.31	0.85	24.16
ADF	%	5.28	3.45	0.21	15.75
NSC	%	57.04	10.21	40.07	85.54
Ca:P		1.14	0.36	0.30	1.84
Energy	Kcal/day	75.57	36.5	24.04	178.00

Number of diets = 47. All values used for the above values were represented by at least 85% of the total dry matter content for each diet. C.F. crude fat, C.P. crude protein, Ca calcium, P Phosphorus, Fe Iron, Mg magnesium, Cu copper, K Potassium, Se selenium, Na sodium, Zn Zinc, NDF Neutral Detergent Fibre, ADF Acid Detergent Fibre, NSC Non-Structural Carbohydrates.

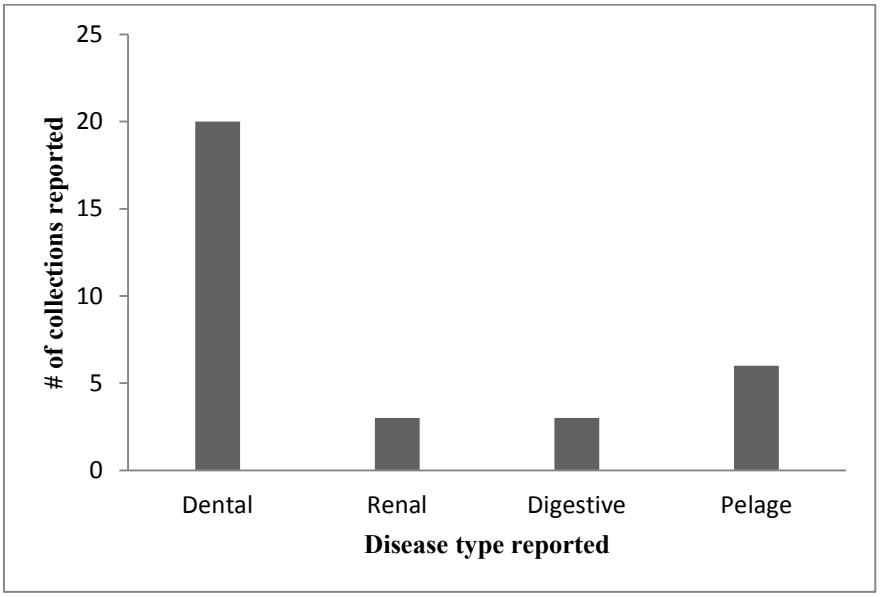


Figure 3.1 Health afflictions reported by zoos and rescue centres worldwide showing the prevalence of dental diseases in captive populations. Results are based on replies from the questionnaire survey (n=39).

We ran a binary stepwise logistic regression analysis using the stepwise "Forward Wald" method to test which variable could have been a predictor of presence/absence of dental disease. Nine possible predictors within 47 different diets were used in the BLR. Overall the BLR model was significant ($X^2 = 38.872$, $p \leq 0.001$), with presence or absence of gum being a significant predictor (Wald = 0.031, df=1, p=0.039) to the occurrence of dental disease (Table 3.4). The model interpreted 56.8% of cases correctly in the first step and 84.1% of cases correctly in the second step.

We further explored the status of fruit in a chi-square test for association between amount of fruit in diet and presence of dental disease. The values used for the different levels of fruit for each collection are as follows: level 1 comprises values including and less than 28.84, level 2 is including and between 28.85 to 52.96 and level 3 is anything equal to and greater than 52.97. All expected cell frequencies were greater than five. There was a statistically significant association between amount of fruit in diet and presence of dental disease $\chi^2_{(2)} = 11.113$, $p = 0.004$ and Cramer's V showed the association to be strong *V=0.486, p=0.004.*

Table 3.3 Results of the principle components analysis rotated components matrix on the nutrient values of *N. pygmaeus* diets

	Component			
Factors	1	2	3	4
Calcium	**0.953**	0.070	-0.064	-0.089
Ca:P	**0.844**	-0.049	0.041	-0.174
Ash	**0.737**	0.293	0.230	0.162
Magnesium	**0.703**	-0.018	0.021	0.276
Vit D	0.066	**0.858**	-0.089	-0.227
Protein	0.089	**0.785**	-0.055	0.197
Gum	0.013	**0.739**	0.094	0.074
Potassium	-0.085	0.126	**0.873**	-0.012
ADF	0.224	-0.17	**0.818**	-0.045
Nectar	-0.145	0.251	0.037	**0.783**
Energy	-0.196	0.146	0.087	**-0.727**
Eigenvalue	2.922	2.068	1.483	1.339
% Variance Explained	26.560	18.800	13.481	12.173

ADF Acid detergent fiber (sum of cellulose, hemi-cellulose and lignin)

Values are loadings of the variables on each of the four principal components derived. Bold values indicate the largest absolute loading per factor.

Table 3.4 Logistic regression analysis of the occurrence of dental disease as a function of diet nutrients and proportion of food items where only presence of gum was a significant predictor.

Variables	**Wald/Score**	**Df**	**Sig.**
Calcium	1.805	1	0.179
Vitamin D	0.091	1	0.763
Magnesium	0.040	1	0.842
Protein	2.655	1	0.103
Potassium	1.369	1	0.242
ADF	1.489	1	0.222
Energy	1.071	1	0.301
Gum	4.281	1	0.039*
Nectar	0.000	1	0.998

Nutrient values used were on a dry matter basis within the diets while the food category proportions within each diet were on a fresh weight basis.

3.4 DISCUSSION

3.4.1 Trends in Nycticebus Husbandry and Health Issues

As a general rule, fruit is a staple part of captive diets of *N. pygmaeus* and exudates are not. Our results show that high amounts of fruit were associated with the occurrence of dental disease and the absence of gum was also shown to be a predictor in its occurrence. Teeth issues were the most abundant health affliction, found in 51.3% of all collections. Streicher (2004) hypothesized that diets high in fruit promote dental problems in captive *N. pygmaeus* and providing them with gouging opportunities could help remove dental plaque and combat these effects. While we cannot comment on the validity of these assumptions, our data support the premise of Streicher's hypothesis. The genus *Nycticebus* has specialist morphological adaptations, similar to the gummivorous marmosets *Callithrix* and *Cebuella* (Hladik 1979, Tan and Drake 2001*).* Their dentition is particularly useful for the harvesting and processing of gum exudates. Their incisors and canines are specialised to form a toothcomb (Nekaris and Bearder 2011, Nekaris et al. 2010). The large procumbent lower premolars are also used in the gouging process, acting as a pivot point for the other teeth to be able to dig out the lignin from trees to stimulate gum flow (Nekaris et al. 2010). The incredible stress placed upon the teeth is indicated by stress fractures that appear in older animals, yet broken teeth have rarely been observed in wild slow lorises that gouge nightly (Nekaris 2014). Dental disease has not yet been reported in wild lorises. Providing gum to captive *N. pygmaeus* had a marked effect in the reduction of gingivitis (Streicher 2004). There are many contact points between the broken cambium of the tree during gouging and/or the tree's gum during intake that may act as a source of friction and remove any plaque. However, gum provided to captive lorises is either spread on braches or placed into drill holes of thick branches (Gray et al. 2015), and may not illicit the same gouging behaviors. It is uncommon for a captive slow loris to gouge offered branches. Instead they are observed only processing the gum with minimal interaction with the wood. An alternate hypothesis is the high amounts of plant

secondary metabolites from the gum in conjunction with the mechanical contact between bark and tooth has a beneficial effect on teeth health (Nussinovitch 2009). *Nycticebus pygmaeus* has been observed feeding largely on insects, gum exudates and nectar in nature (Starr and Nekaris 2013, Streicher et al. 2012). Fruit forms an insignificant part of their natural diet; even if they were to ingest fruit in the wild in an important quantity, fruit found in nature has a significantly different chemical composition than the fruits zoos and rescue centres use in their collections (Oftedal and Allen 1997, Schwitzer and Kaumanns 2003). Cultivated fruits are higher in soluble carbohydrates and lower in protein, fiber fractions and microminerals when compared to "wild" type fruits. The main contribution of fruit in captive primate diets is water and soluble sugars that translates into energy (Plowman 2014). Too much of this may be the root cause of dental disease.

Caries and other dental diseases are known to be caused by bacterial plaque (Sheiham 2001). The microflora that inhabits this yellow plaque produces organic acids that effectively lowers the pH of the saliva and erodes the surface of the teeth, the enamel, to eventually expose in inner dentine layer. This acidification renders the remineralisation activity of saliva non-competitive and leads to open cavities that are prone to infection (Meurman and Cate 1996). This plaque gains a foothold when concentrations of soluble carbohydrates (sugars and starches) are high and also constant (Brathall 1996). Sugars can also augment the production of the plaque matrix itself, as well as fuel the production of organic acids by feeding the problematic acidogenic microflora (Sheiham 2001). Without this intake of sugars, the caries in question would not be produced. Fruit has specifically been identified as a possible cariogenic agent (Moynihan 1998). Dental diseases linked with sugar intake in humans are caries, root caries (which leads to infections), gingivitis and facial and mandible abscesses, all of which have been identified in captive slow lorises (Debyser 1995, Fuller et al. 2014, Fitch-Snyder et al. 2001, Sheiham 2001).

3.4.2 Effect of Diet on Health

As well as a specialised dentition adapted for gouging, the genus *Nycticebus* also possesses specialised gut morphology. They are described as having a shorter duodenum and a relatively long and thick large intestine and a caecum (Stevens and Hume 2001). The presence of these microbial chambers gives reason to assume they possess the microbiota capable of fermenting plant fiber and perhaps even chitin. Gum exudates contain a high amount of soluble fiber such as pectins and fructans that can only be digested with the symbiotic relationship of these microorganisms (Nussinovitch 2009). If *Nycticebus* is adapted to ferment food items high in fiber, not providing gum in lorises' diets could have a detrimental effect on their health. *Cebuella* and *Callithrix* are also gummivorous small primates and are known to suffer from a wasting disease in captivity. Gore et al. (2001) posits that a lack of fiber in the diets is responsible for the inflammation of the intestine that leads to nutrient malabsorbtion. Although wasting disease has not yet been described in captive lorises, the presence of fiber itself may carry out indispensible physiological processes in the gut. There is a condition where lorises which suffer from poor welfare are emaciated, wet and have sunken eyes, however, the entire husbandry of these animals is inadequate so it is difficult to diagnose this as wasting (Nekaris, pers obs). *Nycticebus* have relatively thick masseter muscles that could possibly atrophy in captivity if not given the opportunity to gouge (Perry and Harstone-Rose 2011). Lorises forage by climbing up and down every branch and trunk of gum trees within their territory (Starr and Nekaris 2013). If an already open gouge hole has gum, the gum will be eaten, and fresh scrapes will be made so the hole keeps producing gum, similar to the process described for galagos (Bearder and Martin 1980, Nekaris et al. 2010). It is more energetically expensive to create a new gouge hole than to harvest pre-existing ones, therefore the creation of new gouge holes in an existing territory is rare (Vinyard et al. 2009). Thus, the actual gouging action may have little to do with dental health, and more to do with the actual oral processing of exudates. Insects were caught and ingested

opportunistically throughout the night and wholly ingested (Starr and Nekaris 2013, Streicher 2009, Streicher et al. 2013). When wild born *N. pygmaeus* were confiscated at Customs and sent to a rescue centre for rehabilitation and release, Streicher et al. (2013) fed them a diet of fruit, insects and animal products. Once in soft release, the primates had the opportunity to eat from a large variety of food items. They chose naturalistic "wild" type food items significantly more than most fruits, animal products or dairy. There is ample evidence that *N. pygmaeus* are adapted to ingest and process diets high in gum exudates and insects.

3.4.3 Appropriateness of Nutritional Models

The nutrients from the analyzed diets displayed had a wide range and no sets of guidelines unified them. Fitch-Snyder et al. (2001) produced the only husbandry manual for lorisine primates, and suggested using the nutrient recommendations for Old World monkeys (NRC 2003) that were originally identified from rhesus macaques (*Macaca mullatta*). These recommendations derived from a medium-sized primate with an opportunistic and generalist feeding behavior. *Nycticebus* is at the other end of the feeding continuum (Nekaris 2014). It would seem counter intuitive that these recommendations are fully appropriate for the lorisines, although basic nutrition principles apply across wide differences in feeding behaviours. No evidence based recommendations for lorisines, or closely related species such as galagos, currently exist, particularly under controlled conditions. The average crude protein concentrations in captive diets were 19.64%, and ranged from 6.7 to 30.91%, while the OWM recommendations are stated at 17-28% (NRC 2003). This is also a large range and with a diet high in insects, *Nycticebus* are believed to have high protein requirements although this is purely speculative. Callitrichids may provide a better model; a study byMitura et al. (2012) showed pathologies only occured in *Callithrix* receiving diets less than 6% high quality crude protein. One long term study showed a diet of 15% crude protein (DM basis) is adequate for maintenance, normal breeding and social

behaviours in callitrichids (Flurer and Zucker 1985), thus although insectivorous, protein requirements may not be high for lorises. Calcium is also often thought to be a limiting nutrient in small insectivorous primates such as callitrichids (Smith 2000). The average amount of calcium in the *N. pygmaeus* diets was 0.55% and ranged between 0.04 and 1.04%. OWM recommendations, incidentally, are also 0.55% (NRC 2003). Multiple supplements such as cricket gut loading gel or powders, insect mineral dusts and mineral supplements spread over the fruit, as well as the presence of concentrate feeds ensures minerals are found in abundant concentrations. Gum becomes increasingly important for collections that cannot afford concentrate feeds or supplements. The calcium found in wild gums would naturally help balance out the high phosphorus concentrations found within insects (Bearder and Martin 1980, Heymann and Smith 1999). Calcium is found in the chitinous exoskeleton of some insects, however it has not yet been established if *Nycticebus* can digest chitin in any significant amount. Indeed the gastric mucosa of *N. coucang* was reported to contain chitinolytic enzymes; no information is available on the provenance or effectiveness of these enzymes (Stevens and Hume 1995). Research conducted with callitrichids in the wild have often reported individuals removing the most chitinous parts of an insect such as wings or legs prior to consumption (Heymann and Smith 1999). This activity is not true for wild *Nycticebus,* which ingest insects whole, and suggests they may possibly be better able to utilize chitin, possibly as an energy and/or calcium source (Starr and Nekaris, 2013). Some captive bred individuals may remove the wings of some insects before consumption, but we regard the wild type as the "golden standard". A variety of insects should be presented to *N. pygmaeus* rather than relying on a single species (i.e. crickets or mealworms), as different nutrient contents over a week may help to balance out nutrient intake(s) and assimilation. Wild *Nycticebus* nutrient intake is mostly from a short specialized list of food categories, and their captive diets should reflect those choices.

3.4.4 Husbandry Recommendations

In terms of husbandry, the questionnaire results shed some insight as to the current practices of keeping *N. pygmaeus* in captivity. Free ranging *N. pygmaeus* expend the majority of their activity budgets foraging (Starr and Nekaris 2013). Easily consumed diets in captivity are estimated to take up roughly 10% of their active periods when food was presented once per day, leaving more time for abnormal behaviour patterns to be performed (Cabana and Plowman 2014). The questionnaires received indicated the majority of collections fed their slow lorises once per day. Because of the large disparity between foraging times in the wild and in captivity, we believe this to be inadequate and would recommend two or more feeds per day. The provision of gum also significantly extended feeding time (Cabana and Plowman 2014, Fitch-Snyder et al. 2001, Gray at al. 2015). Bowls or plates were reported to be used in over 92.3% of collections. Ideally there would be many bowls scattered around the enclosure to promote an uneven and random distribution of food to possibly stimulate natural foraging behaviours (Montaudouin and Le Pape 2005). In terms of diet variation, both Starr and Nekaris (2013) and Streicher (2004) reported a very strong seasonal effect on free ranging *N. pygmaeus* diets, accompanied by a period of weight gain and loss. The effects of mimicking these changes in captivity have yet to be quantified so we do not promote or discourage its application at this moment.

Creating an ideal diet for a wild animal in a captive setting can be a challenge, especially for specialist species. It is difficult to recreate a wild diet, however recreating the nutrients found within this diet is possible. Perhaps the nutrients should be used as the structural framework for the diet creation and specific food items should be chosen to be as close as possible to "wild" type food items (Clauss et al. 2008). If a collection cannot provide a large enough variety of insects, replacing a portion of this food category could be attained by using a nutrient dense concentrate such as pellets, eggs, or a canned food. Insects should be gut loaded with a high calcium insect food. Nectar is easily replaced by providing a

small amount of dilute juice or using a bird nectar powder and adjusting the sugar concentration to range between 22 - 30% (pers. obs). Vegetables may be included to ensure the diet will not lead to an energy deficit, and as a source of fiber. Gum exudate is more difficult to replace because of its unique chemical composition of soluble fiber, high Ca:P ratio and cocktail of secondary metabolites (Smith 2000; Smith 2010). However, gum arabic from the *Acacia senegalensis* can be readily sourced in raw or refined form in most countries because of its use in the food, pharmaceutical, and cosmetic industries. In tropical countries where purchasing is an issue, gum can be harvested from trees on site using a variety of techniques (Nussinovitch 2009). As shown by Cabana and Plowman (2014), it is possible for a "wild" type diet of gum, nectar, insects and vegetables to be both palatable and nutritionally appropriate for slow loris.

3.5 CONCLUSION

Anecdotes and history have been the basis for shaping diets of *N. pygmaeus* in captivity, as opposed to scientific evidence. A clear lack of guidelines and nutrient recommendations targeting slow lorses has further led to current feeding practices not catering for the slow loris's morphology, physiology or behaviour. Captive diets evaluated were generally high in fruit and low in exudates, which lorisine primates have evolved to harness, process and digest. Not providing them with this food source could have serious health consequences. Future research should focus on identifying specific nutrient recommendations and identifying ingredients which better meet the adaptations of the loris. Digestive capacities of the slow loris should also be investigated, specifically for soluble fiber and insect chitin.

A causal link was identified between the presence of fruit and lack of gum in the diet and the occurrence of dental disease. Captive diets should not rely on fruits, but rather consider the use of gum Arabic and a variety of insects to better duplicate natural feeding habits and nutrient balance.

Key lessons learned from this work allowed us to engage in a conversation with the zoological community worldwide. By taking part in this study, they could not deny their involvement in our results and similarly, they could not provide a valid excuse. This made them feel invested and open to our future results and evidence based recommendations. Our results were also useful to begin convincing managers of the importance of gum, especially for rescue centre housed animals destined to return to the wild.

Slow lorises are not fed like the published information available to us, and we have our first hint on the importance of gum for their health. Diet was directly linked with their health issues observed in captivity.

CHAPTER IV

“What is the nutrient intake of wild slow lorises and do males and females differ in their nutrient selection?”

or

"GIMME MORE: GUM IS NOT A FALLBACK FOOD IN THE SEASONAL FEEDING ECOLOGY OF THE JAVAN SLOW LORIS"

4.1 INTRODUCTION

4.1.1 Coping with Seasonal Fluctuaitions in Food Availability

Primates have evolved a variety of ways to cope with the seasonality of their habitats, specifically in regards to the flux of available nutrients and energy (Gould et al. 1999; Irwin et al. 2014). Changes in behavior leading to reduced metabolic costs (Gould et al. 2011); reduction of fat reserves (Knotts 1998), changes in physiology i.e. torpor (Nowack et al. 2013; Pereira 1993;); alteration of home range size and/or daily distance travelled (Campera et al. 2014; Pichon et al. 2016; Sato et al. 2015); and prominently, the ingestion of less preferred (fallback food) items (McGraw et al. 2014; Serckx et al. 2015) are all strategies primates may employ to cope with energetically restrictive seasons. The term "fallback food" has been used inconsistently in the primatological literature. Fallback foods are not intrinsic, meaning a plant part is not inherently of low quality, but instead fallback foods are comparatively observed to have a lower nutritional quality (Lambert and Rothman 2015). Following optimal foraging theory (Charnov 1976), the highest quality food items should be selected based on what is available, given their defined constraints such as requiring substantially more handling or processing time, possessing a higher fiber concentration or higher secondary plant metabolite content. Any of these factors associated with fallback foods may ultimately reduce the amount of or absorption rate of nutrients, decreasing the nutritional gains of this particular food in regards to the resources needed to process it (low quality). When compared to a food item with higher gains and/or requiring a lower processing intensity (high quality), the low quality food item is described as fallback and should only be selected when the higher quality food is not available. Thus "fallback" is relative to what other edible items are available. Tougher or more fibrous foods have often been labeled as fallback foods, without regard for consumer species that may have morphological or physiological adaptations to avoid increased processing time (Constantino et al. 2009; Lambert et al. 2004; Moura and Lee 2004). Such a species may select a typical fallback food

disproportionally relative to abundance in a given habitat, using it as a preferred food item (Leighton 1993; Marshall and Wrangham 2007). Fallback foods can be further defined into "staple" and "filler". The staple fallback foods are always available and are a small yet consistent part of the overall diet. This is in opposition to filler fall back foods, which may be available year-round or only seasonally yet very rarely become an important part of the diet, usually when preferred foods are absent (McGraw et al. 2014). Preferred food items, often ripe fruit or young leaves, can be eaten alongside the modified described staple fall back foods (Marshall and Wrangham 2007). Many frugivorous species have to survive drastic changes in food availability, often by choosing to ingest a larger variety of plant parts as well as possibly insects (Beeson 1989; Gould et al. 2011; Norconk et al. 2009; Ossi and Kamilar 2006; Terborgh 1984). Little is known about how exudativorous or insectivorous primates respond to such seasonal changes.

4.1.2 Exudates as Fallback Foods

Exudates as a food resource have been reported to be of little nutritional value, with low levels of crude protein, virtually no lipids and mostly made of soluble structural carbohydrates (Nash and Whitten 1989). The energy content of gums may be limited, unless the species possesses the capacity for fermentation, as well as the ability to cope with plant secondary metabolites (PSM). Given both of these anti-feedants (high fiber and PSMs) contribute to gum being described as a fallback food for many primate species (Smith et al. 2010). Nonetheless, gum is usually available year round and can act as a staple or filler fallback food. Increasing proportions of exudates are consumed by many primate species during the Austral winter when fruits or young leaves are less accessible, such as for the grey mouse lemur (*Microcebus murinus)*, Senegal bushbaby (*Galago senegalensis)*, the giant mouse lemur (*Mirza coquereli*), a number of tamarins (*Saguinus spp.*) and marmosets (*Cebuella* and *Callithrix spp.)*, squirrel monkeys (*Saimiri sciureus*), red-tailed monkeys (*Cercopithecus ascanius)* and yellow

baboons (*Papio cynocephalus*) (Chapman et al. 2002; Dammhahn and Kappeler 2008; Garber 1984; Hladik et al. 1980; Oates 1984; Porter et al. 2007; Raboy et al. 2008; Sugiyama and Koman 1992; Stone 2007). Gum was labelled as a fallback food in these studies.

Slow lorises (*Nycticebus* spp.) display a suite of morphological adaptations that are centered on exploiting exudate food sources, which defines their ecological niche of "exudativore" (Burrows et al. 2015). Although originally suggested to be frugivorous based on comparisons with pottos (Charles-Dominique 1977), wild field studies have clearly demonstrated that slow lorises are specialised exudativores (gum specialists) (Nekaris 2014; Nekaris and Bearder 2007). The largest of the lorisines (1.1-2.4 kg), the Bengal slow loris (*Nyctiebus bengalensis*) has been observed spending the majority of its feeding time on exudates (Pliosugnoen et al. 2010; Swapna et al. 2010), with only 4.45% time spent eating fruit (Das et al. 2014). The smaller bodied pygmy slow loris (*N. pygmaeus*) (350-550 g) has rarely been observed to ingest fruit in the wild, focusing on a diet of gum, nectar and insects (Streicher 2004; Starr and Nekaris 2013). A three-month study of the Javan slow loris (800 g -1 kg), *N. javanicus*, also yielded similar results with the majority of feeding time spent on exudates, insects and nectar (Rode-Margono 2014). In peninsular Malaysia, Wiens et al. (2006) did observe some consumption of fruit, but two-thirds of the diet of wild *N. coucang* (650-850 g) was exudates (Barrett 1984; Wiens et al. 2006). None of these studies, however, quantified the nutritional content of slow loris diets.

4.1.3 The Framework of Nutritional Geometry

Quantitative feeding ecology research has allowed for a deeper understanding of the different strategies of primates to seasonality (Norscia et al. 2006). The framework of nutritional geometry (FNG) alone has allowed for in-depth analyses of how species react during lean seasons as population or refined sex-specific strategies, especially regarding reproductive costs (Ganzhorn 2002;

Pichon and Simmen 2015; Rothman et al. 2008). The FNG's unique characteristic allows the portrayal of an animal's response and resource availability simultaneously. For example, this has been used to empirically define the term 'lean season' as well as identify if any food items are being used as a fallback food with quantitative data (Felton et al. 2009; Simpson and Raubenheimer 2002). It is an integrative framework and allows us to include multiple food components, not limited to two axes. By characterizing two of three nutritional parameters, the third can be implied in geometric space, and a three dimensional model of major nutrient intakes can be displayed. Alternatively, it can also graph the relative importance of one nutrient relative to others through time. It also allows modelling of either one individual's nutrient intake, or that of an entire population. With primates, this methodology has been successfully applied to Peruvian spider monkeys (*Ateles chamek* - Felton et al. 2009), Chacma baboons (*Papio ursinus* - Johnson et al. 2013), mountain gorillas (*Gorilla beringei* - Rothman et al. 2011), Bornean orangutans (*Pongo pygmaeus* - Vogel et al. 2012), guereza (*Colobus guereza* - Johnson et al. 2015) and two strepsirhines, the diademed sifaka (*Propithecus diadema* - Irwin et al., 2014, 2015) and the white-footed sportive lemur (*Lepilemur leucopus* - Droscher et al. 2016).

In this chapter, we aim to quantiatively describe the seasonal feeding strategies of an exudativore, using the Javan slow loris as a model species. We examine the presence of a lean season by using the FNG to graph the energy intake between weather periods and seek to determine if gum is indeed a fallback food by analyzing its usage, -- rather than intrinsically labelling it as such -- by using food intake rates to plot nutrient intake per season. To rule out other possible theories of fallback food usage, we report the seasonal proportions of time spent feeding on all food items, as well as quantify intake and graph both protein and non-protein intake, for both sexes and weather periods to control for sex-specific changes in food habits.

4.2 MATERIALS AND METHODS

4.2.1 General Methods

The field site on this study was described in section 2.2.1 using the observation methods explained in section 2.2.3 and collected and analysed slow loris food samples based on the methods of section 2.2.4. We also collected data within our captive field site (section 2.3.1) in order to calculate gum intake rate (2.3.2). We calculated the intake rate of each food item and nutrient based on the methods described in section 2.2.5. Phenology and insect availability methods are described in section 2.2.3. To determine if food items were being eaten in proportion to their availability, we plotted the mean yearly availability score for each food type against the yearly contribution of that food source for each individual slow lorises diet (based on intake weights) as per Johnson et al. (2013). We used a Spearman's Correlation test to determine if the contribution of food items to the diet correlated with their availability.

4.2.2 Data Analyses

Right angle mixture triangles (RMT) were employed to visualize which energy source was variable and which was controlled between the seasons. The proportions of protein, total carbohydrates and crude fat energy were plotted against each other on a scatter plot in all combinations, leaving the outlier to be interpreted as the implicit axis (Raubenheimer 2011). We plotted the average daily PE versus NPE for both dry and Austral summer. We also plotted the average daily seasonal intake of protein in grams versus combination of fat plus carbohydrates for males and for females in order to determine if they adopt different strategies seasonally. For this graph only adults were used, reducing numbers to n=7 (males) and n=8 (females).

After we checked that there was no multicollinearity between the independent variables by using a linear regression analysis (VIF values between 1.000 an 1.150), we used a Generalized Linear Mixed Model (GLMM) with an identity link function and inverse Gaussian distribution of the response variable (based on visual inspection) to determine the effect of sex and season on nutrient intake and proportion of the diet food items. Nutrients we tested were crude protein, crude fat, NDF, ADF, carbohydrates, TNC, total energy and protein:non-protein energy, obtained from proportions of the various diet items (gum, nectar, fruits, insects, flowers and leaves), all using the seasonal average daily intake data in grams for each individual. Individual was used as a random factor in the GLMM. We conducted all statistical analyses in SPSS 22 (IBM Software).

4.3 RESULTS

4.3.1 Wild Slow Loris Diet

We recorded the slow lorises feeding on six plant species and various insect species (Table 4.1). Each plant species was used for one plant part whether it was gum, fruit, young leaf, etc. We only observed the mesocarp of fruits being eaten; slow lorises discarded the skin and seeds. The insects were ingested in their entirety; legs or wings were not removed. Lastly, we only noticed young leaves being eaten. The activity loggers revealed an average active cycle of 11.95 hours (SD ± 0.12), which we rounded up to 12 hours for the purpose of our average daily nutrient intake.

The average sugar content in *Calliandra* nectar was found to be 22.82 (SD ±5.12) Brix, which equates to 253 g of sugar per L of nectar, which we estimate to be 98% of DM and 22.55% as fed (AF). Average hourly intake rates for each food category under both seasons and also yearly were tabulated in Table 4.2. The average seasonal proportions of each feed category for instantaneous and intake data do not match, presenting different magnitudes of consumption (Figure 4.1). The phenology and proportion of diet correlation were not significantly correlated

(r=0.192, P=0.070, n=84), which points to an unseasonal use of these resources (Figure 4.2).

Table 4.1 Itemized free-ranging Javan slow loris diet with nutrient content where insects were the most concentrated source of macronutrients on a dry matter basis.

Species Name	Part eaten	# Months observed consumed	Crude Protein (%)	Crude Fat (%)	Ash (%)	NDF (%)	ADF (%)	Sugars (%)	TNC* (%)
Acacia decurrens	Gum	12	3.74	0.83	0.94	12.7	0.09	NA	81.79
Calliandra calothyrsus	Nectar	12	NA	NA	NA	NA	NA	98	98
Artocarpus heterophyllus	Mesocarp	1	3.80	0.35	3.14	10.26	10.26	NA	82.45
Various Insects	Whole	12	63.55	7.72	6.79	NA	14.35	NA	7.59
Gigantochloa cf. *ater*	Young Leaf	4	9.71	0.97	4.76	65.17	40.68	NA	19.39
Eucalyptus spp.	Flower	4	4.4	2.62	2.23	42.17	34.18	NA	48.58
Diospyros kaki	Mesocarp	3	8.74	0.50	3.76	26.19	24.40	NA	60.81

***** Total non-structural carbohydrates (TNC) through calculation (TNC = 100-crude protein-crude fat-ash-NDF)
NDF= neutral detergent fibre and ADF= acid detergent fibre

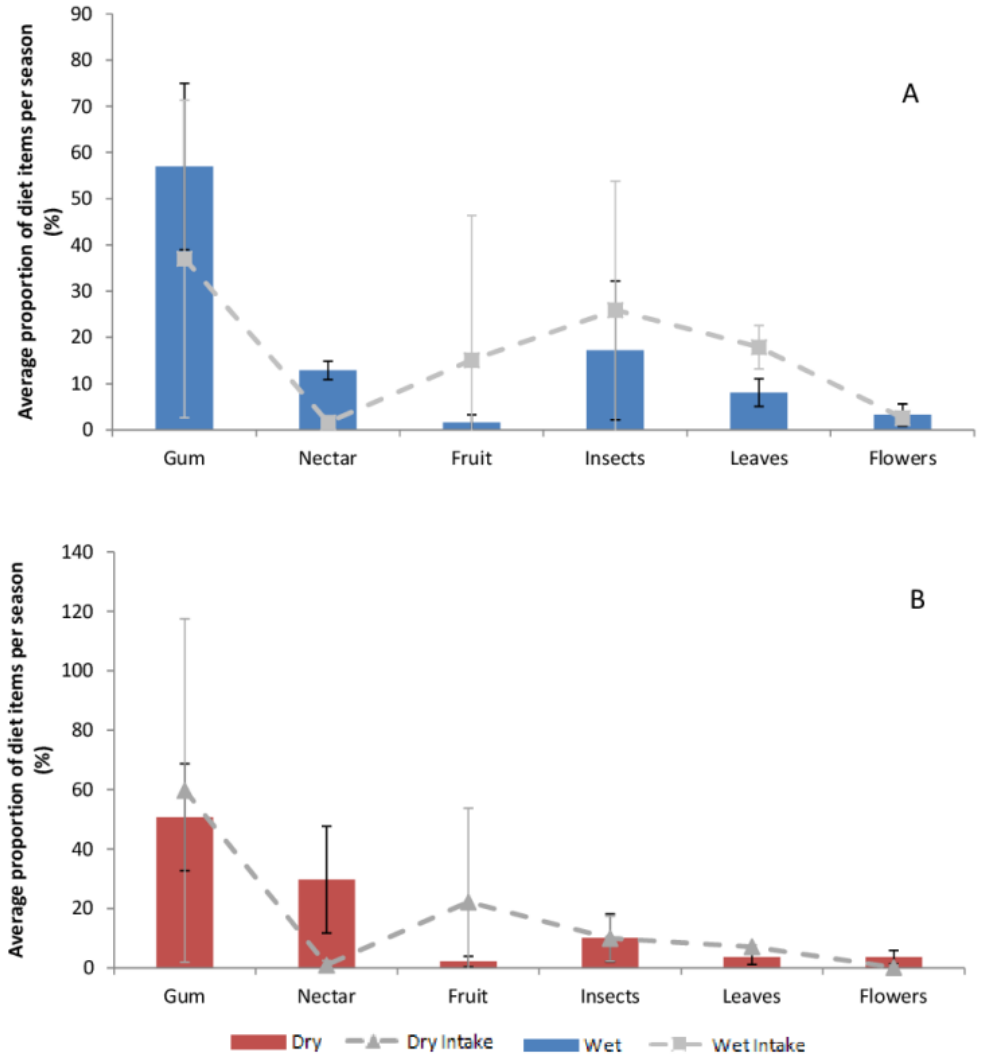


Figure 4.1 Histogram representing the averaged proportion of feeding time for each food category, as well as a broken line graph representing the weighted proportion ingested food items throughout the seasons A) Austral summer B) Austral winter.

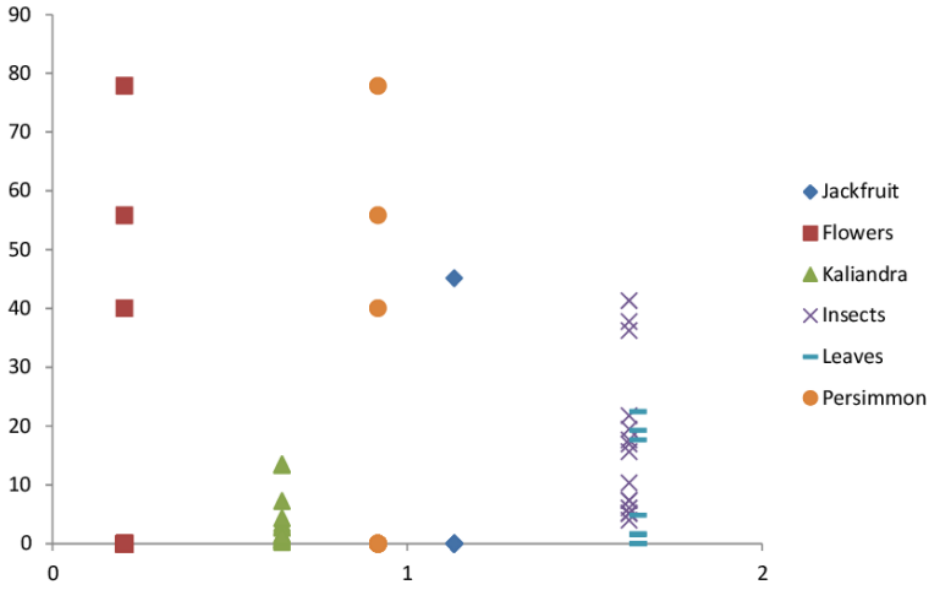


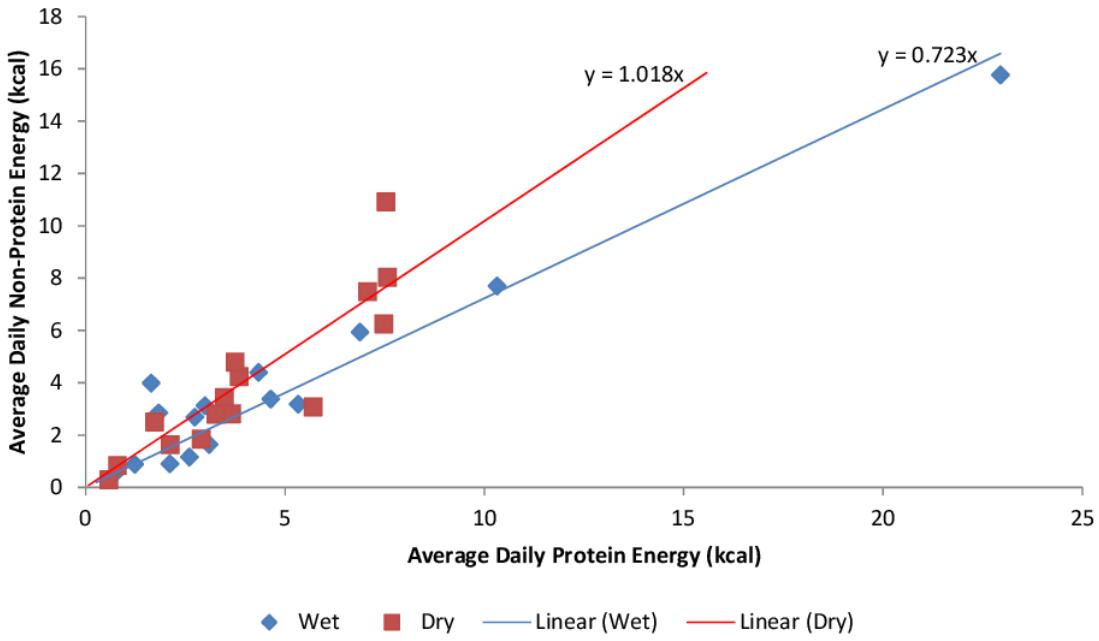
Figure 4.2 Figure 2: Scatter plot of the relationship between availability and percentage contribution of foods to the diets of the Javan slow lorises (*Nycticebus pygmaeus*). The lack of correlation suggests that foods were not eaten in proportion to their availability (Spearman's rho= 0.192, P =0.070). Austral winter was from October until April and Austral summer from mid April until October (See methods). Squares is flowers, triangles is *Calliandra* nectar, circles is persimmons, diamonds are jackfruit, Xs are insects and lines are leaves.

Table 4.2 Average hourly intake rates (g/h) of each food category during the wet, dry and yearly weather period including the proportion (%) of each food item ingested for each period of the year

	Gum	**Nectar**	**Fruits**	**Insects**	**Flowers**	**Leaves**
Yearly ± SD (g/h)	0.907 ± 0.524	0.022 ± 0.02	0.271 ± 0.521	0.34 ± 0.044	0.14 ± 0.266	0.033 ± 0.063
Proportion of Yearly Diet (%)	52.95	1.28	15.82	19.85	8.17	1.93
Austral summer (g/h)	0.709 ± 0.897	0.031 ± 0.029	0.242 ± 0.624	0.496 ± 0.32	0.343 ± 0.985	0.049 ± 0.105
Proportion of Summer Diet (%)	37.91	1.66	12.94	26.52	18.34	2.62
Austral winter (g/h)	1.105 ± 1.275	0.017 ± 0.024	0.246 ± 0.95	0.184 ± 0.057	0.131 ± 0.29	0.002 ± 0.009
Proportion of Winter Diet (%)	65.58	1.01	14.60	10.92	7.77	0.12

4.3.2 Nutrient Intake

The average daily intake ratio of protein (PE) and non-protein energy (NPE = fat + TNC+ NDF) for both the dry and Austral summers for each individual (wet n=15 dry n =15) is depicted in Figure 4.3. Non-protein energy was more important during the Austral winter with a slope of y=1.018x (R^2=0.8057), where y is NPE and x is PE, than during the Austral summer (y=0.723x, R^2=0.8374). The point with the highest protein content has no significant effect on the results of this figure as its removal alters the Austral summer ratio to y=0.793x.



The average nutrient intake values are presented in section 8.3 where they may be discussed thoroughly.

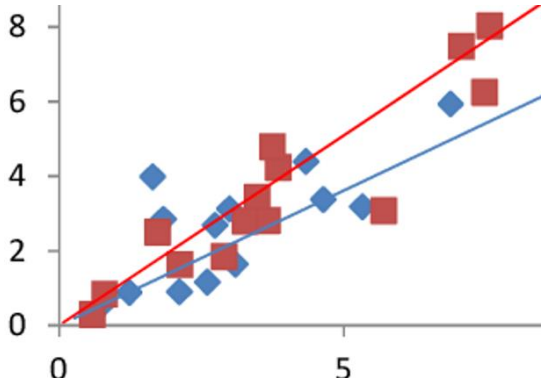


Figure 3.3 Seasonal intake of crude protein versus non-protein (crude fat and total non-structural carbohydrates and 40% NDF) energy intake, in Kcal, for each individual slow loris during the wet (diamonds) (n=15) and dry (squares) (n=15) seasons and a close up of the origin. Notice how much more important protein energy intake is during the Austral summer than the Austral winter.

We used right angle mixture triangles (RMT) to graph which macronutrient was used as a consistent and controlled energy source for the dry and Austral summers, where the implicit axes were fat (Figure 4.4A), carbohydrates (Figure 4.4B) and protein (Figure 4.4C). The proportions were tightly controlled as they demonstrated similar patterns across seasons. The proportion of fat energy was constant throughout the year (~20%). The energy from protein and carbohydrates could be used interchangeably year round. Females had a larger variation between average daily macronutrient ratio intake between seasons (dry: NPE:P = 1.7886 wet: NPE:P = 1.0612) than males which had a narrower seasonal difference (dry: NPE:P =1.5909 wet: NPE:P = 1.2165) (Figure 4.5). The removal of the "dry female" point with the highest protein value reduces the slope to y=1.372x which shows the seasonal values are different, albeit a smaller difference.

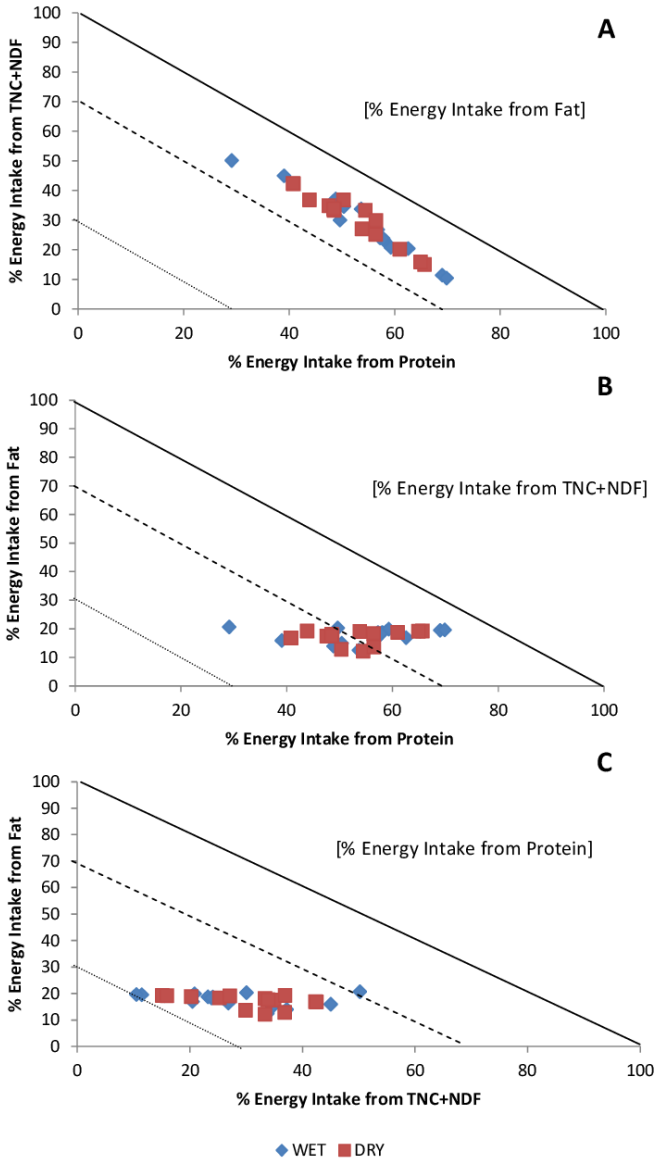


Figure 4.4 Relative contributions of crude fat, carbohydrates (TNC+ 40% NDF) and crude protein to gross energy intake (in Kcal) throughout both the dry and Austral summers using a right-angled mixture triangle (RMT). Diamonds represent the average seasonal energy intake of one individual slow loris during the Austral summer (n=15), and squares the Austral winter (n=15). Crude fat contribution is the implicit axis in A, carbohydrate contribution is the implicit axis in B and crude protein is the implicit axis in C. Carbohydrate amounts were 40% of NDF and TNC was calculated by subtracting the dry matter values of ash, crude protein, NDF and crude fat from 100. The dotted line represents the 20% implicit axis value and the dashed line represents the 60% implicit axis value.

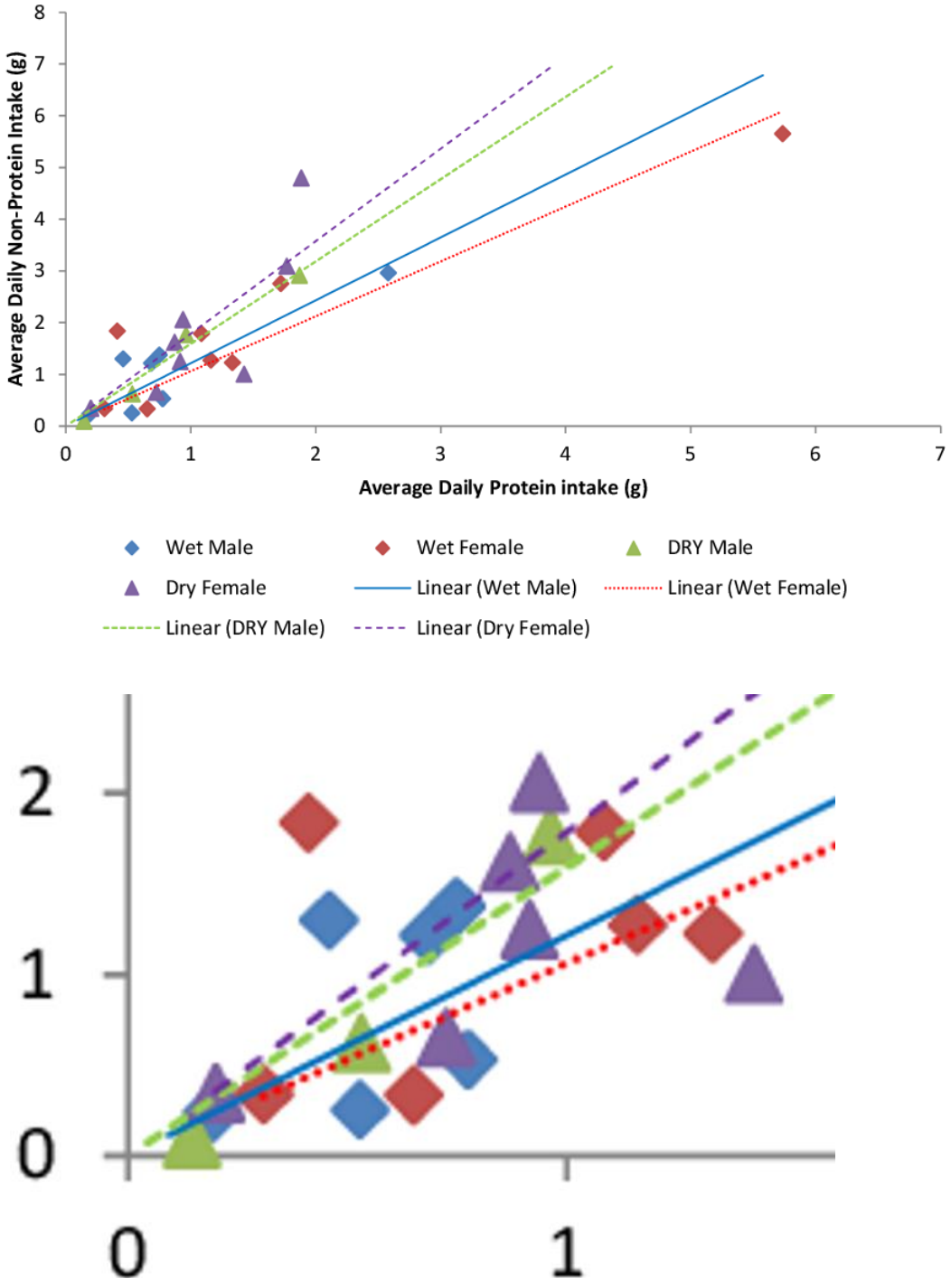


Figure 4.5 Average daily intake of crude protein and non-protein (crude fat+ TNC + NDF) for both the dry (n=15) and Austral summer (n=15) for males (n=7) and females (n=8) including a close up of the origin.

The average weight of adult female Javan slow lorises during the Austral summer was 930.07 g (± 71.28) versus 844.31 g (± 97.46) for the Austral winter

(n.s.). The male's weight was 898.05 g ± 74.01 in the Austral summer, and 887 g ± 80.93 in the Austral winter (n.s.). Overall average weight for our study individuals over 3 years of data was 900.47 g (± 83.46).

The results of the GLMM show that females had a higher intake of crude protein (B=+0.314 SE= 0.7400), gum (B= +15.953 SE= 37.5419), fruit (B= +4.875 SE= 20.0763) and insects (B= +20.081 SE= 17.3900) and males had a higher intake of fiber (ADF) (B= -0.840 SE= 0.4437); season had a significant effect on ADF (B= -1.328 SE=0.4917), gum (B= +18.493SE= 38.8800), insects (B= +25.933 SE= 19.2735), flowers (B= -36.145 SE= 16.25) and leaf (B= 46.45 SE= 13.94) consumption. The interaction between sex and season was significant for ADF (B= +0.994 SE= 0.305), flowers (B= +27.88 SE= 10.0839) and leaves (B= -19.999 SE= 8.6488) (Table 4.3).

Table 4.3 Generalised linear mixed model results of the main effects of sex and season on the average daily nutrient intake of each individual (n=15) and food type within the diet.

	Overall Model			Sex			Season			Sex*Season		
	X^2	df	P	X^2	df	P	X^2	df	P	X^2	df	P
Crude Protein	**74.150**	**16**	**0.000**	**149.104**	**13**	**0.000**	.317	1	0.583	0.394	1	0.541
Crude Fat	23.888	16	0.092	**32.410**	**13**	**0.002**	.406	1	0.524	0.823	1	0.364
CHO	20.826	16	0.185	**25.820**	**13**	**0.018**	1.060	1	0.322	0.565	1	0.466
NSC	21.751	16	0.151	**26.325**	**13**	**0.015**	1.272	1	0.280	0.976	1	0.341
NDF	24.871	16	0.072	**35.300**	**13**	**0.001**	.188	1	0.672	0.242	1	0.631
ADF	**41.272**	**16**	**0.001**	**71.423**	**13**	**0.000**	**7.291**	**1**	**0.007**	**10.616**	**1**	**0.001**
NP:P	26.142	16	0.052	**34.580**	**13**	**0.001**	**15.393**	**1**	**0.000**	2.787	1	0.095
NPE:PE	26.070	16	0.053	**163.736**	**13**	**0.000**	**13.291**	**1**	**0.000**	2.022	1	0.155
Energy	19.264	16	0.255	0.809	13	0.646	.624	1	0.444	0.285	1	0.602
Gum	**74.152**	**16**	**0.000**	**149.104**	**13**	**0.000**	**9.351**	**1**	**0.002**	2.594	1	0.107
Nectar	21.132	16	0.174	**26.061**	**13**	**0.017**	1.010	1	0.315	0.183	1	0.669
Fruit	**31.794**	**16**	**0.011**	**55.636**	**13**	**0.000**	.104	1	0.753	0.216	1	0.650
Insects	**33.656**	**16**	**0.006**	**28.871**	**13**	**0.007**	**14.041**	**1**	**0.002**	0.088	1	0.771
Flowers	**29.790**	**16**	**0.019**	1.206	13	0.370	**4.942**	**1**	**0.026**	**7.644**	**1**	**0.006**
Leaves	**30.720**	**16**	**0.015**	0.992	13	0.506	**11.095**	**1**	**0.001**	**5.347**	**1**	**0.021**

Bold values indicate a significant P value. Definitions are: CHO=carbohydrates, NSC=non-structural carbohydrates, NDF=neutral detergent fibre, ADF=acid detergent fibre, NP:P=Non-protein to protein intake ratio within the diet, NPE:P=non-protein energy to protein energy intake ratios.

4.4 DISCUSSION

4.4.1 Characterizing the Diet of the Javan Slow Loris

Our data indicate that the Javan slow loris consumes a narrow range of food items found within the study area (Table 4.1). As a note of caution, we acknowledge that the nature of our field site, with crops and planted trees and bushes with economic value, may provide different sources of food and nutrients from Javan slow lorises found in primary forests. Plant diversity, nutrient intake and phenology could vary significantly, potentially resulting in different results than observed here. However, since our studied slow loris population is breeding regularly and thus is likely to receive an adequate nutrition we think that our results represent a valid contribution for future comparisons with other *Nycticebus* studies. Although the field site was largely affected by anthropogenic disturbances, our results may be indicative of slow loris evolutionary adaptations rather than a mere result of disturbance. For example, fruit and other food items (flowers and leaves) were available year round and yet the individuals chose to ingest gum as a majority food item. All other studied slow loris species (studied in secondary or primary forests) also reported an exudate and insect based natural diet (Starr and Nekaris 2011; Das et al. 2015; Wiens et al. 2006). Future studies in secondary or primary forest areas are necessary to obtain more robust conclusions in support of the observed trend.

As expected, gum was the staple food of this exudativorous primate ranging from 38 to 60% of diet intake, being exploited in both the wet and Austral winter (Figure 4.1). The slow loris exhibited the same gouging feeding behaviors as described in Nekaris (2014), which is similar to all *Nycticebus* taxa studied thus far. The slow lorises would anchor their lower mandible into the cambium and use the upper maxilla to bite into the tree and remove pieces of cambium. This process is repeated until the desired size and depth has been reached to stimulate gum production. Along with gum, we also recorded insect feeding during the majority

of observation periods. Nectar from the *Calliandra calothyrsus* flower was seasonally consumed, as the flowers mostly bloom during the Austral summer, although a small amount was also present in the diet during the Austral winter.

We observed the slow lorises ingesting a variety of plant parts rarely reported as part of the slow loris diet. They targeted the young leaves of only one of three bamboo species available, *Gigantochloa* cf. *ater* as well as the flower of *Eucalyptus* spp*;* both of which had high levels of NDF (> 40 %). Following the Jarman-Bell rule (Gaulin, 1979), the larger size of *N. javanicus* would allow it to subsist on a diet which contains more fermentable food items when compared to the smaller *N. pygmaeus,* which has a higher amount of insects within its diet (Starr and Nekaris 2013). The total amount of fermentable plant food items (gum, leaves, flowers) in this study is still lower in proportion than that reported for the largest *N. bengalensis*, which theoretically should have the largest fermentation capacity of the slow lorises due to its large size (Das et al. 2014). With an average weight of 844 to 930 g, *N. javanicus* is estimated to have the similar fermentation capabilities as the 1 kg white-footed sportive lemur (*Lepilemur leucopus*), which is entirely folivorous (Droscher et al. 2016). However, differences in gut microflora, surface area and presence of diverticula will impact fiber digestibility. A complete diet necessitating fermentation is predicted to only be energetically sustainable for a primate whose mass is greater than 700 g (Kay 1984). Javan slow lorises are at the cusp of reaching the metabolic weight where it would be very difficult for them to consume enough insects to meet their energetic requirements (Rothman et al. 2014). Both Kay (1984) and Rothman et al. (2014) do not factor in the reduced metabolic rates of strepsirrhine primates, that at this weight, may potentially allow them to flow between a more fermentable diet or a more insect based diet to meet their needs. This is consistent with our observations, as the intake of insects ranged from 12 % of intake during the Austral winter to 27 % during the Austral summer.

Lastly, we observed fruit feeding quite rarely within our field site. Only two fruits were ingested, the domesticated persimmon (*D. kaki)* and jackfruit (*A.*

heterophyllus). Jackfruit was available all year long but we only observed one instance of feeding, and persimmon was heavily abundant between late Austral winters until early Austral summer, yet we only saw four feeding bouts of this food item (Figure 4.2). Indeed the data further reinforce the current body of evidence that *Nycticebus* are not frugivores. Although gum is overall a low quality food item and may fit the description of a fallback food for many species (see Smith, 2010), it comprises the majority of *N. javanicus* diet even when other food items were available, providing evidence that gum is an obligate food source for the Javan slow loris. Being the only venomous primate, slow lorises are hypothesized to ingest specific compounds from one or more of its food items and metabolically alter it to result in venom (Nekaris et al. 2013). Gum or noxious insects may potentially contain the necessary compound(s), perhaps making gum even more important than solely for nutrition. Future ad-hoc analyses are necessary to clarify this aspect.

4.4.2 Seasonal Feeding Ecology

The Javan slow lorises employed two different nutritional strategies between the abundant Austral summer where protein energy was easier to obtain, and leaner Austral winter that necessitated a more important non-protein energy income. The varying seasonal availability of food items such as leaves, flowers, nectar and insects meant that the slow lorises had to alter their feeding intake in order to balance their needs between seasons (Figure 4.3). Although energy amounts may seem low relative to average daily consumption, Figure 3 corresponds to intake ratios, similar to Johnson et al. (2015), who also observed some low intake amounts and did not control for outliers. The Austral winter has lower food abundance than the Austral summer; however it was not as drastic as lean seasons described for other primates since it allowed for the slow lorises to maintain their overall energy intake (Curtis 2004; Felton et al. 2009; Rothman et al. 2006). During the Austral winter, NPE:PE shows a shift towards non-protein

energy, whereas during the Austral summer, protein energy becomes favored. The Austral summer has an abundance of all food types, which would allow the free ranging slow lorises to select their intake from a larger variety of food items. The main source of protein for this population of *N. javanicus* is insects, whose ingested amounts are significantly affected by season, i.e. much higher in the Austral summer (Table 4.3). Feeding on insects during the Austral summers apparently led to both a relatively constant proportion of energy from fat and a higher protein intake than during the Austral winter, when energy needs drove a higher carbohydrate (gum) intake. High NPE foods such as nectar, flowers and leaves, whose average daily-ingested amounts were also affected by season, are also exploited more during the Austral summer.

Diet switching is a strategy used by slow lorises as a response to variations in food availability, also observed by the generalist *Propithecus* (Pichon and Simmen 2015; Sato et al. 2015). *Eulemur* spp*.,* as fruit specialists, did not employ diet switching, but instead resorted to a cathemeral lifestyle and increased total foraging time (Sato et al. 2015). This option is not available to the nocturnal slow loris, as our data-loggers indicate an essential lack of activity during the day. Mountain gorillas (*Gorilla beringei)* over-eat protein to meet carbohydrate energy requirements, which is a strategy that would not apply for slow lorises considering their main food items are exclusively high in either carbohydrates (gum) or in both fat and protein (insects) (Rothman et al. 2011). The overall strategy of diet switching, however, is still possible. Exudativorous primates such as the slow loris should be able to fine tune their nutrient intakes throughout the seasons by balancing the intake of different food items, which is supported by our data. *Nycticebus javanicus* were thus able to manage their nutrient intake during temporary shifts of availability using nutritional strategies similar to generalist leaf-eating primates.

The analysis of the proportion of energy ingested from fat, carbohydrates (TNC and 40% of NDF) and protein in a right-angle mixture triangle depicted a

tightly controlled response to seasonal food availability (Raubenheimer et al. 2015). Fat energy intake is relatively constant throughout the year, while protein and carbohydrates are used somewhat interchangeably without regards for seasonal food availability (Figure 4.4). This is different than what has been observed in *Colobus guereza* where carbohydrates and fats are used interchangeably, both of which are the major energy providing macronutrients (Johnson et al. 2013). Chimpanzees exhibit a trend similar to slow lorises, decreasing protein during the lean season, substituting it with carbohydrate energy and also being able to adjust between nutrient sources (Conklin-Brittain et al., 1998). The main source of fat for the slow lorises is insects, and the stable and relatively low proportion of fat energy of the diet suggests the slow lorises are controlling their intake of this nutrient. The main source of carbohydrates was from gum, which must be consumed as the main source of available energy. The intake of gum and insects seem to be finely balanced throughout the year. The young leaf specialist *Avahi meridionalis* branched out into broader folivory during the lean season, selecting from a large array of lower quality leaves. During this time, they must cope with higher fiber and lower protein intakes, also increasing their structural carbohydrate intake during their lean season (Norscia et al. 2011). Gum intake in lorises was significantly affected by season, which supports these observations (Table 4.3). Although gum is available year round, reliance on it as a food item and source of energy increases during the Austral winter, hence, NPE energy intake is more prominent during the Austral winter. The variation between energy proportions of this triangle mixture may be largely due to the seasonal intake of the nutrient dense insects (also significantly affected by season in the GLMM). The slow lorises' Austral winter is limited in Java, and prioritized by insect consumption to ensure minimum protein and fat intake. During the abundant Austral summer, insects are easily available and their intake balanced alongside flowers, leaves and gum, which may explain variations observed in total NPE:PE ratios. The frugivorous atelines preferred to over-eat food items, therefore over-consuming carbohydrates and fat in

order to meet their protein requirements (Felton et al. 2009), whereas *N. javanicus* can interchangeably use the proportions of foods eaten in order to ensure minimal requirements. Further evidence that *Nycticebus* spp. are employing a mix of strategies to cope with varying seasonal food availability are necessary; however their strategies are more akin to folivores than frugivores.

4.4.3 The Effect of Sex on Nutrient Selection

Our hypothesis of similar nutrient intake between sexes was not supported by our data, where the average daily intake of macronutrients showed some seasonal variation for males, while there is a drastic shift observed for females when compared to males (Figure 4.5). Males and females form pairs where both individuals have their own superimposed territories; hence, it would appear that the seasonal abundances within each individual's territory can explain some amount of these differences or we would have observed similar patterns amongst the sexes from varying territories (Nekaris 2014). Slow lorises are not sexually dimorphic and live under similar abiotic conditions; therefore we might expect their foraging strategies to be identical or at least similar (O'Mara and Hickey 2014). Either the females require a higher protein intake during the Austral summer, where high insect availability may coincides with gestation/lactation, or the females must focus on a higher energy diet to build fat deposits to provide stored energy for gestation/lactation, perhaps even overeating as observed in sifakas (Irwin et al. 2015). Energy and protein demands of mammalian females increase during reproductive events such as gestation and lactation (Jessop 1997). The average weight of a female *N. javanicus* during the Austral summer is roughly 85 g heavier than during the Austral winter compared to an 11 g difference for the males. Based on our anecdotal first sightings of females with offspring, gestating females were more predominant in wet compared to Austral winters. This is consistent with a female strategy of increased nutrient intake related to seasonal reproduction. Female gorillas also ingest more protein per unit metabolic weight when compared

to males; however this was due to an overall higher food intake strategy, rather than a better food quality selection (Rothman et al. 2008). There would be an evolutionary advantage to having juveniles weaned by the abundant Austral summer when they need to be foraging solely, making the Austral winter to early Austral summer more energetically expensive for reproductively-active females, possibly explaining the lower weights observed (Narconk et al. 2009). This is observed with sexually monomorphic *Lemur catta* and *Lepilemur ruficaudatus,* which gestate and begin to lactate during the Austral winter (Ganzhorn 2002; Gould et al. 2011; Sauther 1998). Within the Lorisiformes thus far, seasonal weight gain and loss have been recorded in *N. pygmaeus,* with changes sometimes as extreme as 25 % of body weight (Streicher et al. 2012). A marked seasonal variation in weight is also thought to be widespread amongst the Malagasy strepsirhines (Simmen et al. 2003, 2010). The ratio of male NPE:PE intakes does not vary seasonally, which may indicate that the main source of protein, insects, are still available in large enough quantities to meet their requirements. Male and female *N. javanicus* may have different requirements, probably driven by reproductive needs. Both males and females of *L. leucopus* did not have different seasonal intake amounts of macronutrients or energy, as reproduction was not their main constraint, but instead had to cope with thermoregulation (Droscher et al. 2016). Our population of slow lorises had seasonal variations in intake due to food availability with reproductive events exaggerating the seasonal variation in females.

Crude protein, ADF, gum fruit and insect intakes were all shown to be significantly affected by sex of the slow loris (Table 4.3). Although males and females are comparable in mass, the energetic costs of reproduction may place a burden, which requires a larger demand for females, as shown by O'Mara and Hickey (2014) where female *L. catta* became much more efficient and selective during periods of lactation (and higher energy/nutrient needs). Acid detergent fibers are composed mainly of lignin and cellulose found in all plant food items

except gum. Females increase their protein intake during the Austral summer, though insects as a larger proportion of their diet for that season, while male intake is only slightly altered. While the response of a male may vary, female strepsirrhines in general tend to increase their protein intake seasonally more so than males (Gould et al. 1999; Gould et al. 2011; LaFleur and Gould 2009; Meyers and Wright 1993; Overdorff 1993; Rasamimanana and Rafidinarivo 1993; Sauther, 1994, 1998; Vasey 2002). Fruit intake was similar between seasons yet we observed females consuming more fruit than males (4 bouts versus 1), possibly due to their higher energetic needs, especially during the Austral winter. They increase NPE intake by increasing gum intake, of which a larger amount was ingested during the Austral winter. Although not ingested in particularly large amounts, the seasonal intake of flowers and leaves may also be a significant source of fermentable fiber energy for females, as both, along with ADF, were affected by an interaction between sex and season. Although small overall amounts, we cannot dismiss them as unimportant. Being a potentially available source of energy, leaves, flowers, and gums may have provided essential energy and fiber as well as other micronutrients not measured here (i.e. vitamins, minerals). The results from the GLMM were all supported by our data and observations.

The exudativorous *N. javanicus* seems to follow a foraging strategy more closely resembling a generalist folivore, rather than a frugivore. At our field site, the lean season was characterized by a qualitative decrease of food items (flowers, leaves, insects and nectar) (Ganzhorn 1992). Generalist frugivores should preferentially ingest fruit when it is available, yet broaden their range of food items ingested during the lean season (Norscia et al. 2006). Such diets are expected to result in an overall decrease in protein and digestible carbohydrate intake during the lean season, with recovery in the following season, possibly causing seasonal fattening, with food choices based on nutritional composition.

4.5 CONCLUSION

Slow lorises were able to meticulously manage their nutrient intakes and maintain constant contributions of fat energy while using carbohydrate and protein interchangeably. NPE was prioritized during the Austral winter probably due to higher gum intake, similar to the energy and protein maximization rule described in Altmann (2006). Leaf eating primates will prioritize protein only if protein is limiting in their environment (Ganzhorn et al. 2016). The only significant source of protein for our slow lorises was insects, with their availability decreasing in the lean season. The slow lorises were able to continue ingesting protein to meet their requirements. Their protein intake became more consistent in its proportion with crude fat and carbohydrates when protein was difficult to find. This behavior is also consistent with the behavior of a generalist leaf eating primate. The slow lorises were able to control their NPE:PE as thoroughly as some generalist species (Johnson et al. 2012, 2015), which is consistent with slow lorises following an energy and protein maximization strategy.

This chapter enabled us to calculate the nutrient intake of free ranging slow lorises to use in our future captive diet trials. However, we must still know how energy is expended within the slow lorises, especially between the sexes.

CHAPTER V

Do males and females expend a similar amount of energy throughout the year? How does their energy expenditure affect their nutrient selection?

or

"GET TO WORK: SEASONAL DIFFERENCES IN TOTAL ENERGY EXPENDITURE BETWEEN THE SEXES IS LINKED TO REPRODUCTIVE COSTS"

5.1 INTRODUCTION

5.1.2 Strategies for Coping with Energetically Difficult Periods

Animals must balance their energy intake versus energy expenditure by modifying their activity budgets to compensate for seasonal shortages of food, temperature and day length fluctuations, humidity, breeding and infant care costs and territory and mate guarding (Chaves et al. 2011; Erket and Kappeler 2004; Kobbe et al. 2014; Mojolo et al. 2013; Porter and Garber 2012; Reinhardt et al. 2016; Rode and Nekaris 2014). Energy budgets are becoming more common when testing hypotheses about assessing the benefits and costs of particular behaviours (Miller et al. 2006). The cost of performing certain behaviours across taxa may not be equivalent, which is why species- or group-specific basal metabolic rate (BMR) equations are used to estimate the total energy expenditure (TEE) when assessing energy budgets of free ranging animals (McNab 1988; Leonard and Robertson 1997). BMR measures the resting energetic costs of an animal under thermoneutral conditions (Thompson et al. 1994). This resting metabolic rate increases allometrically, however when compared to species-specific experiments rather than general equations, large variations are sometimes observed (Genoud et al. 1997). This variation can be explained by specific conditions related to the natural history of the species such as sex, trophic level and diet, body composition, body temperature, socioecology, nocturnal habits, etc. (Raichlen et al. 2009).

Some species have adaptations specifically aimed at reducing their BMR and coincidentally, their TEE, during either periods of low food availability or heightened metabolic costs which are not necessarily apparent during metabolic studies (see Dausmann 2014); such adaptations have been shown to significantly reduce TEE (Schmid 1998). Behavioural adaptations such as increased resting time (Knott 1998; Levine et al. 2000) or selecting different sleeping sites (Schmid 1998) have also been shown to be effective in some situations at reducing overall energy spent during trying times. Daily torpor and hibernation are well-studied energy-saving strategies in primates and ursids (Geise 2004, Heldmaier et al. 2004; Reinhardt et al. 2016; Ruf et al. 2015; Dausmann, 2014). Hibernation is defined as suspended thermoregulation over a long period of time and is known for ursids and

other mammals and birds, yet only known for two primate families: the Cheirogaleidae (see Dausman 2014 for a review) and Lorisidae (Ruf et al. 2015). Others primate species only enter a state of torpor when extreme climate conditions prompt them to such as *Galago moholi* (Nowack et al. 2013).

Another effective behavioural adaptation to reduce TEE includes modulating the daily path length. The contribution of travel behaviours to energetic costs can be measured by calculating TEE across seasons.

5.1.2 Methods Used to Estimate TEE

Estimating TEE in animals has led to three validated methods being used: doubly labeled water (Speakman 1998), body motion vigor analysis (Shepard et al. 2009) and time-energy budget (Goldstein 1988). A more precise but also invasive method of measuring energy expenditure is the doubly labeled water technique (DLW), which requires the injection of oxygen and hydrogen isotopes into an animal followed by an immediate body water sample, then again after a chosen time period a follow up sample is taken. The amount of excess oxygen out flow, equal to carbon dioxide production can then be measured as a representative for energy expenditure (Speakman 1998; Xiao et al. 2010). Although considered accurate, this method is not feasible for a number of species due to logistical and/or ethical constraints. After comparing the two methods in wood mice (*Apodemus sylvaticus*) and loggerhead shrikes (*Lanius ludovicianus*), the time-energy budget underestimated the TEE when compared to the DLW values (Corp et al. 1999; Weathers et al. 1984). The analysis of body motion vigor has been used as an accurate estimate of energy expenditure for certain behaviours in magellanic penguins (*Spheniscus magellanicus*), lemon sharks (*Negaprion brevirostris*), leatherback turtles (*Dermochelys coriacea*), whale sharks (*Rhincodon typus*) and imperial shags (*Phalacrocorax atriceps*) (Shepard et al. 2009). This method is reserved for aquatic animals as the resistance offered by the water is a key variable in the equations used. Lastly, constructing a time -energy budget is regularly used for TEE research with birds and mammals (Goldstein 1988; Knott 1998). This

method requires a great knowledge of the animal's behaviour and physiology, while also making a number of assumptions including summation of energetic costs of various behaviours over the course of a day. An even more detailed study can be developed when one of the above mentioned TEE methods considers the energy intake of a species. Although possibly widening the margin of error, this comparison can illustrate how species mediate energetically expensive life stages (pregnancy, lactation), seasons (colder or dryer seasons), or periods of low food availability (Houston and McNamara 2014) as well as differential responses between sexes in similar conditions (Key and Ross 1999). By using a combination of methods, differences between males and females within the same social groups have been discovered (Gilbert et al. 2007). Especially for primates, where males and females often have different metabolic constraints and possess large behavioural repertoires, this is often an important method in uncovering coping strategies (Knott 1998).

5.1.3 Energy Saving Adaptations of the Slow Loris

Slow lorises (*Nycticebus* spp.) have long been described as having a host of adaptations that heavily reduces their BMR and TEE (Nekaris 2014). For example, the pygmy slow loris (*N. pygmaeus*) has a BMR 52 % of the generic Kleiber equation developed for placental mammals, while *N. coucang* has a BMR of 40 % of the Kleiber equation (Kleiber 1961; Muller 1979; Whittow et al. 1977; Xiao et al. 2010). The body temperature of *N. coucang* is 1-2 degrees lower than the haplorhine average (Muller 1985). There is also a significant difference between slow loris' core body and surface temperatures which can differ up to 11°C lower than the core average of 35.5°C (Muller, 1985). Vascular bundles in slow lorises, termed "retia merabilia", allow vice-like grips of the limbs, resulting in less energy expenditure (Muller et al. 1984). The coat of *Nycticebus* is also well insulated for a tropical animal (Muller 1979). This suite of adaptations aimed at reducing energy expenditure should allow slow lorises to survive in spite of

energetic constraints year round. Despite this realisation, there are some possible inconsistencies due to the numerous taxonomic changes amongst *Nycticebus* spp. and past species identification. The study subjects of Muller (1979) may not have been *N. coucang* but actually *N. bengalensis.* Because *N. bengalensis* is the largest slow loris species, with a body weight range of 1050 to 2010 g rather than 650 to 850 g for *N. coucang*, misidentification may explain why the larger species has a lower metabolic rate per body weight when compared to the smaller species. The Javan slow loris (*N. javanicus)* is the second largest slow loris species, for which no metabolic studies have been conducted. To further understand the constraints placed upon slow lorises by their seasonal environment and reproductive costs, this chapter aims to compare TEE of male and female free-ranging *N. javanicus*, to understand the significant reproductive investments made by females versus males by using the time-energy budget method. Like other primates, slow lorises should fine-tune their time allocation and travelling behaviours to their energetic needs and constraints, such as food availability and climate (Grueter et al. 2013; Reinhardt et al. 2016). Theoretically, a primate should only increase its path length for high reward food resources, resulting in a net gain or decreasing deficits, with females facing reproductive pressures perhaps having a different strategy than males. Other less costly behaviours such as resting should be observed for larger proportions of the active period if energetic constraints are observed.

5.2 MATERIALS AND METHODS

5.2.1 General Methods

The field site used is described in section 2.2.1 using the observation methods of section 2.2.3.

5.2.2 Daily Path Length

In order to calculate TEE, we calculated the average minimum linear nightly path length for each individual for each night of observation. Once converted the GPS points into UTM coordinates, we used the Pythagorean Theorem (Equation 5.1) in Excel 2011 to calculate the straight-line distance between each point collected throughout the night (Suarez 2006).

(Equation 5.1) $A^2 + B^2 = C^2$

Where A is the distance between the northing and easting of the first GPS point, B is the distance between the northing and easting of the next consecutive point and C is the distance between the first GPS point and the next consecutive point, representing the distance traveled between two locations. Then we calculated the average linear distance traveled per hour, and multiplied it by the average number of active hours for males and females (results of activity loggers) to produce a total nightly distance travelled. This calculation method does not account for vertical movement between canopy levels or back and forth movements during the 15-minute sample interval periods, since it only calculates linear distances between GPS points. We have utilized this method to estimate only the minimum nightly path length of the Javan slow loris, thus rendering it still appropriate method to use in our minimimum TEE estimations (Alba-Meija et al. 2013).

5.2.3 Total Energy Expenditure

We calculated the total energy spent for each individual slow loris. The BMR equation used for slow lorises (Equation 5.2) was a modification of Kleiber (1961), reduced by 60% as per Muller (1979) and Whittow et al. (1977) where BMR is the amount of energy in Kcal per day and W is body mass in kilograms.

(Equation 5.2) $BMR = 42W^{0.75}$

Following Coelho (1974, 1986), the TEE was calculated by the sum of the energetic costs for each individual activity performed by the slow lorises. Equation

5.3 describes how to calculate the energetic costs of an individual activity where A was the energetic cost (kcal) of individual activity *i*, T was the percentage of the day spent performing activity *i* and D was the energy constant for each activity. The energy constants for various activities are outlined in Table 5.1. Eating and grooming were allocated the same energy constant by Miller et al. (2006).

(Equation 5.3) $A_i = (D_i \text{ X } BMR \text{ X } T_i)100$

Table 5.1 Energy constants used in the TEE equations to estimate how much energy is spent during each behaviour.

Activity/Behaviour	Energetic constant *D*
Inactive (sleep)	1.0
Stationary rest	1.25
Eating/grooming	1.38
Social	2.35

Locomotion energy costs depended upon speed and distance travelled. We use the equation of Taylor et al. (1970) and Key and Ross (1999) to determine the energetic cost of locomotion of slow lorises (Equation 5.4).

(Equation 5.4) $A_{loc}=0.40[(0.041 \text{ X } W^{0.60})R_D + (0.029 \text{ X } W^{0.75})T_{loc}]$

Where W was body mass in grams, R_D was the day range in km and T_{loc} was the time spent moving in hours. The energetic costs of locomotion were not significantly different between taxa according to Taylor (1980), thus we use this as our best estimate.

To incorporate the estimated energetic costs of gestation and lactation we follow Key and Ross (1997), however modify its use. This original equation was used to estimate costs of reproduction year in general using published values (Equation 5.5).

(Equation 5.5) $E_r = (TEE((T_{gest} \text{ X } 1.25) + (T_{lact} \text{ X } 1.5)+(T_{ibi}))/180$

Where E_r was the energetic costs associated with reproduction (gestation and lactation), T_{gest} was the length of gestation in days, T_{lact} was the length of lactation in days and T_{ibi} was the interbirth interval in days where no gestating or lactating is taking place. We used the maximum values from Izard et al. (1988) and Zimmerman (1989) where T_{gest} = 192, T_{lact} = 183 and T_{ibi} = 478 d, values of each slow loris were calculated individually. We used the actual number of days within our year of observation where our females had young babies as T_{lact} and the days prior to first sighting of the baby as T_{gest}, with a maximum of 183 and 192 days respectively. Within our seven observed adult females, six were observed with a baby within our study period. One was first observed with her baby in August 2014, three in September 2014, one in January 2015 and one in March 2015.

5.2.4 Statistical Analyses

We used two Generalized Linear Mixed Model (GLMM) analyses to determine if the fixed factors sex and season had main effects on 1) the average monthly proportion of each behaviour for each individual and 2) on the average daily path length for each individual for each month. None of the dependant variables were normally distributed and instead we observed an Inverse-Gaussian distribution for the behaviours and a gamma distribution for the daily path lengths.

5.3 RESULTS

5.3.1 Activity Budgets per Sex and Season

Free-ranging *N. javanicus* were observed for a total of 256 days over the course of 12 months for 1470 hours (5.8 hours/night average), totaling 7191 instantaneous points of data. Although not significantly different between sexes, we observed males being stationary, grooming and performing social behaviours more often in the Austral winter (Figure 5.1A), and females were also more social and stationary during the Austral winter (Figure 5.2A). The GLMM revealed a significant main effect of season on overall behaviour (B=4.467, df=5, P=0.001)

and specifically a larger proportion of time spent foraging during the Austral summer (B=7.165, df =1 P=0.008) with no other factors having a significant effect. Stationary was observed proportionally more during the Austral winter (B=15.533, df=1, P=0.0001) and foraging more in the Austral summer (B=7.165, df=1, P=0.008).

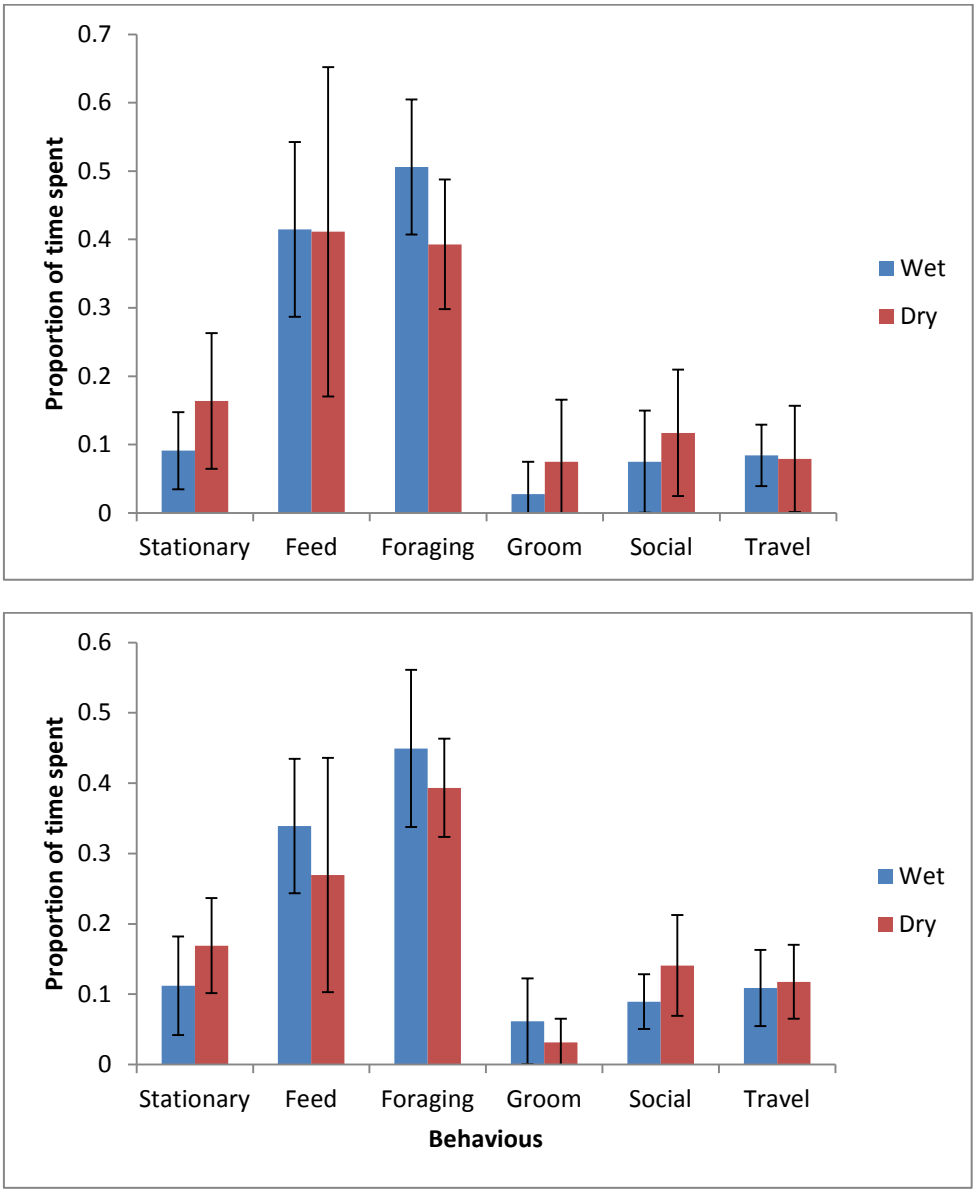


Figure 5.1 - Average activity budget for male (A) and female (B) slow lorises during the wet and Austral winters showing an increase in stationary and decrease in foraging behaviours during the Austral winter.

5.3.2 Daily Path Lengths

Males and females had similar minimum average nightly path lengths of 257 m/night (SD ± 239) n=296 and 249 m/night (SD ± 309) n=334, for males and females respectively. Males varied between 290 m/night (± 166) during the Austral summer and 245 m/night (± 103) during the Austral winter. Females varied between 326 m/night (± 271) during the Austral winter and 207 m/night (± 39) during the Austral summer. The overall model was significant (X^2=10.009 df=3 P=0.018) and the only significant fixed effect was the interaction between sex and

season (X^2=4.860, df=1, P=0.027). Specifically, the average path length of females was longer in the Austral summer than in the Austral winter by 125.83 (P=0.024).

5.3.3 Total Energy Expenditure

The average daily energy expenditure of males was 68.64 (±26.23) Kcal during the Austral summer and 52.47 (±2.58) Kcal during the Austral winter (n=5 each). Females spent 60.81 (±5.31) Kcal during the Austral summer and 57.96 (±5.47) Kcal during the Austral winter (n=8 each). After adding the estimated reproductive costs of females, the average increased to 74.48 (±2.91) kcal during the Austral summer and 70.83 (±3.47) kcal during the Austral winter (n=6 each) which is an average increase of 28%. The most important behaviors in terms of energy expenditure (besides sleeping which was calculated as 12 hours daily), were foraging and travelling (Figure 2).

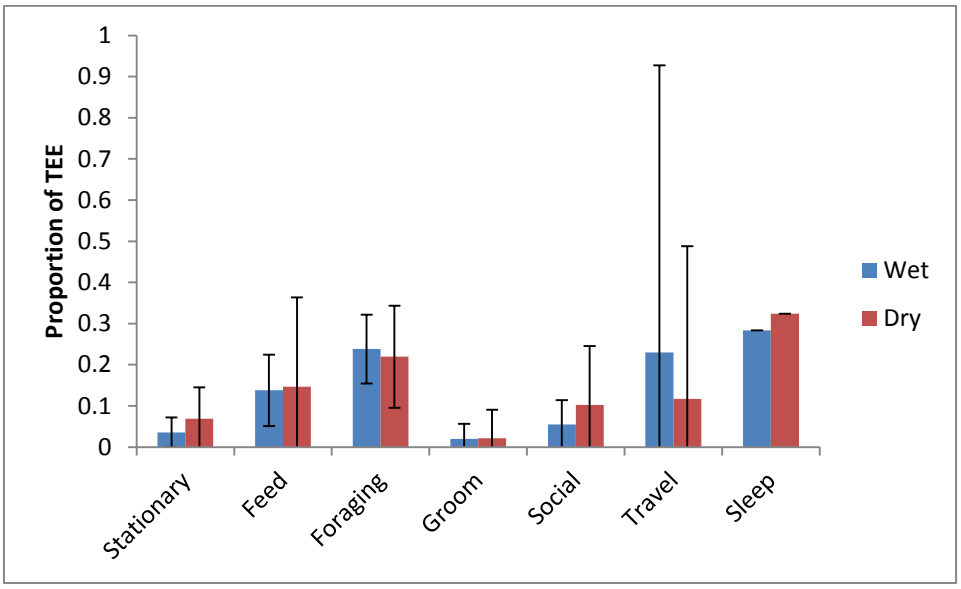


Figure 5.2 Proportion of each behaviours contribution to average TEE for the wet and Austral winter

5.4 DISCUSSION

5.4.1 Activity Budgets

While there were no significant differences between the activity budgets of male and female Javan slow lorises, seasonality had an effect on the performance of foraging behaviours. This behaviour became more important during the Austral summer, where food abundance and diversity is at its highest. Stationary behaviours were shown to be performed more often during the Austral winter, which is expected to occur as an energy saving adaptation. The Austral winter is also the "lean" season of the field site, where slow lorises are generally restricted to subsist on a diet of gum and insects (Chapter IV). Due to possible energy constraints during this period, as well as the thermoregulatory stress of lower temperatures, stationary behaviours may be employed in order to reduce TEE. Stationary behaviours have an increased contribution to TEE during the Austral winter because it is a larger proportion of the activity budget. The slow lorises in this study chose to be stationary more often, which means the contribution of TEE by feeding, foraging and travelling are lower, even if marginally (Figure 5.2). Being the most energetically conservative behaviour, remaining stationary reduces the occurrence of more expensive behaviours. This strategy is also observed by collared lemurs during their lean season (Donati et al. 2011) but differ from the Yunnan snub-nosed monkey (*Rhinopithecus bieti*) and the Himalayan grey langur (*Semnopithecus ajax)* which reduce their overall resting times to spend more time foraging on fallback foods in order to decrease their energetic deficits (Grueter et al. 2013; Minhas et al. 2013). The primates in these examples are placed under extreme climatic and energetic stress unlikely to compare to the seasonality of our field site. Reproductive events are unlikely to have influenced the strategy observed in Javan slow lorises as this was observed for both males and females. We also observed nearly as many total reproductive events (lactation and/or gestation) during both seasons. Physiologic torpor is reportedly employed by the

Javan slow lorises (Reinhardt et al. 2016) there would be even further energetic savings, further reducing TEE (Dausmann 2014). Mouse lemurs are able to save 37.7% of energy costs due to torpor (Schidt 1998). *Nycticebus* sleep in a curled up position known as the sleeping ball, reducing their body surface-to-volume ratio, which is known to conserve heat and favour energy savings (Donati et al. 2011). Being an exudativorous primate, large amounts of time spent feeding and foraging is common. Such behaviour is also observed in another gum eater, the common marmosets (*Cebuella pygmaeus*), where foraging and feeding can make up as much as 55% of their diurnal activity budgets (Correa et al. 2000). Similar to the slow loris, other primates also employ behavioural modifications during the lean season to reduce overall TEE.

5.4.2 Minimum Path Lengths

The minimum path lengths of the slow lorises were generally short compared to those of other similar sized primates (Razafindratsima et al. 2014). The limitations attached to our methods must be considered when comparing with other studies. Our data do not reflect vertical movement, nor capture information in-between 15-minute sample points. Our estimates of path lengths and TEE must be viewed as absolute minimums however, due to the relatively small travelling energy costs, our data can still be effectively used to estimate TEE (Alba-Meija et al. 2013). The context of DPL within this study is to compare our approximations of TEE with our calculations of BMR for captive individuals. This comparison still holds as the majority of slow loris enclosures in captivity are usually constructed to have a large amount of horizontal movement but very limited for vertical movement (Fitch-Snyder et al. 2001). The proportion of energy spent using our DPL approximations would therefore be in line with what we would expect in captivity. The short path lengths cannot be ruled out as an "exudativorous rule" due to common marmosets having a daily path length between 830 and 1498 m (Ferrari 1988, Correa et al. 2000, Ferrari et al. 2004, Abbehusen et al. 2007; Rylands 1989).

Path lengths of the more insectivorous white-footed tamarins (*Saguinus leucopus*) ranged from 1671 to 1975 m (Alba-Meija et al. 2013). Marmosets tend to have a shorter DPL than tamarins, even when both increase their DPL during the abundant seasons (Digby et al. 2011; Vilela and de Faria 2004). The tamarin's larger reliance on patchy fruit resources may require them to have a longer DPL.

5.4.3 Seasonal Differences Differ Between the Sexes

Our results support the hypothesis of Thompson et al. (2013) that primates that are able to exploit fixed and reliable exudate food resources will have a reduced energetic cost of traveling. Male and females did not statistically differ in their average path lengths, and although males did not differ, females had a longer path length in the Austral summer compared to the Austral winter. Female slow lorises in this study had a larger overall TEE due to reproductive costs. We would expect foraging and nutrient balancing to be of higher importance to the female than the male and/or energy conserving mechanisms to be more important for females (Vedder 1984). Females should move less during the Austral winter to conserve energy during an energetically stressful season which is seen in slender lorises, yet it seems to be more advantageous for our studied slow lorises to increase their path lengths to find scattered food sources during this period (Radhakrishna and Singh 2002*)*. During the Austral summer, when food is abundant and diverse, extra energetic costs of a longer path length may be made up for by a higher energy return. This is a similar strategy employed by female chimpanzees which reduce their daily path lengths during the lean season (N'guessan et al. 2009). Male chimps and slow lorises do not show a significant variation, which may again be explained by their lack of reproductive costs. We expected females slow lorises to have a decreased path length during lactation with a young baby, however this was shown not to occur. *Nycticebus* are known to "park" their infants on branches and to continue foraging, only to return every few hours (Nekaris 2014). This is another potential energy saving adaptation of the

Lorisidae. By contrast, callitrichids are known to be energetically constrained during weaning, and family units must share the infant carrying burden, even though depending on location and/or season, some *C. jacchus* populations do not alter path length (Miller et al. 2006, Pinheiro and Pontes 2015). Strepsirrhine milk is known to be denser than callitrichid milk, which may incur extra energetic production costs; however, infants are fed less often which may even out energetic costs over time (Nievergelt and Martin 1999). If energy was easier to come by during the abundant season, this adaptation may be for other purposes rather than energy saving. Northern pig-tailed macaques (*Macaca leonina*) also increased their DPL during the abundant season from averages of 1588 to 1804 m (Albert et al. 2013). Similarly to our female Javan slow lorises, they were able to adjust their diet according to an increased energetic burden. Macaques are generalist feeders, which means often have a large diversity of food items at their disposal including numerous high quality fruits during the Austral summer. Finding a patch resource, such as, a fruiting tree would easily justify the energetic costs of increasing your DPL. Although gum itself is patchy (multiple gum holes being found on one tree), the trees are spread out, similarly to insects and therefore not patchy, which may mean that female slow lorises under energetic constraints must wander within their territory to find the less abundant insect protein they require. This strategy often leads to increases in energy expenditure (Cunningham and Janson 2013).

5.4.4 Energetic Contribution of Travelling

The energetic costs of traveling in arboreal primates are relatively small (Porter and Garber 2012). Squirrel monkeys (*Saimiri sciurus*) have a DPL of 9500 m/day and yet this contributes to only 10% of their TEE. Their seasonal increase only minimally increases the proportion of TEE (Steudel 2000). The average seasonal increase of TEE is less than 3 kcal/day for squirrel monkeys, which is consistent with a hypothesis proposed by Porter and Garber (2012). The increased path length may not be an energetic burden itself, therefore the more important

constraint involves possibly not being able to ingest enough food due to timing of the lean season. Specialist folivores such as the Yunnan snub-nosed monkey (*Rhinopethicia bieti*) also increased their DPL during the abundant season by 66% (Grueter et al. 2013). However, this is not a general rule for folivorous primates as *S. ajax* increased its DPL during leaner seasons (Minhas et al. 2013).

5.4.5 Differences in Energy Expenditure

Reflective of their similar activity budgets, males and females did not have significantly different average TEE values, and they also did not vary according to season. This was expected as the TEE is based on body weight and activity budgets, none of which are particularly affected by the sex of the slow loris. The estimated BMR of a 900 g Javan slow loris is 38.80 kcal/day with the coinciding FMR estimated to be at minimum 44.41 when using a multiplication ratio of factor of 1.25. This factor underestimates the calculated TEE of 49-50 kcal/day. The 700g golden lion tamarin had a TEE of 92.7 kcal (Miller et al. 2006). Using the nutritional data from (Chapter 4), slow lorises would need to ingest approximately 30g of tree gum and 6g of insects daily, assuming the Austral winter (where insects are the limiting food item). Although possible, it will make collecting insects during the Austral winter more difficult which should render this strategy less plausible. Indeed the slow lorises are adapted to reduce their TEE according to their ecological niche of exudativore, which follows the predictions of Genoud et al. (1997) that primates ingesting difficult to digest diets, such as gum, will require adaptations to lower their TEE. This holds true for some non primates as well such as the giant panda (Nie et al. 2015). The slow lorises have a 37-45% lower TEE than expected as an adaptation to their low quality diet. Other nocturnal primates such as the owl monkey (*Aotus*spp.) also have a lower BMR than expected, without counting any behavioural adaptations the animal may have (Leonard and Robertson 1997).

5.4.6 Influence of Reproductive Costs on TEE

Reproductive costs were significant in the female slow lorises, adding on average 28% of their TEE. Golden tamarins needed to increase their energy intake by 2.8 times (280%), perhaps indicating the inadequacy of the equations used in this study (Miller et al. 2006). Possibly a more suitable comparison with the slow loris, the exudativorous common marmoset did not show different energy intakes during gestation, perhaps through increasing digestive efficiency (Kemnitz et al. 1984). With lactation being a significant energetic cost, females must either feed for longer or choose higher quality food items (Hiraiwa-Hasegawa 1997). Female orangutans altered their time spent feeding and dietary composition according to season and their reproductive state (Knott 1998). Energetic costs of slow slow lorises were higher during the Austral summer than the Austral winter, which can be explained as a larger proportion of the adult females were lactating during this period of time. Juveniles are also expected to be weaned during this period in order to provide them with the best possible ecological conditions for sub adult survival (Nakagawa 2000). After weaning, sub adults stay within the territory of their parents for another year and have been documented playing with their younger siblings and father, yet are entirely independent in terms of feeding (Nekaris 2014).

The Javan slow loris has been found to display a climate-mediated activity budget, with a positive correlation of increased foraging with humidity (Reinhardt et al. 2016). This is presumed a result of increased activity of insects at higher humidity (Fadamiro and Wyatt 1995), allotting individuals to forage more frequently for insects. In this geographic region, percentage of relative humidity is higher during the Austral summer, which also may be implication for why females are increasing foraging during this season.

5.5 CONCLUSION

Like other primates, the Javan slow loris demonstrated the ability to modulate their activity budget based on season, specifically resting and foraging behaviours. Their path lengths were relatively short due to our methods. Females

appeared to have an overlying energetic constraint due to the costs of gestation and lactation, which may explain why their seasonal strategies are different compared to males. Comparing TEE to energy intake would allow us to measure in greater detail the difference between male and female strategies, and further quantify the reproductive constraints of female Javan slow lorises.

The results of this chapter can inform us on the energy requirements of slow lorises in captivity. We now know that males and females spend a very similar amount of energy year round, except perhaps when the female is in her final terms of gestation and when she is caring for her youngster. During this time her energetic needs will increase. In captivity this means more food is required and combined with what we learned in Chapter IV, the ratio of protein to non protein energy should also become more specific.

Now that we have a good framework for building the new diet in terms of nutrients and energy, we must now focus on the actual food components.

CHAPTER VI

"What effects does gum have on the physiology of the slow lorises?"

or

SLOW LORISES REALLY ARE SLOW: A COMPARATIVE STUDY INTO FOOD PASSAGE RATES OF WILD-CAUGHT AND CAPTIVE INDIVIDUALS

6.1 INTRODUCTION

6.1.1 Food Passage Rate

The length of time food remains inside the gastrointestinal tract (GIT) of an animal can impact many interrelated biological functions such as the concentration and composition of intestinal microflora (Bailey and Coe 2002); the extent of nutrient breakdown/absorption, especially structural carbohydrates (Flores-Miyamoto et al. 2005); energy yield (Blaine and Lambert 2012); metabolic rate (Muller et al. 2013) and detoxification of secondary plant metabolites (Cork and Foley 1991). Depending on the food ingested, some mammal species modulate this rate of passage (Edwards and Ullrey 1999a; Kuijper et al. 2004) to enhance digestibility of poor quality food, to speed up intake of food items high in easily digestible nutrients (Caton 1997; Sawada et al. 2011), or to eliminate non-digestible food items (Dierenfeld et al. 1982; Power 1991; 2010). The most informative measurements used to estimate the food passage rate are transit time (TT) and mean retention time (MRT) (Warner, 1981). MRT values in wild animals are very difficult to measure, therefore MRT in captive animals have been used to infer information about their wild feeding ecological niche, revealing vital information about their energetic needs (Blaine and Lambert 2012; Lambert 2002). This has been studied in detail on a number of primate species.

6.1.2. Fibre's Effect on Food Passage Time

When similarly sized primates undergo changes in their feeding regimes, changes in MRT values can be grossly predicted depending on their feeding ecology. For example, with the introduction of more fibre in the diet: folivores should increase their MRT (proboscis monkeys *Nasalis larvatus*: Dierenfeld et al. 1992, gorilla *Gorilla gorilla gorilla*: Remis and Dierenfeld 2004), frugivores should decrease (red ruffed lemur *Varecia rubra*: Edwards and Ullrey 1999b), granivores should decrease (white-faced saki monkey *Pithecia pithecia*: Norconk et al. 2002) and exudativores should increase (common marmoset *Callithrix*

jacchus: Power and Oftedal 1996) although there are exceptions if the animal ingested an entirely indigestible bolus such as whole seed. These observed changes in MRT are mainly due to the assumed importance of plant fibre in the diets of species from different feeding guilds. Plant fibres include structural carbohydrates that must be fermented by the microbial populations inhabiting the digestive system of primates. The folivorous colobine primates have been observed to have the highest MRT (up to 49 h) with folivorous hindgut fermenters varying greatly in their digestive capabilities, from 12 to 37 h (Lambert 2012). Such values were expected, as colobine primates ingest food items very high in fibre content and must have a long MRT in order to allow their symbiotic microbes' ample contact time to convert the cellulose and hemi-cellulose fibres into energy (Lambert and Fellner 2011). The hind gut fermenters such as gorillas (*Gorilla* sp.) are in a similar situation to colobines, requiring a longer MRT when fed high fibre foods (Remis and Dierenfeld 2004). Frugivorous species such as the spider monkeys (*Ateles* spp.), on the other hand, do not exploit the fibre portion of the diet as much as the soluble nutrients found in fruit (proteins and sugars) and therefore do not require a long passage rate (Milton 1984). There have been numerous comparisons between the passage rates of frugivorous versus folivorous primates within the literature with other types of primates being fit into one of these moulds (Lambert 2002). One such primate group are the exudativores.

6.1.3. The Digestion of Exudativorous Primates

Exudativorous primates also ingest other food items, such as insects, reproductive plant parts and nectar (Coimbra-Filha and Mittermeier 1977). Much of our understanding of exudativory in primates has come from the New World marmosets (*Calithrix* and *Cebuella* spp.*),* which are known, throughout the year, to gouge trees and trigger gum production that they later harvest (Smith 2010). Gums comprise soluble structural polysaccharides that require microbial fermentation for digestion (Ushida et al. 2006). They are high in minerals, namely calcium, and

once fermented, gums provide a good source of energy, however they are low in most other nutrients such as protein and lipid (Hladik 1979). Among exudativores, *Callithrix jacchus* have been show to exhibit a longer MRT to accommodate the opportunity for fermentation and energy gain (Power and Oftedal 1996). Related tamarin species, which consume more insects and fruits than marmosets, did not alter their MRT when gum was added to their diet (Power and Oftedal 1996). Until now, studies of MRT in relation to exudativory have been limited to platyrhine primates, despite the prevalence of this diet amongst Strepsirrhini.

Numerous wild field studies have now concurred that exudates play a vital role across slow loris species (*Nycticebus pygmaeus, N. coucang, N. bengalensis* and *N. javanicus*), with some taxa spending 43-87% of their feeding time on exudates (Chapter IV; Das et al. 2014, Starr and Nekaris 2013, Wiens et al. 2006). In the past, however, Asian lorises were classified as frugivores on the basis of comparisons to African pottos (Charles-Dominique 1977) and a few days' field study (Barrett 1984). This misconception has led to a captive diet comprised largely of fruits, which has led to diminished reproduction and high incidence of diseases (Chapter III). The aim of this study was to compare the MRT of slow lorises subsisting on a traditional captive diet of fruits, vegetables and insects compared to a wild type diet of gum, insects, vegetables and nectar. We predict that slow lorises should show a similar MRT response to common marmosets and that they will adapt their MRT according to the presence of gum in the diet to maximise fermentation opportunities (exudativorous/folivorous response), rather than have no changes in their MRT (frugivorous response). We also expected *N. bengalensis* to have the longest MRT and *N. pygmaeus* to have the shortest MRT due to the allometric relationship of body mass and MRT. Understand exudativorous physiological response to fibre would permit us to infer how important gum may be to their metabolism, which can have an evidence based impact on improving captive conditions of these endangered primates.

6.2 Materials and Methods

6.2.1 General Methods

We conducted the study at two locations, the first at Cikananga Wildlife Rescue Centre (CWRC) which is described in section 2.3.1. Animals housed at the centre and used in the study included: *N. coucang* (n=15)*, N. menagensis* (n=4) and *N. javanicus* (n=15)*.* All animals at CWRC were wild born and had been at the centre, confiscated from traders or markets, from 14-20 months. We performed the second set of trials at Shaldon Wildlife Trust (SWT), Shaldon, United Kingdom with (n=2) *N. pygmaeus* and *(n=2) N. bengalensis* (Table 6.1). Both *N. pygmaeus* were captive born, and both *N. bengalensis* were wild born. The CWRC individuals were put under all three diet treatments (captive and wild) while the SWT were only fed their current captive diet. We conducted a food passage rate study using the methods explained in section 2.3.3 on two diet treatments: Diet 1 (current captive diet), and gum using Diet 2 (naturalistic diet). We always fed markers first, before the rest of the diet. At CWRC we fed markers at 1800 hr when the slow lorises awoke, and at SWT we fed slow lorises at 0800 due to the reversed light cycle of their nocturnal enclosures.

Table 6.1 Details of the food passage rate study population of five *Nycticebus* species at two locations.

	N. javanicus	*N. coucang*	*N. menagensis*	*N. pygmaeus*	*N. bengalensis*
Sample size (n=males.females)	7.8	6.9	2.2	1.1	0.2
Average weight (± SD)(g)	1050 (±236)	936 (±312)	702 (±53)	423(±25)	1020 (±93)
Location	CWRC	CWRC	CWRC	SWT	SWT

CWRC = Cikananga Wildlife Rescue Centre (Indonesia)
SWT = Shaldon Wildlife Trust (United Kingdom)

6.2.2 Intake Study

We conducted intake studies with the captive slow lorises fed two different diet regimes simultaneously with the MRT data collection (Britt et al. 2015). Diet 1 at the CWRC was their current diet comprising (per individual): katydids (*Scudderia* spp. - 3.4 g), peeled oranges (18.3 g), peeled banana (44.0 g), mealworms (*Tenebrio molitor* - 4.9 g), crickets (*Acheta domestica)* (1.3 g), peeled rambutans (*Nephelium lappaceum* - 12.2 g), hardboiled chicken egg without shell (2.2 g), sapodilla without seeds (*Manilkara zapota* - 17.1 g), honey (4.0 g), mangosteen (*Garcinia mangostana* - 12.9 g) and sago worms (*Rhynchophorus ferrugineus* - 2.1 g). We weighed food items offered and weighed the uneaten left over food from the enclosure the following morning at 700 hr. Desiccation dishes of food items were also set up and measured at feeding time and at time of clean-up. They comprised the same portions of the diet and were kept in a pest proof area with the same temperature and climate as the enclosures. The decrease in weight was entirely attributed to evaporation which allowed us to correct the diet intake values. Diet 2 was a naturalistic type diet based on the nutrient intake framework of Chapter IV. This diet was aimed to mimic a "natural" diet and was fed to the individuals of all species for seven days (with a progressive diet change over seven days and an extra week long acclimatisation period). We used only food items that were affordable and available for the rescue centre. The average daily new diet, as offered, consisted of 20 g of various insects (including mealworms, crickets, wild caught katydids, sago worm larvae and pupae mix), carrot (10 g), green bean (10 g), young bamboo leaves (*Gigantochloa* cf. *ater*) (5 g) and gum from wild *Acacia decurrens* (20 g). Components of both diets were analysed for primary nutrients and fiber fractions (moisture, ash, crude protein and crude fat as well as acid detergent fibre (ADF), neutral detergent fibre (NDF)) as well as soluble fibre and soluble sugars to calculate the nutrients ingested on both diets, as per section 2.2.2. Diets at SWT consisted of 50 g of watery vegetables (broccoli, peppers, cucumber), 50 g of various root vegetables (carrots, sweet potato, parsnip, swede),

2 g of nectar powder (Sunbird Nectar, Mazuri Europe, UK), 3 g of locusts (*Schistocerca gregaria*), 3 g of mealworms, 1/2 hardboiled egg with shell, and 5 g of gum arabic powder from *A. senegalensis*.

6.2.3 Statistical Analyses

We conducted all statistical analyses using SPSS version 22.0 (IBM). We used a Generalized Linear Mixed Model (GLMM) to test if there was a main effect of species and diet composition on both the TT and MRT. The assumptions associated with GLMMs were considered and not violated. We used a gamma distribution for the response variables (TT and MRT), individuals as a random factor and diet (captive and wild) and species (*Nycticebus javanicus, N. coucang* and *N. menagensis*) as fixed factors.

6.3 RESULTS

6.3.1 Transit and Mean Retention Times

The average food transit time for CWRC *Nycticebus* spp. on Diet 1 (captive diet) ranged between 24.2 (*N. menagensis) to* 25.6 hours (*N. javanicus*), and on Diet 2 (wild type diet) ranged between 24.4 (*N. coucang)* to 25.9 (*N. javanicus*) (Table 6.2). The mean retention time of *Nycticebus* at CWRC on the original diet ranged between 29.7 (*N. coucang)* to 33.4 h (*N. javanicus*) and on the wild type diet ranged between 34.1 (*N. menagensis)* and 38.5 hours (*N. javanicus)*. SWT slow lorises had TTs of 25.3-29 h and MRTs of 42.6-58 h. No overall models had a significant effect on the TT, however MRT was significantly affected by diet (χ^2=710.276, df=1, P=0.0001), species (χ^2=17.531, df=2, P=0.0001), and the interaction between species and treatment (χ^2=710.276, df=1, P=0.0001). Diet 1 was associated with a lower MRT overall (B=-4.750 df=1 P=0.0001). Both *N.*

javanicus (B=4.600 df=2 P=0.0001) and *N. coucang (B=4.000 df=2 P=0.0001)* had larger MRT values than *N. menagensis*. Although when *N. coucang* was fed Diet 1, they had significanty lower values than the other species (B=-4.000, df=1 P=0.001).

Table 6.2 Gastrointestinal tract transit time and mean retention time (with standard deviation and max-min range) of five *Nycticebus* species under two dietary treatments, where the wild type diet has led to an increase in mean retention time.

	Diet	*N. javanicus* N=15	*N. coucang* N= 15	*N. menagensis* N= 4	*N. pygmaeus* N=2	*N. bengalensis* N=2
Transit Time (hours)	Original Captive Diet (range)	25.6 (±2.6) (23.0-31.5)	25.00 (±3.5) (21.5-29.0)	24.2 (±3.2) (21.0-27.5)	29.0 (±2.0) (27.0-31.0)	25.3 (±2.2) (22.75 - 30.0)
	Wild type diet (range)	25.9 (±3.4) (24.0 - 29.0)	24.4(±2.1) (24.0 - 26.5)	24.5 (±2.9) (22.5- 27.0)		
Mean Retention Time (hours)	Original Captive Diet (range of max transit times)	33.40 (±1.0) (51.0-52.5)	29.70 (±1.5) (47.0-49.5)	32.88 (±3.1) (48.0-53.4)	39.75 (±1.5) (56.5-58.5)	24.32 (±0.5) (42.8-42.5)
	Wild type diet (range of max transit times)	38.50 (±2.0) (54.5-59.0)	38.0 (±2.5) (54.0-57.5)	34.13 (±4.1) (50.0-54.8)		

6.3.2 Nutrients Ingested by Slow Lorises

The average nutrient ingestion on both the original CWRC and SWT diets differed slightly between species, yet was dissimilar to the average nutrient intake for the wild type diet (Table 6.3). Notable nutrient differences include soluble fibre which ranged from 0.71 -- 0.78 % in the original captive dietto 3.09 --3.24 % on the wild type diet (all values on a dry matter (DM) basis). Both ADF and NDF increased, 4.35 -- 8.41 % versus 16.13 -- 17.56 %, and 8.56 % -- 10.50 % versus 17.72 % --19.01 %, respectively. At SWT, *Nycticebus pygmaeus* and *N.*

bengalensis had intermediary fiber intake levels for ADF and NDF of 9.24-12.34% and 12.04-13.56%, respectively. All nutrients ingested except for iron were significantly different between diets 1 and 2. Diet 1 resulted significantly higher intakes of ash (Z=-3.170 P=0.002), soluble sugars (-7.729 P=0.0001) and copper (Z=-6.772 P=0.0001). Diet 2 resulted in significantly higher intakes of calcium (Z=-9.616 P=0.0001), crude fat (Z=-9.379 P=0.0001) , crude protein (Z=-8.940 P=0.0001), energy density (Z=-4.588 P=0.0001), magnesium (Z=-5.295 P=0.0001), phosphorous (Z=-8.385 P=0.0001), sodium (Z=-8.144 P=0.0001), soluble fibre (Z=-7.729 P=0.0001), ADF (Z=-7.992 P=0.0001) and NDF (Z=-7.484 P=0.0001).

Table 6.3 Average daily nutrient intakes and statistical results of the five *Nycticebus* species under two dietary treatments showing a wild diet higher in fibre fractions and lower in soluble sugars.

	N. javanicus		***N. coucang***		***N. menagensis***		***N. pygmaeus***	***N. bengalensis***
Nutrient	**Original**	**Wild Diet**	**Original**	**Wild Diet**	**Original**	**Wild Diet**	***Original***	***Original***
Ash (%)[1]	2.90 (±2.51)	2.64 (±0.53)	3.11 (±2.65)	2.44 (±0.43)	2.88 (±2.44)	2.76 (±0.62)	5.67 (±2.34)	5.43 (±2.21)
Crude Protein (%)[2]	12.79 (±4.59)	26.23 (±5.58)	12.11 (±4.91)	25.64 (±5.48)	13.69 (±4.34)	24.35 (±6.01)	24.08 (±3.56)	22.56 (±3.31)
Crude Fat (%)[2]	7.58 (±2.03)	10.41 (±2.09)	7.81 (±1.98)	11.15 (±2.37)	8.30 (±2.00)	9.62 (±2.56)	14.75 (±6.75)	13.65 (±5.23)
Energy (Kcal/g)[2]	3.92 (±0.68)	4.17 (±0.61)	3.91 (±0.74)	4.31 (±0.48)	4.25 (±0.51)	4.09 (±0.73)	4.02 (±0.34)	3.96 (±0.12)
Soluble fibre (%)[2]	0.72 (±1.27)	3.11 (±2.71)	0.71 (±1.11)	3.09 (±2.31)	0.78 (±1.19)*	3.24 (±3.01)	NA	NA
ADF (%)[2]	5.28 (±4.05)	17.04 (±6.73)	4.35 (±3.93)	16.13 (±5.19)	8.41 (±3.99)	17.56 (±6.87)	9.24 (±2.59)	12.34 (±2.46)
NDF (%)[2]	8.56 (±3.00)	18.72 (±6.81)	7.31 (±3.16)	17.72 (±6.27)	10.50 (±2.69)	19.01 (±7.23)	12.04 (±2.99)	13.56 (±2.64)
Sugars (%)[1]	9.60 (±6.86)	3.88 (±10.76)	9.20 (±5.12)	4.10 (±10.32)	9.14 (±6.73)	3.56 (±11.38)	NA	NA
Ca (%)[2]	0.17 (±0.04)	0.33 (±0.09)	0.14 (±0.10)	0.35 (±0.12)	0.15 (±0.12)	0.31 (±0.11)	0.35 (±0.09)	0.37 (±0.11)
P(%)[2]	0.19 (±0.06)	0.30 (±0.08)	0.16 (±.09)	0.32 (±0.10)	0.20 (±0.11)	0.28 (±0.13)	0.40 (±0.13)	0.38 (±0.06)
Mg (%)[2]	0.27 (±0.13)	0.54 (±0.24)	0.29 (±0.17)	0.49 (± 20)	0.24 (±0.11)	0.51 (±0.29)	0.10 (±0.02)	0.09 (±0.03)
Fe (mg/kg)	59.47(±13.71)	123.00 (±38.17)	57.26 (±11.57)	113.45 (±39.62)	69.12 (±13.56)	119.57 (±41.67)	43.69 (±9.16)	46.97 (±8.82)
Na (%)[2]	0.43 (±0.63)	0.11 (±0.10)	0.36 (±0.72)	0.10 (±0.15)	0.12 (±0.59)	0.11 (±0.07)	0.24 (±0.14)	0.20 (±0.12)
Cu (mg/kg)[1]	7.45 (±2.88)	6.67 (±1.46)	6.96 (±2.81)	6.79 (±)1.86	7.2 (±2.63)	6.41 (±1.75)	3.70 (±1.04)	3.98 (±0.94)
Ca:P	0.89	1.10	0.88	1.09	0.75	1.11	0.875	0.975

6.4 DISCUSSION

The food passage rate of *Nycticebus* species was relatively long for their body sizes and showed a hyper-folivorous type response, similar to the exudativorous marmosets. Now referred as the exudativorous response, adding gum to their diet significantly increased MRT by up to 42% but did not change TT values. The diets were significantly different for every nutrient except one, which also reflects how different the captive diet truly was compared with wild slow loris diets. Although transit times of the slow lorises did not vary depending on diet, the already long MRT values (ranging from 29.7 to 33.40) increased by a further 4 to 29% when fed the wild type diet. This diet contained significantly more fibre fractions (soluble fibre, ADF and NDF) which may be the major reason for this altered gut passage rate, although other reasons for variation in MRT are technically possible (Lambert 1998).

6.4.1 Slow Loris Food Passage Rates

Anatomy, physiology, behaviour, body mass and diet are possible factors which may explain variance observed among the MRT of slow loris species (Blaine and Lambert 2012; Lambert 1998; Martin et al. 1985; Warner 1981). Behaviour and overall activity may be dependent upon the enclosure the slow lorises were inhabiting (both size and complexity of environment), meaning it will be very difficult to compare CWRC's slow lorises with SWT's slow lorises without accompanying activity budgets. The most important factor within this study appears to be the nutritional intake of the animals because diet was the only factor that significantly changed before and after treatment.

We predicted *N. bengalensis* to have the longest MRT and *N. pygmaeus* to have the shortest MRT, however our data were not consistent with this outcome. Rather, the longest MRT was seen for *N. pygmaeus* and the shortest, N. *bengalensis*. These two species were fed different diets that were not altered during

the study, raising difficulties in comparing values. We expect the NDF values of the captive diet of *N. pygmaeus* to be quite similar to, or higher than, their wild diet comprising insects and gum (Starr and Nekaris 2012) while *N. bengalensis* was likely receiving a diet much lower in fibre fractions than wild conspecifics (Das et al. 2014). Fermentable food items, such as gum and leaves, comprise the largest proportions in the diets of wild *N. bengalensis* (Das et al. 2014). The larger Bengal slow loris should theoretically have a longer MRT to exploit its lower quality diet fully, when compared with the pygmy slow loris, which has a higher quality insect-based diet (Lambert 2002; Parra 1978). This may be explained by our study population where the Bengal slow loris actually did not possess the largest body weight.

6.4.2 The Exudativorous Response

The typical model species for exudativorous primates have been the gouging marmosets, allowing a direct comparison between slow lorises and callitrichids (Smith 2010). The marmosets, *Cebuella pygmaeus* and *Callithrix jacchus,* also displayed an increase in their MRTs when gum was added to their diet (Power 1991). This is contrary to the results seen with non-gouging tamarins, *Leontopithecus rosalia, Saguinus oedipus* and *S. fuscicollis*, which were not able to modify their gut physiology to exploit gum more efficiently (Power and Oftedal 1996). The MRT of *C. jacchus* with gum ranged between 14 and 15 h, which is much lower (faster passage) than all of the slow lorises, which were on average 24.3 to 39.8 (Caton et al. 1996; Power and Myers 2009). Slow lorises possess morphological adaptions that allow them to subsist on an exudate based diet such as specialized dentition, modified capillary system, and enlarged hind gut and caecum (Nekaris 2014). The TT and MRT values observed in the slow lorises are even slower than anticipated or predicted, not following the expected body size to MRT rule. Cercopithecines do not seem to conform to the theoretical digestive rate to body weight linear rule of Lambert (2002). Similarly, maintaining a small body

size (for slow lorises and certain cercopithecines) may be helpful in detoxifying plant metabolites with which larger primates may not be able to cope. The gum and insects ingested by the slow lorises have been shown to contain high levels of toxins and secondary plant metabolites, providing support to this argument (Nekaris and Bearder 2007; Rode-Margono et al. 2015). Slow lorises must also cope with lean seasons in their natural habitats, where even insects are less available (Chapter IV). A larger and flexible MRT may also allow them to fall back onto poorer quality food resources more easily during lean seasons (Lambert 2002).

6.4.3 Morphological Adaptations Lead to Variations in Food Passage Rates

Morphology is one of the determinants of passage rate. Marmosets have a large caecum, similar in structure to *Nycticebus* (Caton et al. 1996), which may explain their relatively longer average TT values when compared to tamarins who lack a complex caecum and large intestine: 4.6 and 6.3 h for *C. jacchus* and *Cebuella pygmaeus* respectively versus 2.7, 3.6 and 3.9 h for *S. fuscicollis, S. oedipus,* and *L. rosalia* respectively (Power and Oftedal 1996; Crissey et al. 1990). Callitrichids are often described as omnivores, feeding on fruits, gums, invertebrates and sometimes small vertebrates (Power 2010). The marmosets differ from tamarins, with significantly longer feeding time spent on gums. Also similarly to *Nycticebus*, the dental adaptations of *Callithrix* and *Cebuella* enable them to gouge trees, actually classifying them as exudativores rather than insectivores/omnivores (Coibra-Filha and Mittermeier 1977). As a rule, the strepsirrhines that feed on fermentable food items such as leaves have an enlarged large intestine, such as the sportive lemur (*Lepilemur leucopus)* (Perrin 2013). This reflects their efficient use of high fibre diets due to the active microbe populations within the enlarged intestines and caecum. Slow lorises are no exception to this rule, although are able to survive on lower fibre diets as well. They seem to be

quite adaptable as was made obvious by the large increase in MRT values on their wild type diets. Howler monkeys (*Allouatta spp.*) also eat a very fibrous diet and have a TT of 20.4 - 35.0 h and MRT of 49.5 - 57.0 h, versus the 5.3 h of the frugivorous spider monkey (Crissey et al. 1990; Espinoza-Gomez et al. 2013; Milton 1984). This enables the spider monkeys to allow indigestible materials to be passed rapidly through their less complex digestive tract, similar to the mechanism used by tamarin species, which can pass whole seeds within 2.2-2.5 h (Heymann and Smith 1999; Knogge 1998). This frugivorous response allows speciesto ingest more food and exploit the easily absorbable nutrients within the diet at faster rates. Even if preferred foods are not available in large quantities, higher intakes of poorer quality food can substitute in these instances. This strategy is useful for frugivorous *Saguinus* and *Ateles* spp*.,* Japanese macaque (*Macaca fuscata*), white handed gibbon (*Hylobates lar)* and de Brazza's monkey *(Cercopithecus neglectus),* whom decrease their MRTs with increasing dietary fibre (Sawada et al. 2011). Although not primates, the frugivorous binturong (*Arctictis binturong)* and kinkajou (*Potos flavus*) display a very short MRT, of 6.5 and 2.5 h respectively (Lambert et al. 2014). They have little to no fermentation capabilities in a simple digestive system, yet ingest mostly plant matter with occasional vertebrate prey. A fast passage rate is necessary for them to meet their metabolic needs. This adapation is also similar with the maned wolf (*Chrysocyon brachyurus*), which does not increase its TT when dietary fibre is increased (Child-Sanford and Angel 2006). The dichotomy between folivorous and frugivorous responses is further exemplified in the lemurs. The frugivorous *Eulemur spp.* have a rapid TT of 1.6 to 3.3 h, yet the fermenting *Hapalemur griseus* has a much longer TT of 18.2 h (Overdorff and Rasmussen 1995). Slow lorises had an even longer TT then the related *Galago crassicaudatus,* with reported values of 10.5 (Nash 1986). Larger fermenting species such as *Pongo pygmaeus* had an MRT of 37 h, *Gorilla gorilla* of 36.6 - 97 h, and *Pan troglodytes* of 37 - 48 h (Milton 1984; Milton and Demment 1988; Remis 2000; Remis and Dierenfeld 2004). Indeed the slow loris

had a similar MRT to the 80 times larger *P. pygmaeus*. There is a trend for frugivores to down modulate their MRT when ingested fibre fractions increase, while folivores and exudativores increase their MRT.

6.4.4 Why are Slow Lorises so Slow?

Nycticebus spp. have unique traits and a unique life history, that may explain the exagerrated slow passage rates observed (Nekaris 2014). Their low quality, gum-based diet may require a long MRT to assimilate sufficient nutrients and energy required. If so, their gut physiology should be relatively plastic in allowing maximal use of good and bad quality diets, and MRT should increase depending on overall fiber (NDF and soluble) amount. Alternatively, the longMRT may allow gut microbes to detoxify the secondary plant metabolite load found in plant exudates (Anderson 1990). The gum given in this study was collected from the field; it was expected to contain a high amount of metabolites (Anderson 1973, 1990). However, the gum given at SWT was purified and contained no toxins, and yet resulted in a long MRT for *N. pygmaeus* although shorter (yet still longer than other similarly sized primates) for *N. bengalensis.* Our results are more consistent with the low quality diet hypothesis, rather than long MRT as a means of anti-predation defenses. More importantly, we must also include the low basal metabolism of the *Nycticebus.* They have a basal metabolic rate up to 60% lower than similarly sized mammals (Muller 1979). This slow MRT may simply be an effect of their low metabolism. Until comparative *Nycticebus* spp. metabolic rates are calculated, support for this hypothesis will be difficult to acquire.

6.4.5 Particle and Fluid Sorting

Gums may be selectively fermented in the caecum, leaving more easily digested food items to pass through the intestine quickly (Lambert 1998). This strategy theoretically would allow small bodied primates to live off a diet of difficult to digest polysaccharide, while simultaneously taking advantage of high quality resources to meet immediate energy needs (Caton et al. 1996). While more research is needed to confirm this within *Nycticebus*, our results do not contradict this. One adaptation some herbivores have, mostly Artiodactyl ruminants and rodents, is the ability to selectively retain particles or liquid digesta. Depending on the species, this could theoretically allow the nutrient rich liquid digesta to be kept in the intestines longer while expelling the nutrient poor cellulose particles (Espinosa-Gomez et al. 2013). Conversely, herbivores may maintain the particles for fermentation while passing the liquid fractions more rapidly. Separate methodologies are necessary to evaluate liquid vs particulate digesta rates, and were not undertaken in this study. Lowland anoas (*Bubalos depressicornis)* have an average particle MRT of 39 h and 25 h for fluid digesta (Flores-Miyamoto et al. 2005). These small bovines preferred to keep the fibre particles that they could ferment and expel the liquid to not waste precious space that could be filled with fermentable fibres. There was no difference in the liquid compared to particle passage rates for herbivorous *Propithecus coquereli*, *P. tattersalli*, *Ateles spp., Gorilla gorilla*, *H. griseus* nor all studied callitrichids (Campbell et al. 1997; Edwards and Ullrey 1999, Espinosa-Gomez et al. 2013; Perrin 2013; Power 1991; Remis and Dierenfeld 2004). By inference, we did not expect slow lorises to show a significantly different particle to fluid digesta passage rate. The increase in fibre led to a significant increase in MRT, but not in TT, which may allude to there being a slight difference in how slow lorises sort their digesta.

6.4.6 Slowest Relative MRT of all Primates

Foregut fermenting primates (*Colobinae*) had a TT ranging from 14.0 to 38.0 h (Dierenfeld et al. 1992; Kay and Davies 1994; Edwards and Ullrey 1999; Kirkpatrick et al. 2001; Sakaguchi et al. 1991). This is necessary since their diet is composed almost entirely of high fibre plant parts requiring fermentation. Increasing the fibre content in their diet also increases MRT according to Edwards and Ullrey (1999a,b). With fibre being their main source of energy, we expect their gut physiology to be able to adapt according to the fibre contents of their diet. However long the MRT of colobine primates may be, cercopithecines have the longest reported gut retention times (according to trials contrasting both absolute and relative values across the primate order), reaching up to 43.0 h for a 5.9 kg *C. mitis (*Lambert 1999). With the results of this study, we now posit that the slow lorises may now have the slowest gut passage relative to size within Primates, with a last appearance of marker at 59 h for a 1.05 kg *N. javanicus* and an MRT of 39.5 h for a 0.450 kg *N. pygmaeus*. This has large implications for their captive care which is low or absent in gum fibre (Chapter III).

6.4.7 Captive Husbandry Implications

Nycticebus primates are fully adapted to a high fibre diet, and possible benefits of including gum in the diet, may be significant to their captive management (Campbell et al. 2001). Slow lorises in captivity suffer from many health issues including obesity, dental disease and kidney diseases as shown by our results of Chapter III (Cabana 2014; Cabana and Nekaris 2015; Debyser 1995; Fuller et al. 2013). Overall, captive diets are lacking or low in gum (and coincidentally fibre) and are high in soluble carbohydrates. A longer MRT value means an increased opportunity for fermentation, resulting in potentially higher concentrations of available short-chain fatty acids (Blaine and Lambert 2012; Lambert and Fellner 2012). This longer digestion time may also lead to more time for digestive enzymatic action and more contact time with intestinal villi. Not only

can this result in better digestive efficiencies for many nutrients, but also the physical properties of volatile fatty acids created at the end point of fermentation have long been associated with gut health benefits (Plaami 1997). Acetate, propionate and butyrate are created by lactate metabolising bacteria (Lambert and Fellner 2012). Optimal presence of these acids may contribute to positive gastrointestinal cell proliferation and increased substrate for cellular energy production, as well as a more stable luminal pH which allows bacterial metabolic functions to be most efficient (Walker and Bucklet 2006). It can also have a protective effect against potential pathogens and diarrhoea, and reduce the negative effects of high soluble carbohydrates within the diet (Bailey and Coe 2002; Johnson et al. 1984). Coincidentally, the components which most affected the MRT in *Alouatta palliata mexicana* were soluble sugars and condensed tannins (Espinosa-Gomez et al. 2013). In our study, sugar content decreased by an average of 5 %, perhaps also explaining why we observed such high MRT increases. Increasing fibre within the diet may also increase animals' gut fill and satiety, possibly reducing stereotypies and other abnormal health patterns (Britt et al. 2015; Remis and Dierenfeld 2004). There are no obvious downsides to increasing fibre in the diets of captive exudativorous primates, and a plethora of possible benefits.

6.5 CONCLUSION

Slow lorises, like marmosets, show an exudativorous response which is similar to the folivorous response of increasing MRT values when fibre is increased in the diet. However these results do come with caveats. The markers used were not as sensitive as other available methods. Due to the dearth of physiological knowledge about *N*ycticebus, these results are nonetheless useful for future comparisons and to influence captive care. Future studies should consider repeating diet manipulations with exudativorous primates by only modifying fibre and not other nutrients to have more robust conclusions.

We have now begun to link our wild and captive slow loris research. Fibre that the wild slow lorises eat is an important component of captive diets as well and every effort must be made for gum to be used. Will insects also show to be equally as necessary within the diet?

CHAPTER VII

"Can slow lorises use insect chitin as an energy/nutrient source?"

or

CHITIN DIGESTING MICROBES FOUND IN THE GUT MICROBE COMMUNITY OF SLOW LORISES

7.1 INTRODUCTION

7.1.1 Chitin as a Polymer

Chitin is a complex structural carbohydrate that consists of linked N-glucosamine monomers (Gkargkas et al. 2004). It serves as the main component in the exoskeleton of arthropod animals, and is also an important component of fungal cell walls. Serving as protective and structural in nature, chitin is very stable due to beta linkages, and can only be digested by targeted chitinolytic enzymes. Chitinase is one such enzyme. Some organisms secrete their own chitinolytic enzymes (endochitinase) while others use the enzymes gernerated by symbiotic microbes inhabiting their large intestines (exochitinase). These enzymes are necessary for the complete degradation of chitin polymers to the end point of N-acetyl D-glucosamine solution that can be used as energy (Gkargkas et al. 2004).

7.1.2 Chitinolytic Enzymes

Chitinolytic enzymes can be obtained from several groups of organisms, such as bacteria, fungi, plants, protozoa, insects, and vertebrates (Adrangi and Faramarzi 2013). Chitinases are among a group of proteins that insects use to digest the structural polysaccharide chitin in their exoskeletons and gut linings during the moulting process (Fukamizo 2000). Some vertebrates have been confirmed to digest at least a small portion of chitin found in their arthropod prey, such as rats (Crane 1968), mice digesting up to 27.6% (Jeuniaux and Cornelius, 1978), musk shrews (*Suncus murinus*) and pygmy hedgehog tenrecs (*Echinops telfairi* and *Atelerix albiventris*) digesting up to 19.8% (Allen 1989; Graffam et al. 1998). Out of these four species, only the hedgehogs (*Atelerix* spp.) are known to ingest insects whole, including the more chitinous legs and wings, whereas the rats, mice and shrews are known to select less chitinous body parts (Jeuniaux and Cornelius 1978). Seabirds and fish are reported to have very active chitinolytic activity, being able to digest up to 56.8% of ingested chitin (Jackson et al. 1992). Thus far, the only primate confirmed to have chitinolytic ability is the Goeldi's

monkey, *Callimico goeldii* (Macdonald et al. 2013). These small New World primates are known to hunt insect prey as well as consume fungi in the wild, rendering them ideal models for chitin digestion studies.

Some microorganisms possess genes to produce chitanase enzymes (Khan and Khan 2011). Microorganisms known to produce chitinase enzyme thus far include: *Aspergillus terreus* (Ghanem et al. 2010), *Serratia marcescens* SMG (Das 2011), *Aeromonas* (Sitrit 1995), *Alteromonas* bacteria groups (Tsujibo et al. 1993), *Bacillus* (Watanabe et al. 1990), *Serratia* (Jones et al. 1986), *Streptomyces* (Blaak and Schrempf 1995), *Enterobacter* (Chernin et al. 1995) and *Vibrio* species (Bassler et al. 1991). Chitinase production has been isolated from different environments including soil (Kuddus and Ahmad 2013; Khan and Khan 2011; Wang and Chang 1997), clam shell wastes (Wang and Hwang 2001), sea sediments (Annamalai et al. 2010), soil samples from hot springs (Dai et al. 2011), rhizopores of the chili plant (Narasimhan and Shivakumar 2012) and faeces, as secondary metabolites of bacteria.

7.1.3 Chitinolytic Activity in Primates

An ideal group of primates to study chitinolytic activity are the slow lorises (*Nycticebus* spp.). These primates spend between 20 and 45% of their feeding time consuming insects with the Javan slow loris *N. javanicus* being on the lower end of this spectrum (Chapter 4, Nekaris 2014). Contrary to other primate species, they ingest the entire insect, and do not remove the chitinous wings or legs, as I observed in Chapter 4. With their main food item comprising tree exudates (gum), which require microbial fermentation for digestion, the slow lorises are expected to have a diverse gut flora, perhaps capable of chitin digestion as well (Cabana et al. in press). The presence of chitinolytic activity in mammals, including humans, has been described as an immune defence system against pathogenic fungi, and the role chitinolytic enzymes may play in mammalian nutrition warrants further investigation (Bussink et al. 2006; Boot et al. 1995), particularly if, like the

Goeldi's monkey, the slow lorises are able to exploit this resource. Assuming their slow metabolism is an adaptation to survive on a low quality diet (Nekaris 2014), using microorganisms to help digest food without necessitating any extra energy may be a valid strategy for these primates.

With exoskeletons of arthropods representing a substantial source of potential energy, calcium and nitrogen, which may be coveted resources in the slow loris ecology due to their low metabolism, this study aims to determine if slow lorises (using *N. javanicus* as a model species) possesses gut microbes capable of degrading chitin, like we predict they possess. We will also estimate the potential of their chitinolytic activity to inform us on how important an energy source chitin may be for them.

7.2 MATERIALS AND METHODS

7.2.1 Study Location

We collected faecal samples opportunistically from two wild *Nycticebus javanicus* (one male and one female) from the agroforests surrounding the village of Cipaganti, Garut District, West Java, Indonesia while the animals were handled to change radio tracking collars. Individuals defecated directly into the sterile sample storage container. A further two samples were obtained from two wild born *N. javanicus* currently kept in captive conditions at Cikananga Wildlife Rescue Centre, Sukabumi, West Java, Indonesia, and resident there for the previous two years. Fresh faecal samples were collected from the center of the bolus on the ground while wearing sterile latex gloves using a sterile toothpick, approximately five minutes after defecation, and placed inside the sterile sample container. Immediately after collection, collection vials were placed in a cooler box and brought to the laboratory at the Department of Mikrobiologi at the Universitas Negeri Jakarta Department of Biology, Faculty of Mathematics and Natural Sciences for storage at -20 ºC pending analysis. Captive individuals were fed a diet composed mainly of fruit (400g of various fruits including bananas, guava, papaya,

mangosteen, rambutan and pear) and two portions of insects (5g each portion of various insects including sagu larvae, katydids, grasshoppers, crickets and mealworms).

7.2.2 Preparation of Colloidal Chitin and Colloidal Chitin Agar

A colloidal chitin substrate was used to induce chitinase activity from microorganisms as previously demonstrated by Jagadeeswari et al. (2012) and Widyastuti (2010). Twelve grams of shellfish chitin powder (Himedia Inc., Indonesia) were added into 400 ml of concentrated HCl (37%) for 2 hours, stirred continuously in an ice bath. NaOH (10 N) was then added, stirring rapidly, and the solution kept at 4°C overnight. The precipitate was collected by centrifugation at 4800 rpm for 30 min at 4°C, and then washed with sterile distilled water until the colloidal chitin became neutral (pH 7.0). Colloidal chitin agar (CCA) was made with composition KH_2PO_4 0.1%, $MgSO_4.7H_2O$ 0.05%, yeast extract 0.1 %, colloidal chitin 1%, agar 2% and 1 litre Aqua Dest.

7.2.3 Isolation of Chitinolytic Bacteria and Assessment of Chitin Degrading Potential

Bacteria were isolated from the faecal samples by placing one gram of faeces into 30 ml sterile distilled water (in a 50 ml conical tube) and vortexing at 2000 rpm for 10 min (Sjamsuridzal et al. 2013; Zhang et al. 2013). A suspension of 0.1 ml of faeces was innoculated directly onto 1% colloidal chitin agar medium in three replicates. The isolation medium contained 1% colloidal chitin, 0.1% peptone, 0.1% KH2PO4, 0.05% MgSO4.7H20 and 2% bacto agar modification. Plates were incubated at 30°C for three days, after which all single colonies were picked up using sterile toothpicks and placed into new plates to create colony libraries. The representative colonies of each isolate were purified at least twice on nutrient agar (NA), being maintained on NA slants. The completed isolates were preserved at – 20 ^{0}C. Due to many known chitinolytic bacteria being facultative

anaerobes, experiments were conducted in an aerobic environment, following Macdonald et al. (2013).

Preliminary testing was done on 1% colloidal chitin agar (CCA) medium. Bacterial isolates that were 24 hours old were inoculated onto a petri dish containing the 1% CCA medium, forming 0.5 cm X 0.5 cm streaks. Each petri dish was separated into 4 quadrants, and cultures were incubated for 48 hours at 30°C. If a clear zone formed around a colony, the colony was identified as chitin degrading. The representative bacteria were scored according to the following scale: + (the clear zone ranged between 0.1 and 0.79 cm); ++ (the clear zone ranged between 0.80 and 0.89 cm); +++ (the clear zone ranged between 0.90 and 0.94 cm); ++++ (the clear zone ranged between 0.95 and 1.91cm); - (there was no clear zone). The chitinolytic index equation (Equation 7.1) was used to estimate the chitinolytic potential of the different isolates (Pratiwi et al. 2015).

(Equation 7.1) $CI=d_c-d_b$

Where CI is the chitinolytic index, d_c is the diameter of the clear zone formed around the colony in mm, and d_b is the diameter of the bacterial colony. The clear zone demonstrates the number of N-acetyl glucosamine monomers formed from the lysis of chitin by chitinase (Pratiwi et al. 2015).

The bacterial population was assessed by optical density using a spectrophotometric technique. The bacterial cell density was calculated by culturing the bacteria on slanted agar and suspended using Aqua Dest. The bacterial suspension was then assessed at a wavelength of 600 nm.

To determine the level of chitinolytic activity, potential isolates were grown in 1% CC broth at 30 °C, and incubated for 24h at 30 ^{0}C (Soeka and Sulistiani 2012). Each sample was centrifuged at 10.060 × *g* for 5 min to create the supernatant. Chitinolytic activity was determined by incubating 0.5 mL of culture supernatant with 0.5 mL of 1% colloidal chitin in a 0.05 M phosphate buffer, pH 7.0 at 50 °C for 30 min. When enzymatic reaction ceased, made obvious by a static

clear zone, the mixed supernatant was boiled at 100 ^{0}C for 5 min. Each sample was then centrifuged at 10.060 × *g* for 5 min. 250 µl supernatant was added with 50 µl potassium tetraborat and boiled at 50 °C for 3 min. The tubes were cooled and 1.25 mL of p-dimethylaminobenzaldehyde was added to each tube. This supernatant was incubated at 37 ^{0}C for 20 min. Absorbance was read within 10 min at 585 nm against the control solution of distilled water without enzymatic action. One unit of chitinase was defined as the amount of enzyme which released 1 µM N-acetyl-D-glucosamine per hour under the conditions of the study. The pH of all samples was also recorded.

The macroscopic morphology of the bacterial colonies was observed in NA medium by incubating them for 24 hours at 28 ˚C. Shape, color, margins, gram staining and elevation of the colonies were all recorded.

7.2.3 Statistics

Shapiro-Wilks test for normality was used to determine the distribution of the CI values for each isolate of the wild and captive samples. Since the data were not normally distributed, the non-prametric Mann-Whitney U test was used to determine if there was a significant difference between the captive and wild samples. All statistical tests were conducted using SPSS version 23.00 (IBM, USA).

7.3 RESULTS

7.3.1 Isolation of Bacteria from Nycticebus Javanicus

1,085 isolates were successfully cultured from Javan slow loris faecal samples, with 430 of those isolates being from wild slow lorises and 655 isolates from captive slow lorises. The clear zone seen on 1% CCA medium demonstrated that the bacteria had the ability to produce a chitinolytic enzyme. The isolates with the strongest potentials were chosen, which totals 71 representative isolates from the wild (34 isolates) and captive (37 isolates) faecal samples. The ability to

produce chitinase (identified by the length of the clear zone in cm) was high for 43 isolates (6 wild and 37 captive) (Tables 7.1and 7.2).

Table 7.1 Bacterial isolates with the ability to produce chitinase from free-ranging Javan slow lorises (*N. javanicus)* for each of the first four days, incubated at 30^0C in 1% colloidal chitin.

No.	Isolate code	Length of clear zone (cm/day)				Chitinase Potential
		H1	H2	H3	H4	
1	T67	0.2	0.47	0.76	1.06	++++
2	T65	0.22	0.42	0.73	1.05	++++
3	T37	0.26	0.44	0.84	1.05	++++
4	T63	0.16	0.58	0.67	1	++++
5	T68	0.12	0.54	0.72	0.97	++++
6	T56	0.15	0.48	0.7	0.95	++++
7	T50	0.19	0.42	0.75	0.94	+++
8	T51	0.17	0.52	0.83	0.93	+++
9	T77	0.25	0.47	0.76	0.93	+++
10	T57	0.14	0.45	0.7	0.93	+++
11	T61	0.21	0.45	0.73	0.92	+++
12	T79	0.22	0.53	0.71	0.92	+++
13	T60	0.25	0.57	0.8	0.92	+++
14	T4	0.24	0.48	0.71	0.92	+++
15	T59	0.21	0.44	0.67	0.91	+++
16	T49	0.23	0.5	0.77	0.9	+++
17	T74	0.18	0.46	0.76	0.9	+++
18	T71	0.19	0.48	0.83	0.9	+++
19	T73	0.12	0.45	0.64	0.89	++
20	T52	0.19	0.49	0.72	0.89	++
21	T75	0.18	0.51	0.8	0.88	++
22	T69	0.25	0.48	0.83	0.88	++
23	T54	0.18	0.58	0.75	0.86	++
24	T78	0.25	0.53	0.66	0.85	++
25	S39	0.2	0.51	0.65	0.83	++
26	T55	0.17	0.4	0.81	0.82	++
27	T72	0.19	0.48	0.62	0.82	++
28	T58	0.24	0.47	0.7	0.82	++
29	T53	0.17	0.39	0.68	0.81	++
30	T70	0.14	0.48	0.76	0.77	+
31	T64	0.23	0.28	0.62	0.76	+
32	T76	0.14	0.41	0.61	0.72	+
33	T66	0.19	0.47	0.69	0.64	+
34	T62	0.16	0.39	0.63	0.53	+

Table 7.2 Bacterial isolates with the ability to produce chitinase from captive Javan slow lorises (*N. javanicus)* for each of the first four days, incubated at 30^0C in 1% colloidal chitin.

NO	Isolates code	Length of clear zone (cm/day)				Chitinase Potential
		H1	H2	H3	H4	
1	C1	0.60	1.25	1.51	1.91	++++
2	C56	0.60	1.25	1.51	1.91	++++
3	C100	0.70	1.42	1.59	1.91	++++
4	C97	0.56	1.09	1.51	1.90	++++
5	C25	0.63	1.15	1.51	1.90	++++
6	C13	0.62	1.12	1.55	1.90	++++
7	C3	0.62	1.19	1.52	1.83	++++
8	C30	0.55	1.12	1.44	1.83	++++
9	C6	0.72	0.52	1.32	1.82	++++
10	C26	0.62	1.08	1.50	1.81	++++
11	C40	0.55	1.21	1.45	1.80	++++
12	C92	0.64	1.25	1.50	1.76	++++
13	C11	0.61	1.17	1.51	1.75	++++
14	C19	0.57	1.08	1.42	1.73	++++
15	C63	0.13	0.83	1.21	1.63	++++
16	C88	0.18	0.63	1.11	1.61	++++
17	C59	0.20	0.83	1.18	1.61	++++
18	C60	0.23	0.75	1.23	1.59	++++
19	C85	0.42	0.68	1.15	1.59	++++
20	C69	0.27	0.80	1.12	1.57	++++
21	C39	0.24	0.67	1.11	1.54	++++
22	C17	0.21	0.82	1.17	1.54	++++
23	C53	0.18	0.74	1.06	1.54	++++
24	C22	0.33	0.74	1.02	1.53	++++
25	C2	0.20	0.74	1.07	1.53	++++
26	C442	0.20	0.75	1.16	1.52	++++
27	C61	0.26	0.76	1.11	1.52	++++
28	C90	0.27	0.77	1.16	1.51	++++
29	C68	0.18	0.76	1.16	1.50	++++
30	C87	0.21	0.78	1.08	1.48	++++
31	C73	0.23	0.77	1.07	1.47	++++
32	C15	0.13	0.72	1.06	1.46	++++
33	C55	0.42	0.87	1.09	1.44	++++
34	C70	0.12	0.54	0.92	1.43	++++
35	C12	0.52	0.83	0.90	1.21	++++
36	C10	0.49	0.84	1.28	1.13	++++
37	C33	0.47	0.93	0.98	1.11	++++

7.3.3 Assessment of the Bacterial Isolates' Ability to Produce Chitinase Enzyme Based on the Chitinolytic Index

We assessed 22 bacterial isolates with the largest CI values (14 isolates from captive slow lorises and 8 isolates from wild slow lorises). There were variations in the results of the chitinolytic index assessment based on clear zone diameter: 2.23-3.27 cm range (4.55%), 1.52-2.22 cm range (22.73%), 1.02-1.50 cm range (13.64%), 0.88-0.99 cm range (9.10%), 0.50-0.59 cm range (18.18%), 0.42-0.49 cm range (9.10%), 0.32-0.36 cm range (9.10%), and 0.21-0.27 cm range (13.64%) (Table 7.3). The average chitinolytic index after 48 hours of incubation revealed that isolates C60, C1, C25, C90, C33, C97, and C59 produced chitinolytic enzymes that could degrade the chitin substrate faster than the other 15 isolates. The CI values were not normally distributed (P=0.0001) and were significantly different with the captive isolates having a significantly higher CI than the wild group ($U=60.062$, $n_1=14$; $n_2:8$, $P=0.0001$).

Table 7.3 Chitinolytic index of bacterial isolates from wild and captive Javan slow lorises (*N. javanicus*) in colloidal chitin agar medium at1% concentration, incubated for 48 hour at 30 ^{0}C

No.	Isolate code	CI 1	CI 2	CI 3	CI 4	Average CI (cm)±SE
1	C60	3.41	3.85	2.85	2.97	3.27±0.23
2	C1	1.89	2.50	3.31	1.19	2.22±0.45
3	C25	1.66	2.45	1.93	1.88	1.98±0.17
4	C90	2.38	2.28	1.78	1.36	1.95±0.24
5	C33	2.10	1.79	1.08	2.32	1.82±0.27
6	C97	1.48	1.54	1.55	1.52	1.52±0.01
7	C59	1.77	1.39	1.41	1.42	1.50±0.09
8	C2	1.13	1.27	1.31	1.09	1.20±0.05
9	C73	1.00	1.09	0.93	1.00	1.01±0.03
10	C56	0.50	1.16	1.43	0.89	0.99±0.05
11	C85	0.79	1.02	0.81	0.91	0.88±0.05
12	C100	0.26	0.31	0.41	0.29	0.32±0.03
13	C13	0.26	0.33	0.10	0.31	0.25±0.05
14	C6	0.22	0.23	0.20	0.19	0.21±0.01
15	T.68	0.72	0.53	0.58	0.54	0.59±0.04
16	T.65	0.63	0.53	0.54	0.60	0.58±0.02
17	T.63	0.56	0.64	0.57	0.53	0.57±0.06
18	T.50	0.65	0.50	0.41	0.44	0.50±0.05
19	T.51	0.59	0.44	0.56	0.39	0.49±0.05
20	T.56	0.34	0.44	0.54	0.37	0.42±0.20
21	T.67	0.29	0.25	0.39	0.49	0.36±0.45
22	T.37	0.32	0.29	0.30	0.16	0.27±0.04

Note: C: Isolated bacteria from Bogor (Captive); T isolated bacteria from Garut (Wild); CI: Chitinolytic index; SE= standard error

7.3.4 Assessment of Chitinase Activity of Bacteria from the Faeces of the Javan Slow Loris

Twelve bacterial isolates were selected based on their chitinolytic index: the isolates with the highest indices from the two locations (6 isolates from captive and 7 isolates from wild slow loris) were chosen. The crude chitinase activity of the six bacterial isolates of captive origin (C90; C97; C25; C60; C1; and C33) showed the following activity: 21.64, 11.64, 9.67, 7.68, 6.88 and 2.84 (10^{-3} unit/mL) respectively. The crude chitinase activity of the six bacterial isolates isolated from the wild animals (T51, T56, T65, T68, T50 and T63) showed the following

activity: 1.12, 0.51, 0.51, 0.49, 0.28 and 0.07 (10^{-3} unit/mL) respectively (Table 7.4). The best results for protein content and chitinase activity were demonstrated by isolate C90. The bacterial isolates C97 and C90 had a tendency for higher values than the averages of all assessment parameters. Isolates T51 and T50 from the wild individuals showed the highest results of all the bacterial isolates collected from wild individuals (Table 7.4). The bacterial isolate C97 had higher results than the average in all the assessment parameters and was from a captive individual.

Table 7.4 Measurement parameters for bacteria producing chitinase, isolated from slow loris (*N. javanicus*) faeces in Indonesia.

Isolate from	Isolate code	Chitinase activity ($X10^{-3}$U/ml)	Chitinoytic index (cm)	Cell density (OD 600nm)
Captive	**C97^{++++}**	**11.64**	**1.52**	**0.08**
	C90^{+++-}	**21.64**	**1.95**	**0.06**
	C60^{+++-}	7.68	3.27	0.05
	C25^{++--}	9.67	1.98	0.05
	C1^{++--}	6.88	2.22	0.06
	C33^{+---}	2.84	1.82	0.05
Wild	**T.51^{++--}**	**1.12**	**0.49**	**0.11**
	T. 50^{++--}	**0.28**	**0.50**	**0,11**
	T.63^{+---}	0.07	0.57	0,08
	T.65^{----}	0.51	0.58	0.05
	T.56^{----}	0.51	0.42	0.05
	T.68^{----}	0.49	0.59	0.04
Average parameter test		5.28	1.33	0.07

7.3.5 Macroscopic and Mcroscopic Assessment of the Bacterial Isolates from Slow Loris Faeces

Observations of the macroscopic and microscopic morphology of the bacterial isolates were conducted on the 12 bacterial isolates that had the highest chitinolytic potential (Table 7.5). The macroscopic morphological observations of the bacteria included the color, shape, and margins of the colonies. The bacteria within the captive samples are more homogenous than the wild samples which show more

diversity. The faeces from the wild had a pH ranging between 6 and 7, while faecal samples from captivity had a pH ranging between 5 and 6.

Table 7.5 Macroscopic and microscopic characteristics of 12 chitinase bacterial isolates obtained from slow loris (*N. javanicus*) faeces in Indonesia.

No.	Isolate code	Colour	Colony Form	Margin	Cell Form	Gram staining	Spore
1	T67	White	Curled	Ciliate	Rod	Positive	Present
2	T65	White	Curled	crenate	Rod	Positive	Present
3	T37	White	Curled	undulate	Rod	Positive	Present
4	T63	White	Circular	undulate	Rod	Positive	Present
5	T68	White	Curled	entire	Rod	Positive	Present
6	T56	White	Circular	entire	Rod	Positive	Present
7	C97	Dark carmine	Circular	entire	Coccus	negative	Absent
8	C25	Dark carmine	Circular	entire	Coccus	negative	Absent
9	C1	Dark carmine	Circular	entire	Coccus	negative	Absent
10	C90	Light carmine	Circular	entire	Coccus	negative	Absent
11	C60	Dark carmine	Circular	entire	Coccus	negative	Absent
12	C33	Pink madder	Circular	entire	Coccus	Positive	Absent

7.4 DISCUSSION

This investigation shows that it is likely that the Javan slow lorises are able to degrade some amount of chitin from their insect prey. Having chitin degrading bacteria as part of the slow loris gut microbial community would allow for the utilisation of chitin as a source carbon and potentially nitrogen (Woo and Park 2003). The wild and captive individuals investigated in this study have different chitinolytic gut microbe populations, with the captive populations having a stronger capacity for degrading chitin. Our captive population of slow lorises appear to be able to harness a larger amount of energy from ingested chitin. This was expected as the small amounts of insect chitin found within faeces were barely enough to identify insects and never reconstituted the entire insect (Rode-Margono et al. 2014). We have observed the slow lorises ingesting insects whole (Chapter IV) which means some chitin must have been degraded within their digestive system. We also know that they can digest fibre and therefore must possess a

specialised gut microbe community (Chapter VI), perhaps with some capable of digesting chitin.

7.4.1 Javan Slow Lorises Digesting Chitin

It was expected that the exudativorous Javan slow loris have chitin digesting potential. They have a slow food passage rate, on average 32 hours, (Cabana et al. in press), perhaps allowing even weak chitinolytic activity to be significant enough to contribute to their overall nutrient and energy intake. Insects may contain up to 75% chitin, which provides ample evolutionary advantage to insectivorous species capable of digesting chitin (Strobel et al. 2013). With insects being on average 20% of annual dietary intake for slow loris, if 100 g were ingested nightly, up to 38 extra kcal/night may be harnessed if it is assumed that half of the chitin is digested (Whitaker et al. 2004). This is a significant amount, especially for female lorises that face additional reproductive energetic costs. Chitinolytic activity in the hind gut could also serve an immune function, however its activity in the hind gut may be limited as any potential fungal pathogen would have had ample opportunities to infect the host slow loris before arriving in the hindgut (Boot et al. 1995; Bussink et al. 2006). Also, the clear zone obtained from the chitinolytic microorganisms may still contain chitosan, cellobiose, or other derivatives which were not assessed (Zarei et al. 2012). Although chitin may have been degraded, the bulk of the energy and nutrients may still remain unavailable unless cellulobiase(s) are also being secreted. If chitinolytic microorganisms are present, the odds of having cellulobiase producing organisms are highly probable (Jackson et al. 1992). Some species are able to digest all compounds sequentially (Chen et al. 2014). Our results are conservatively consistent with our hypothesis that slow lorises are able to digest chitin.

One of the most common chitinolytic groups found in nature and the intestines of animals are the *Bacillus* spp (Khan and Khan 2011). They are known to produce a large amount of extracellular chitinase which has been shown to hydrolyse chitin, glycol chitin and chitosan with relative activities of 76%, 34%

and 23% (Chen et al. 2014). These bacteria are widespread throughout nature (Khan and Khan 2011; Seo et al. 2014). Most often they are found in soil, digestive tracts, and mangove ecosystems (Kamil et al. 2007). All identified species of *Bacillus* thus far produced the largest amount of chitinase, which is consistent with the chitinolytic bacteria found in other mammalian hindguts (Ivanov et al. 2003; Usharani and Gowda 2010; Zulfaidah et al. 2013).

7.4.2 Captive versus Wild

The isolates with the largest CI ability were from the captive slow lorises. The bacterial isolates from captive individuals were faster and/or more efficient in degrading the chitin substrate in the 1% CCA medium. The bacterial isolates from the wild lorises had a degrading ability that ranged between 0.36 and 0.59 cm. The difference in the number of isolates obtained from the wild and captive samples may be due to the differences in treatments of the faecal samples during both storage and sample handling. Storage temperatures strongly affect the number of microorganisms (Carroll et al. 2012; Sukmawati 2014); due to the different locations and larger distance to the lab, the wild samples may have reached higher temperatures than the captive samples. The captive samples were collected from the ground where they may have been for a maximum of five minutes. Although a relatively short amount of time, this time may have been enough to also collect chitinolytic microbes that lived on the ground that moved into the faeces.

The chitin degradation rate of a bacteria would be most efficient under conditions for favourable for this bacteria (temperature, amount of oxygen, pH, substrate available etc.). The study conditions outlined in this paper were standard for experiments measuring chitinolytic activity; however, it is possible that bacterial species were unintentionally selected for that were optimally adapted for 30°C and1% substrate, cultured under aerobic conditions. The strong chitinolytic potential from the captive slow lorises may also have been influenced by the faecal pH. The wild samples had a near neutral pH and the captive samples had a more acidic pH, possibly due to a high fruit diet. The bacteria's environmental pH would

have influenced the species of microbes thriving and effectiveness of the enzymes, in turn affecting each isolate's chitinolytic index (Das et al. 2012; Hou et al. 2014). The nutrients ingested by an animal would directly affect the available substrates for microbes, creating a selection pressure, but may also modify the gut lumen environment, further altering the species capable of thriving (Knarreborg et al. 2002). The wild slow lorises were ingesting diets of gum, insects and occasionally other plant parts (Cabana et al. in press) while the captive slow lorises had a diet comprising fruit and insects. These two diets potentially led to different nutrients being absorbed, with substrates favoring different chitinolytic microbes (Jackson et al. 1992). The wild lorises may have ingested more fibre fractions (due to gum) and less soluble carbohydrates, cultivating a more neutral pH while the highly soluble carbohydrate concentrations in the captive slow loris diets led to a more acidic environment. Chitin can improve the dietary efficiency of protein degradation which supports the use of insects in captive diets (Spreen et al. 1984). The more acidic environment of the captive slow loris fecaes appeared to select for bacteria with strong chitinolytic potential that are able to be cultured aerobically.

7.5 CONCLUSION

We discovered the presence of chitinolytic bacteria in the faecal samples of both wild and captive Java slow lorises. With the limitations of the methods, it is acknowledged that although there will be a difference in microbial populations between wild and captive populations, the results presented here may be biased. However, the Javan slow loris possesses the potential to digest some amount of chitin, which is reflective of its feeding ecology. Future research should attempt to quantify how much energy may be extracted, using the slow loris microbiome(s). Captive slow lorises may benefit from the feeding of insects rather than concentrate feeds, eggs, or meat to fulfill their protein requirements.

Slow lorises indeed may digest chitin which may act as substrate for microbial degradation and have further effects on disease prevention and nutrient absorption,

similar to the effects of a healthy gut mentioned in Chapter VI. Now we understand that providing both gum and insects are important food items within the diet of the slow lorises. Now we must combine chapters III to VII to conduct the diet trials which has been our goal all along.

CHAPTER VIII

"Can free-ranging slow loris nutrient intakes be used as a framework for an appropriate captive diet which recreates the same physiological reactions as those of wild slow lorises?"

or

BREAKING THE ICE: THE VALIDATION OF FREE-RANGING SLOW LORIS *(NYCTICEBUS)* NUTRIENT INTAKE TO CREATE NUTRIENT RECOMMENDATIONS FOR CAPTIVE NUTRITION

8.1 INTRODUCTION

8.1.1 Primate Nutrition

Feeding wild animals in captivity is a definite challenge due to their estimated nutritional needs being based on models species (O'Sullivan et al. 2013). Nutrient recommendations have been developed primarily for domestic animals or laboratory species because of the extensive sample size(s) available. Non-human primates were originally designated to one of two nutritional models, old world monkey (OWM) nutrient requirements which is based on the rhesus macaque (*Macaca mulatta*) or the new world monkey (NWM) which is based on the common marmoset (*Callithrix jacchus*) (NRC 2003). Both of these original groupings comprise many species which are distantly related and have very different physiologies, behaviour and ecology. The OWM group, in particular, includes a large variety of primate taxa which have been shown to not fit the original OWM model, such as Lemuridae (Junge et al. 2009; Donadeo et al. 2016; Dierenfeld and McCann 1999), Colobinae (Nijboer and Dierenfeld 1996), Hominidae (Crissey et al. 1999; Hoffer 2016; Less et al. 2014) and Lorisidae (Williams et al. 2015). There is increasing evidence that the majority of primate taxa may havespecific /unique nutritional requirements and using a "one model approach" may not be appropriate (NRC 2003). Stepsirhines are particularly to be considered, especially *Nycticebus* due to their unique exudativorous feeding ecology and abundance of health issues observed in captivity (Cabana and Nekaris 2015).

8.1.2 Challenges of Feeding Exudativores

Slow loris primates (*Nycticebus spp.)* have a morphology and physiology adapted to consume and exploit plant gums as a staple food source (Nekaris 2014). Their dentition is specialised to incisiform canines to form a tooth comb, as well as procumbent tusk like pre-molars (Kubota and Iwamoto 1967). They have a long narrow tongue able to lap up nectar, or gum that has not yet dried (Coimbra-Filho

and Mittermeier 1978). Their gastrointestinal tract (GIT) is also described to be specialised, with a wide large intestine and a voluminous caecum, suggesting their capability for fermenting plant structural carbohydrates (Stevens and Hume 1995). These adaptations are convergent within the gum feeding marmosets (*Cebuella, Callithrix spp.*) (Smith 2010). Field research also confirms that gum is a year long staple food for the pygmy slow loris, which on average spends 30% of its foraging time on gum (*N. pygmaeus*: Starr and Nekaris 2013), 66% for the greater slow loris (*N. coucang*: Wiens 2002), 96% for the Bengal slow loris: (*N. bengalensis:* Das et al. 2014) and 52% of intake for the Javan slow loris (*N. javanicus*: Cabana et al., in review). These primates are maintained in captivity as illegal pets, popular within Japan, Russia, Indonesia, the Czech Republic and the United States (Nekaris and Jaffe 2007) and more importantly in zoos worldwide as well as Asian rescue and rehabilitation centres. In spite of the evidence for their exudativorous feeding ecology, this has not been represented in their captive husbandry.

8.1.3 Issues with current Nycticebus Feeding Regimes

Nycticebus primates are found in 79 accredited zoos worldwide (Zoological Inventory Management System, Species360, USA), most of which are being fed diets which do not optimally support their morphology, physiology or reflect wild diets or feeding ecology, which our data in Chapter III demonstrates (Cabana and Nekaris 2015; Fitch-Snyder et al. 2001; Fuller et al. 2013). Zoological institutions worldwide primarily feed these gummivores as frugivores with high amounts of fruits and concentrate feeds, and little if any, gum or insects (Chapter III; Cabana and Nekaris 2015). However, multiple studies have found a link between diet and health issues including kidney, dental, coat and gastrointestinal problems (Debyser 1995; Fuller et al. 2014). Approximately 60% of captive-held *N. pygmaeus* in European facilities may have dental health issues; and as we further explored in Chapter III, a further 51% of zoos and rescue centres worldwide holding slow lorises appear to have at least one affected individual (Cabana 2014; Cabana and

Nekaris 2015). Evaluated diets were high in sugars and starches, and contained low levels of fermentable fibres (acid detergent fibre: ADF; neutral detergent fibre: NDF; gums), which have been linked with the occurrence of dental disease (Chapter III; Cabana and Nekaris 2015). A controlled diet study trialing a naturalistic diet of gum, insects and nectar produced evidence that these primates are able to thrive on naturalistic diets (Cabana and Plowman 2014). The slow lorises in the study maintained a healthy weight and had an activity budget more similar to wild slow lorises, however no nutrient recommendations were used as developmental guidelines for this diet (Cabana and Plowman 2014).

There are currently no published nutrient recommendations for slow lorises. These Asian primates are classified generically as old world primates (Nekaris and Bearder 2011) and diets have thus been developed based on generic nutritional requirements for old world primates, primarily data derived from other African and Asian cercopithecine, pongid, and colobine primate species. The aim of this chapter was to determine more appropriate nutrient recommendation for slow loris species using feeding data from wild individuals. A sample diet based on the wild feeding data was trialed on captive slow lorises. As a proxy for wild animals, captive animals were fed the same food items we observed wild individuals ingesting, in similar proportions.

We measured the validation markers (apparent digestibility, food passage rate, and nutrient intake) throughout controlled feeding studies to determine if varying nutrients resulted in quantifiable differences between typical captive diets and diets based on natural feeding history. Diet suitability was evaluated with respect to best meeting the physiologic as well as behavioral needs of *Nycticebus* spp. Further, we aim to reproduce similar physiologic responses with the new diet as found in wild individuals.

8.2 MATERIALS AND METHODS

8.2.1 Study Groups

This chapter uses the intake rates calculated in Chapter IV (Table 4.2) to calculate the average daily nutrient intake of the javan slow loris (see section 2.5) to be used as the nutrient targets for captive diet trials. The second (experimental) component of this chapter consisted of controlled feeding trials with diet manipulations based on the observational data, utilizing captive slow lorises within CWRC (see section 2.3.1) and measuring changes in digestive physiology parameters, and forms the basis of this report.

8.2.3 Diet Trials

We collected data on the CWRC individuals during three diet interventions We recorded data on diet ingredients and nutrient intake, food passage rates and apparent digestibility while animals were offered three separate diets. Diet 1 consisted of their original diet, therefore no acclimatisation period was needed. Daily average amounts offered, per individual (regardless of species or weight), of Diet 1 included: katydids (*Tettigonidae* spp.) (3.4g), peeled oranges (18.3g), peeled banana (44.0g), mealworms (*Tenebrio molitor*) (4.9g), crickets (*Acheta domestica)* (1.3g), peeled rambutans (12.2g), hardboiled chicken egg without shell (2.2g), sapodilla without seeds (17.1g), honey (4.0g), mangosteen (12.9g) and pine beetle larvae (*Rhynchophorus ferrugineus*) (2.1g). We transitioned the slow lorises to Diet 2 over a one-week period, and animals were fed the full diet for two weeks before collecting any data. Diet 2 consisted of a wild-type diet, approximately 49% gum, 20% mixed insects, 2% nectar and 29% plant parts as per Cabana et al. (in review). We phased the animals to Diet 3 over one week, and fed it for a further 3 weeks before collecting data. Diet 3 comprises a new diet based on the nutrient intake of wild slow lorises, yet composed of food items readily available and affordable to Asian zoos and rescue centres. Diet 3, as offered per individual daily, consisted of mealworms (2.6g). crickets (6.9g), hardboiled chicken egg with shell (1.3g), palm

beetle larvae, pupae and adult mix (6.5g), sweet potato (8g), peeled, semi boiled cassava (6.8g), green beans (9.7g), semi-boiled carrots (2g) and gum arabic (10g made with 2:1 parts powder to water) – essentially replacing fruit with vegetable ingredients plus added gum. We regarded the data gathered during Diet 2 trials as providing “physiological targets" since diets provided the closest approximation for wild slow lorises. We begin with the assumption that wild physiological values are optimal. Intake studies were conducted with the captive lorises fed their current diet as baseline data for seven days as per section 6.2.2. All food items offered in the original diets at CWRC were sampled for nutritional analyses as per section 2.2.4.

8.2.5 Passage Rate Study

The food passage rate study was conducted based on section 2.3.3.

8.2.6 Apparent Digestibility Study

Feces were collected every day at clean-up time (1000 hr) and individual species’ feces were pooled to ensure adequate quantities for chemical analysis to determine apparent digestibility. We used the passage rate studies to link the correct faeces with the correct daily food intake quantities. We compared the total amount of macronutrients within the faecal samples versus the amounts ingested and used the equations described in Graffam et al. (1998) to calculate apparent digestibility (Equation 8.1). Where D_N is the apparent digestibility of nutrient N and N_i is the amount in g of nutrient N ingested, N_o is the amount in g of nutrient N in the faeces.

(Equation 8.1) $D_N = \frac{N_i - N_o}{N_i} \times 100$

8.2.7 Statistical Analyses

All statistical analyses were performed on SPSS version 22 (IBM, USA). We used a Generalised Linear Mixed Model (GLMM) analysis to determine if

species or diet had a main effect upon the nutrient intake data. The interaction between species and treatment was also analysed. The data were not normally distributed and assumed a Gamma distribution for all nutrients and analysed with a link identity function. Species and diet were used as fixed factors and cage number was a random factor. Factors which were significant were further analysed in a pairwise post-hoc test with Bonferroni corrections. The TT and MRT data were also not normally distributed, therefore a non-parametric Friedman test was administered to search for significant differences among the three diet treatments. All species were combined within this analysis as values were similar amongst the three species within the three different interventions, and there are no significant physiological differences between the three species (Nekaris 2014). Any significant results from the Friedman ANOVA were then analysed using a post hoc Wilcoxon Signed Rank Test.

8.3 RESULTS

8.3.1 Nutrient Intake of Wild Slow Lorises

The nutrient content of all food items analysed, including the items ingested by wild slow lorises, are shown in Table 8.1. Each main staple food item was obtained from one or two plant species. Gum was from an Australian acacia tree, *Acacia decurrens*, nectar from red fairy duster flower (*Caliandra catothyrsus)*, fruits from jackfruit (*Arctocarpus heterophyllus*), and persimmon (*Diospyros kaki*), flowers from eucalyptus (*Eucalyptus spp.*) and leaves from bamboo (*Gigantochloa cf. ater*). The average nutrient intake for free ranging *N. javanicus* is relatively high in protein and fibre fractions and low in fat and sugars (Table 8.2). Complete intake data available in appendices IV through VI.

Table 8.1a Nutrient content of food items analysed in the field. All values (except moisture) are on a dry matter basiS.

	Giganochloa cf. ater	***Eucalyptus spp.***	***Acacia Decurrens***	***Diospyros kaki***	***Arctocarpus heterophyllus***	**Insects**
Description	Young bamboo leaves only	Flowers only	Gum	Persimmon: Peel and pith removed	Jackfruit: Fruit flesh only, no seeds	Mixture of all species, in same proportion as ingested
Energy (kcal/100g)	3.38	3.55	1.81	3.85	3.93	4.94
Moisture (%)	71.5	63.72	54.64	79.31	72.66	54.53
Ash (%)	4.76	2.23	0.94	3.76	3.14	6.79
Protein (%)	9.71	4.4	3.74	8.74	3.8	63.55
Crude Fat (%)	0.97	2.62	0.83	0.5	0.35	7.72
WSC (%)	19.39	48.58	81.79	62.81	82.45	7.59
Soluble fibre (%)	0.66	0.92	10.55	0.58	x	x
ADF (%)	40.68	34.18	0.09	26.4	10.26	14.35
NDF (%)	65.17	42.17	12.7	24.19	10.26	14.35
Sugars (%)	1.4	6.4	<0.28	x	x	2
Vit A (IU A/g)	<0.5	<0.5	356.72	<0.5	11.09	2.16
Vit D (IU D/g)	<0.10	2.01	<0.268	0.91	x	x
Vit E (mg/kg)	<0.01	2.78	<0.37	2.28	5.6	438
Ca (%)	0.1	0.35	0.26	0.18	0.22	1.19
P (%)	0.17	0.1	0	0.06	0.01	0.66
Mg (%)	0.17	0.13	0.06	0.04	0.09	0.15
Cu (mg/kg)	9.6	11.2	3.2	2.6	5.8	36.1
Fe (mg/kg)	233	114	40.3	170	276	368
Na (%)	0.15	0.1	0.02	0.16	0.16	0.81

X: Not analyseD

Table 8.1b Continuation of nutrient content of food items analysed in the rescue centre for captive diets. All values (except moisture) are presented on a dry matter basis.

	Banana	**Tomato**	**Carrot**	**Passion Fruit**	**Green Melon**	**Snake Fruit**	**Papaya**	**Mango**	**Apple**	**Eggplant**	**Orange**
Description	Peel removed	Whole	Whole	Peel removed	Rind removed	Peel and seeds removed	Seeds removed	Skin removed	Whole	Whole	Peel removed
Energy (kcal/100g)	3.34	3.23	3.33	3.67	3.14	3.39	3.34	3.44	3.47	3.34	3.98
Moisture (%)	81.06	94.83	90.48	84.02	94.07	90.38	87.91	99.04	86.81	43.49	90
Ash (%)	4.83	12.67	7.8	6.58	10.41	3.34	5.01	2.93	1.98	5.78	4.43
Protein (%)	5.25	18.32	6.59	13.25	10.61	2.55	5.08	3.14	2.09	13.81	7.11
Crude Fat (%)	0.35	3.49	2.07	8.09	0.25	0.17	0.43	0.9	0.98	1	3.15
WSC (%)	78.41	48.02	68.39	37.69	71.58	88.61	83.58	89.12	79.25	58.62	74.97
soluble fibre (%)	0.68	0.21	0.5	0.31	0.95	0.45	0.3	0.75	0.33	0.31	6.79
ADF (%)	10.36	18.65	13.41	30.78	7.81	3.02	7.21	4.92	12.8	16.28	8.4
NDF (%)	11.16	17.5	15.15	34.39	7.15	5.33	5.9	3.91	15.7	20.79	10.34
Sugars (%)	14.6	2.28	4	6.59	5.55	5.25	9.59	9.19	9.39	3.23	x
Ca (%)	0.01	0.01	0.25	0.12	0.08	0.01	0.09	0.02	0.02	0.1	0.24
P (%)	0.04	0.43	0.28	0.12	0.47	0.03	0.07	0.07	0.01	0.24	0
Mg (%)	0.13	0.21	0.11	1.13	0.26	0.06	0.09	0.09	0.04	0.16	0.01
Cu (mg/kg)	4.9	14.3	6.5	9	7.4	7.4	3.7	4.3	5.6	10.6	0.58
Fe (mg/kg)	32	184	243	345	40.9	16	32.7	6.1	21.5	28	11.3
Na (%)	0.03	0.27	0.03	0.01	0.02	0.15	0.09	0.02	0.04	0.07	0

Table 8.1c Continuation of nutrient content of food items analysed in the rescue centre for captive diets. All values (except moisture) are presented on a dry matter basis.

	Mangusteen	**Guava**	**Gum Arabic**	**Sapondilla**	**Long Bean**	**Sweet Potato**	**Cassava**	**Mealworms**	**House Crickets**	**Grasshoppers**
Description	Peel removed	Whole	Refined; white powder	Peel and seeds removed	Whole	Whole	Skin removed	Farmed	Farmed	Wild caught
Energy (kcal/100g)	4.05	4.15	3.59	3.72	3.43	3.76	3.92	5.18	5.22	4.57
Moisture (%)	81.77	86.35	9.13	7.66	11.71	68.12	75	62.7	54.2	60
Ash (%)	1.38	3.38	4.42	2.54	3.56	14.73	4.01	5.27	4.58	4.5
Protein (%)	3.27	4.15	1.17	2.11	24.92	3.5	3.39	48.8	58.8	60
Crude Fat (%)	6.04	2.49	0	6.33	1.14	1.33	0.21	27.9	28.1	15
WSC (%)	49.74	47.48	81.71	45.03	48	59.14	82.19	5.24	-9.62	-0.52
soluble fibre (%)	5.18	30.71	0.21	10.5	1.27	8	0.95	1.66	0.58	0.2
ADF (%)	10.92	37.25	0.09	31.23	8.44	10.57	4.79	5.82	9.04	14.79
NDF (%)	39.57	42.5	12.7	43.99	22.38	21.3	10.2	12.79	18.14	21.02
Sugars (%)	x	x	x	13.9	3.3	4.5	3.4	0.42	0.2	2.1
Ca (%)	0.02	0	0.92	0	0	0.38	0.03	0.55	0.32	0.44
P (%)	0.01	0.01	0.01	0	0.12	0.52	0.7	0.78	0.8	0.23
Mg (%)	0.02	0.01	0.13	0.42	1.68	0.18	0.06	2.11	8.33	0.66
Cu (mg/kg)	1.48	1.2	3	1.5	6.6	10	4.6	11.3	15	28.2
Fe (mg/kg)	20.8	16.9	22	39.2	151	21	77	258	12.6	176
Na (%)	0	0	0.05	0	0	0.13	0.15	0.01	0.05	0.84

X: Not analysed

Table 8.2 Average daily nutrient intake of wild Javan slow lorises (*N. javanicus;* n=15) with a diet consisting mainly of gum, insects and nectar. These nutrient values also reflect the proposed dietary nutrient recommendations for *Nycticebus spp.*

Nutrient	Concentration (DM basis)	Nutrient	Concentration (DM basis)
Energy (Kcal/g)	3.15 (±0.48)	Ca:P Ratio	2.8:1
Crude Protein (%)	23.50 (±8.35)	Cu (mg/kg)	11.22 (± 1.4)
Crude Fat (%)	2.37 (± 1.04)	Fe (mg/kg)	69.16 (± 9.34)
Soluble Fiber (%)	10.67 (±7.86)	Mg (%)	0.37 (± 0.09)
ADF (%)	10.95 (±7.02)	Na (%)	0.38 (± 0.10)
NDF (%)	19.14 (±5.5)	Vit A (IU A/g)	2.06 (± 0.56)
Ash (%)	2.24 (±.94)	Vit D (IU A/g)	0.53* (± 0.23)
Ca (%)	0.45 (±0.23)	Vit E (mg/kg)	0.97* (± 0.36)
P (%)	0.16 (±0.11	Soluble Sugars (%)	3.33 (± 1.52)

*Data represented by less than 80 % of the ingredient

8.3.3 Intake Sudy of Three Dietary Treatments

The average daily nutrient intake of *N. javanicus* (n= 15)*, N. coucang* (n= 15) and *N. menagensis* (n= 4) on all three diet interventions (Diet 1=original diet reflecting diets fed in rescue centres and zoos, Diet 2= wild type based on the proportions of food items eating by wild slow lorises, Diet 3 = new diet based on proposed nutrient intakes) are shown in Table 8.3. Overall, new diets were highest in protein, fibre and minerals and lower in sugars and fat. The GLMM revealed that diet treatment had a significant effect on all nutrient intakes (crude fat: χ^2= 601.6 df= 2 P= 0.0001, crude protein: χ^2= 519.7 df= 2 P= 0.0001, energy: χ^2= 19.686 df= 2 P= 0.0001, soluble fibre: χ^2= 117.9 df= 2 P= 0.0001, ADF: χ^2= 137.3 df= 2 P= 0.0001, NDF: χ^2=78.5 df= 2 P= 0.0001 and WSC: χ^2= 34.2 df= 2 P= 0.0001, ash: χ^2= 104.7 df= 2 P= 0.0001, calcium: χ^2= 395.0 df= 2 P= 0.0001, copper: χ^2= 92.410 df= 2 P= 0.0001, iron: χ^2= 30.4 df= 2 P= 0.0001, magnesium: χ^2= 21.73 df= 2 P= 0.0001, phosphorous : χ^2= 633.1 df= 2 P= 0.0001, sodium: χ^2= 74.5 df= 2 P= 0.0001,). According to post hoc tests, Diets 2 and 3 were more similar to each other when compared to Diets 1 and 2, or Diets 1 and 3 (Table 8.4). Diets 2 and 3 were not significantly different in their amounts of calcium, energy, ADF, NDF, soluble fibre or WSC. Species was not shown to have a significant effect for any nutrient intake.

Table 8.3 Average nutrient intake of *N. Javanicus, N. coucang* and *N. menagensis* at CWRC under three different dietary regimes, with Diet 1 the original captive diet, Diet 2 a naturalistic diet and Diet 3 a diet based on derived nutrient recommendation values.

Nutrient	*N. javanicus* Diet 1*	*N. javanicus* Diet 2*	*N. javanicus* Diet 3	*N. coucang* Diet 1*	*N. coucang* Diet 2*	*N. coucang* Diet 3	*N. menagensis* Diet 1*	*N. menagensis* Diet 2*	*N. menagensis* Diet 3
Ash (%)	4.16	2.46	5.37	3.83	2.93	5.54	3.40	6.21	5.58
Crude Fat (%)	6.15	10.49	12.68	6.21	12.57	14.08	4.94	8.82	12.17
Crude Protein (%)	14.26	20.57	23.85	13.80	26.52	27.03	12.66	20.87	23.33
WSC (%)	58.99	46.36	36.87	58.88	37.10	32.18	62.80	30.34	35.09
Soluble Fibre (%)	2.83	5.95	4.21	2.97	5.66	4.13	2.49	3.51	4.69
ADF (%)	6.94	8.66	7.54	3.27	7.67	4.76	6.00	4.94	6.56
NDF (%)	6.43	20.11	18.24	7.28	23.68	19.18	6.20	16.12	18.83
Calcium (%)	0.12	0.21	0.50	0.21	0.34	0.51	0.35	0.46	0.60
Phosphorous (%)	0.19	0.20	0.45	0.18	0.32	0.46	0.16	0.45	0.52
Ca:P	0.61	0.79	1.01	1.18	1.06	1.00	2.15	4.64	1.16
Copper (%)	10.09	6.92	8.42	9.95	8.06	8.80	10.07	13.08	9.18
Iron (mg/kg)	56.76	48.13	71.99	53.59	39.43	75.44	55.01	103.10	77.96
Magnesium (%)	0.37	0.36	0.27	0.36	0.60	0.36	0.34	6.38	0.34
Sodium (%)	0.06	0.21	0.27	0.07	0.18	0.26	0.05	28.98	0.23
Gross Energy (kcal/g)	2.99	3.18	3.30	3.05	3.62	3.25	2.94	4.08	3.29

*Data also used in Chapter VI

Table 8.4 Post hoc test results indicating significant differences in specific nutrient concentrations among Diet treatments fed to 3 species of slow loris in Indonesia. Diet 2 (diet based on wild feeding ecology and natural food ingredients) and Diet 3 (diet comprising locally available ingredents formulated targeting nutrient content of wild type diet) are more similar compared to Diet 1 (original diet at rescue center).

	Nutrients >		Nutrients <	
	Diet 2	**Diet 3**	**Diet 2**	**Diet 3**
Diet 1 (Captive rescue center)	Ash			Ash
	Cu	Cu	Ca	Ca
	Mg		Fat	Fat
		ADF	Protein	Protein
	WSC	WSC	Energy	Energy
				Fe
			P	P
			Na	Na
			Sol. Fibre	Sol. Fibre
			ADF	
			NDF	NDF
	Diet 1	**Diet 3**	**Diet 1**	**Diet 3**
Diet 2 (based on wild feeding ecology and natural foodstuffs)		Ash	Ash	
	Ca		Cu	Cu
			Fat	
		Fat	Protein	Protein
				Fe
	Energy			
	Mg	Mg		P
	P			Na
	Na		WSC	
	Sol. Fibre			
	ADF			
	NDF			

All results above are significant at P=0.0001

8.3.4 Food Passage Rates

The food passage rate was relatively slow and showed little variation between species or individuals. Transit time values did not increase significantly based on the new diets; however, MRT values increased significantly comparing Diet 1 with Diet 2, or Diet 1 to Diet 3 (Table 8.5). Passage of Diets 2 and 3 were not dissimilar. The Friedman test revealed that TT was not significantly different among the three diet treatments, however, MRT showed significant differences

amongst diet treatments χ^2= 49.81 P=0.0001. Wilcoxon Signed Rank post hoc tests with Bonferroni corrections showed that MRT for Diets 1 and 2 (Z= -5.239, P=0.0001), or Diets 1 and 3 (Z=-5.213 P=0.0001) were significantly different, while the MRT resulting from Diets 2 or 3 did not differ.

Table 8.5 Average food passage rates (TT=transit time and MRT= mean retention time) of *N. Javanicus, N. coucang* and *N. menagensis* at CWRC under three different dietary regimes, with Diet 1 the original captive diet, Diet 2 a naturalistic diet and Diet 3 a diet based on derived recommendation values.

	Diet Time	# of trials	*N. javanicus* n=15	*N. coucang* n = 15	*N. menagensis* n = 4
Transit Time (hours)	Diet 1 (± SD) (range)*	4	25.6 (±2.6) (23.0-31.5)	25.00 (±3.5) (21.5-29.0)	24.2 (±3.2) (21.0-27.5)
	Diet 2 (± SD) (range)*	4	25.6 (±3.4) (24.0 - 29.0)	24.4(±2.1) (24.0 - 26.5)	24.5 (±2.9) (22.5- 27.0)
	Diet 3 (± SD) (range)	4	25.1 (±4.1) (23.0 - 28.8)	24.7 (±2.7) (22.0 - 28.3)	24.4 (±2.3) (22.0- 27.66)
Mean Retention Time (hours)	Diet 1 (± SD) (range)*	4	33.40(±1.0) (31.0-32.5)	29.70 (±1.5) (27.0-29.5)	32.88(±3.1) (28.0-33.4)
	Diet 2 (± SD) (range)*	4	38.50(±2.0) (34.5-39.0)	38.0(±2.5) (34.0-37.5)	34.13 (±4.1) (30.0-34.8)
	Diet 3 (± SD) (range)	4	37.50 (±2.0) (34.0-38.3)	37.60 (±2.0) (33.0-37.75)	34.75 (±3.25) (30.0-34.8)

*Data also used in Chapter VI

8.3.6 Apparent digestibilities

Due to the small weight of faecal matter excreted by the slow lorises, we had to pool the faecal samples for enough dry matter for digestibility analyses, with only 2 pooled samples achieved for each species. We only collected enough *N. menagensis* faecal samples for ADF and NDF analyses. The slow loris species were able to digest protein at relatively similar efficiencies when fed all three diets (76-83%, Table 8.6), although protein digestibility tended to decrease with increasing dietary fiber from Diet 1 to 2 or 3. Fibre digestibility was also similar amongst species (30-51% for ADF, 52-80% for NDF). Insoluble fiber digestibility slightly increased with the increased ADF values of Diets 2 and 3. Calcium was the only nutrient to display a striking change (~40% to 50-60%) in its digestibility when animals were fed Diets 2 and 3.

Table 8.6 Apparent digestibility values for crude protein (only 2 of 3 spp), acid detergent fibre (ADF), neutral detergent fibre (NDF), and calcium (only 2 of 3 spp.) for slow lorises (*Nyticebus* spp., n=3) at CWRC under three different dietary regimes, with Diet 1 the original captive diet, Diet 2 a naturalistic diet and Diet 3 a diet based on derived recommendation values.

		N. javanicus	*N. coucang*	*N. menagensis*
Crude Protein	Diet 1 (%)	82.60	81.80	-
	Diet 2 (%)	80.44	79.28	-
	Diet 3 (%)	78.34	76.05	-
ADF	Diet 1 (%)	38.70	44.60	30.30
	Diet 2 (%)	43.54	49.28	40.46
	Diet 3 (%)	46.40	51.93	42.82
NDF	Diet 1 (%)	58.45	51.69	59.05
	Diet 2 (%)	79.65	71.72	65.61
	Diet 3 (%)	77.35	69.56	68.27
Calcium	Diet 1 (%)	37.60	35.90	-
	Diet 2 (%)	61.03	63.75	-
	Diet 3 (%)	50.07	52.41	-

*It was not possible to collect enough faecal sample material to conduct more than one replicate of the tests for each species.

8.3.7 Health Monitoring of Captive Slow Lorises

The initial BW of the captive slow lorises varied considerably, and some gained weight while others lost throughout the feeding trials. Nonetheless, all individuals ended the experiment at what was considered a healthy weight based on wild averages: *N. pygmaeus*: 360-580 g, *N. coucang*: 635-850 g, *N. menagensis*: 265-800 g, *N. javanicus*: 750 - 1150 g, *N. bengalensis*: 1140-2100 g (Nekaris 2014). Overweight individuals lost on average 77.68 g (SD ± 56.50), and underweight individuals gained 85.12 g (SD ± 76.28) (Figure 8.1).

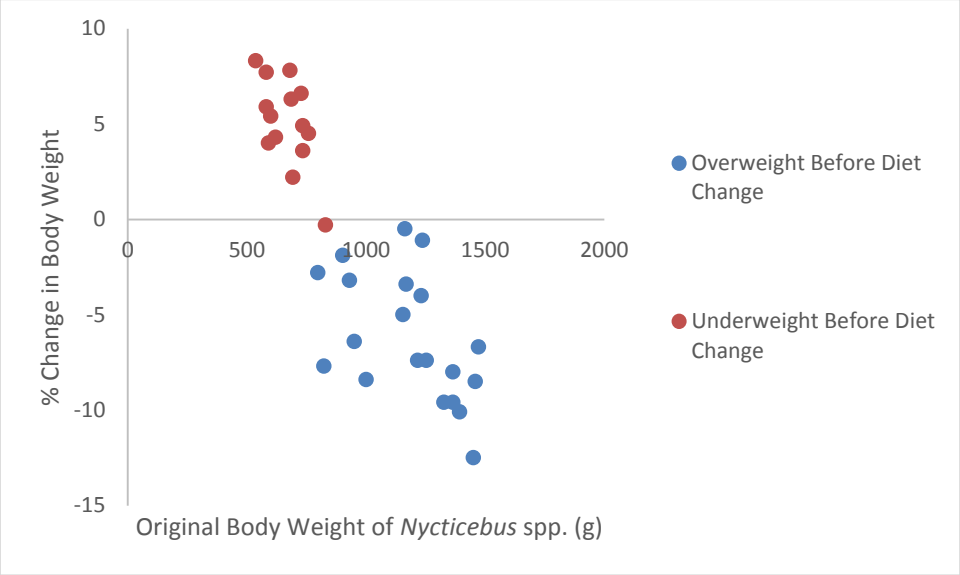


Figure 8.1 The percent weight change from three species of *Nycticebus (N. javanicus, N. bengalensis and N. menagensis*) after a change from a high fruit diet to naturalistic high fibre diet, showing a general trend of overweight individuals reducing weight and underweight individual gaining weight.

8.4 DISCUSSION

8.4.1 Diet Compositions and Nutrient Intake

The current captive diet was significantly different than the wild diet of slow lorises; by using Diet 1 as a proxy for most current diets being fed to slow lorises (Cabana and Nekaris 2015), slow loris captive diets' lead to significantly different physiological parameters such as food passage time, nutrient intake and digestibility than those of wild slow lorises. The wild diet of our model species, the Javan slow loris, *N. javanicus*, did not compositionally resemble the typical captive diet (Diet 1). The wild diets of *N. javanicus* was surprisingly low in fat (average of 2.37 %) for a diet which contains roughly 20% insects. Fibre fractions were high for such a small primate (~11 % soluble fibre, 11 % ADF and 19 % NDF), however this was expected due to the high amounts of plant matter, chitin, and gum within the diets of free-ranging lorises. These values are low compared to some folivorous primates such as *Hapalemur* spp. which has a diet of 30+ % NDF (Overdorff and Rasmussen 1995). Insect chitin is also included in the total ADF values, although we do not yet know how important it is to their physiology or metabolism. Simple sugars and water soluble carbohydrates are very low within the wild-type diet (~3 % and 42% of DM, respectively) which is why the main goal of Diet 3 was to reduce WSC and increase fibre fractions within the diet. The original captive slow loris diet (Diet 1) was heavily based on fruit and honey, with some insect or egg protein, leading to to a diet that was very high in WSC (average 59 % of DM), and moderate in protein (14 % of DM), fibre (ADF: 7%, NDF: 11%, soluble fibre: 3%) and calcium (0.1%), with an inverse Ca:P ratio.

The oldest slow lorises resided in this rescue centre for five years, and their original reason for being confiscated (customs seizure, ex-pet, market rescue etc.) has a large effect on their long term health (Moore 2012). Some have developed stereotypic behaviours and received different diets before arriving at the rescue centre, which may explain how many of the slow lorises are able to subsist on this

diet, some better than others. Considering this diet may be sub-optimal in comparison with the nutrients proposed as recommendations and NRC values for primates (Table 8.2), animals may meet minimum requirements for already healthy and non breeding adults, made evident by their long term survival at the centre, albeit with some health problems (dental issues and hypocalcaemia). The protein, fibre fractions and Ca:P ratio were below our recommended levels based on values derived from the wild diet, with fat and WSC being found in higher concentrations in captive compared with wild diets. Low fibre and high WSC concentrations are typical of captive *Nycticebus* diets, although often very high protein content diets are observed, possible leading to other health complications such as renal pathologies (Cabana 2014; Cabana and Nekaris, 2015).

Our newly formulated diet (Diet 3) was significantly closer to the wild diet of slow lorises in terms of nutrients ingested; concentrations wereattained through a diet comprising gum arabic, insects, eggs and vegetables. The gum arabic itself was purified into a white powder and did not smell or resemble the wild gum of *Acacia decurrens*. Although its texture was different, the slow lorises still found it palatable and the gum Arabic maintained its mineral properties, which makes it a suitable food to pair with insects. Insects eaten by lorises are high in protein and phosphorous while gum is high in carbohydrates, calcium and other minerals (Table 8.1) which may explain why they are eaten in similar proportions by wild *Nycticebus* (Cabana et al. 2016a). The goal of creating a new captive diet resembling the nutrient intake of the wild diet was accomplished, based on information fromthe wild diet data to establish nutrient targets in this study.

8.4.2 Food Passage Rate Validation

The MRT values of all slow lorises fed Diet 3 were similar to the retention times for animals fed wild-type Diet 2. Thus our target duplicated for food passage rate, as both TT and MRT values responded the same manner; responses were as expected due to the higher (and similar) ingested fibre fractions of Diets 2 and 3.

Nonetheless, small differences between fibre contents of both diets reflected small, yet detectable differences within the MRT values. Both the Javan and greater slow lorises fed Diet 3 consumed diets containing 2-4 % less overall fibre fractions than when consuming Diet 2, which apparently reducted the average MRT values for the species (Table 8.4). Yet when the fibre fractions increased by 3 % for the Philippine slow loris, consequently their MRT increased by 0.60%.

Reductions in dietary WSC concentrations had no obvious effect on MRTs across loris species, which suggests the anatomy of *Nycticebus* may be responsive to the mechanical presence of soluble fibres within the gum, but not other solube carbohydrates. The disparity across species with regard to changes in MRT with diets alsosuggests that the microbial communities may have a small (or no) influence on MRT. Given the long periods of time for adjustment/adaptation to higher fiber substrate diets in these trials, one might have expected identical responses across loris species if microbes played a substantial role in passage.

However, with addition of dietary fibre, the MRT values increased subtantially in lorises, with extended retention in hours comparable to much larger colobine monkeys: namely guerezas (*Colobus guereza:* Kay and Davies 1994), the silvered langur (*Trachypithecus cristatus*: Sakaguchi et al. 1991) and the proboscis monkeys (*Nasalis larvatus*: Dierenfeld et al. 1992*).* These results are consistent with our hypothesis that high fibre (both soluble and insoluble) content diets are important in slow loris digestive physiology. The observed increase in MRT with added dietary fibre is also reported for the exudativorous pygmy and common marmosets (*Cebuella pygmaea* and *Callithrix jacchus) (*Power 1991; Power 2010; Power and Oftedal 1996). This effect was not seen in related frugivorous/insectivorous callitrichids, who do not need to rely on gum for nutrients/energy and therefore never evolved to exploit this food item fully. The longer MRT values for the slow lorises on Diets 2 and 3 allow the better breakdown and assimilation of not only fibre, but other nutrients as well. This

would only hold true if the digestive rates of such nutrients alerted to allow a greater overall mass of nutrients to be assimilated.

8.4.3 Apparent Digestibility Validation

The more naturalistic diets (Diets 2 and 3) allowed all three species of loris to digest and assimilate an overall larger amount of each nutrient measured. The amount of protein in Diets 2 and 3 was almost double the amount of protein found in Diet 1, however the apparent digestibility of protein remained similar and only decreased slightly when animals were fed Diet 3. Like all primates, *Nycticebus* has the capacity to digest and assimilate protein to a great extent. In our study, when the majority of protein derived from insects, efficiency seemed to decline above ~23% protein (DM basis). In captivity the minimum protein requirements are surely lower due to reduced physical strain and exertion in daily activities (Flurer et al. 1987).

Apparent digestibility of fibre fractions increased by 5-10% for ADF and 9-19 % for NDF with the addition of more dietary fiber (Diets 2 and 3), with fiber digestibility coefficients similar to the larger and highly folivorous sifakas (Schmidt et al. 2005a). The longer MRT values associated with Diets 2 and 3 may have increased the opportunity for the slow lorises to ferment and digest the cellulose in their large intestine and caecum. The fibre in captive diets could possibly be further increased, at least until a maximum digestibility is achieved, which may benefit gut health. Orangutan NDF digestion began to drop when NDF increased >53% of dietary DM (Schmidt et al. 2005b), thus suggesting a maximum dietary fiber content for this species. The maximum combination of soluble plus insoluble fiber examined in the slow lorises in this study was only about 30% of DM; future studies should be designed to quantify the physiologic scope and benefits of even higher fiber levels consumed by slow lorises.Diets higher in fibre and lower in WSC are also conducive to a change of gut microbial communities, to species with higher cellulolytic abilities (Amato 2016). We posit that the gut

responded to the increased fibre fractions, which led to the gut microbes having a longer amount of time to act upon a larger amount of fermentable substrate. This selection pressure caused a shift in the microbialcommunities, which then led to a higher proportion of fermenting species, further increasing fermentation capabilities. This reflects the wild feeding ecology of the slow lorises which is largely based on gum (soluble fibre) as an energy source.

Lastly, and unexpectedly, calcium uptake from in diet increased by up to 50% with increased dietary fiber. It is possible the longer MRTs also helped the assimilation of calcium, either through normal active uptake processes, but perhaps also through more chances for chitonolytic bacteria to hydrolyze chitin and release calcium that might have been chemically bound in the insect exoskeleton, allowing it to be assimilated. The results from Table 8.6 must be interpreted conservatively due to the pooling of faecal samples and small sample sizes, however this information is still useful when used to compare between diets. Diets 2 and 3 both led to similar amounts of nutrients being digested, and differed substantially from Diet 1.

8.4.4 Health Impacts Validation

The largest effects (or impacts) on health were related to the increase in dietary fibre fractions, and reduction in sugars and starches between Diet 1 compostion compared to Diets 2 and 3 (which were similar). Besides previously observed links between increased dietary fibre and satiety leading to a reduction in abnormal behaviours (Less et al. 2014; Remis and Dierenfeld 2004), the addition of fibres may help modulate the glucose tolerance of the slow lorises, buffering hunger and reducing food intake rates (Jenkins et al. 2000). Anecdotally, the overweight animals were more dominant over food resources, displacing the smaller, thinner individuals. This may be why we observed the overweight individuals losing weight, concommitent with a reduction in dominance behavior (over food), which then allowed underweight subordinate individuals to ingest

more and gain. We observed a tendency that food was less guarded once fruit was removed from diets, and less displacement occurred in social groups.

In other hindgut fermenters, the addition of fibre to standard diets reduced the overall rate of starch digestion (Vervuert et al. 2009). Perhaps the inclusion of root vegetables, typically higher in soluble carbohydrates than other vegetable types, to a diet high in gum may not lead to the harmful effects of WSC on gut microbial communities reported in some dietary studies (i.e. Amato 2016). Stool quality should also be improved on higher fibre diets (Sunvold et al. 1995). Although we did not quantify these aspects, we anecdotally did see more solid faeces from animals fed Diets 2 and 3 compared to Diet 1 when on more than one occasion, scraping was required to gather faecal samples. Both the black and white colobus (*C. guereza)* and the spectacled leaf monkey (*T. obscurus*) also benefited from better formed faeces under a higher fibre diet (Nijboer et al. 2006), as do apes (Remis and Dierenfeld 2004). This may also reflect a healthier overall gut function and more cohesive and responsive gut microbial community (Amato 2016). The lowered WSC content of Diets 2 and 3 would potentially promote a luminal pH more consistent with one of optimal short chain fatty acid production (Gomez et al. 2016). Coupled with the increased fibre substrate particles, this should shift the population of gut microbes to one more adapted for structural carbohydrate fermentation (Clayton et al. 2016). Predominantly cellulolytic gut microbial communities have been linked with enhanced protection from pathogenic microbes, modulating the immune function, and optimising energy conversion and harvesting efficiencies (Gomez et al. 2015).

8.4.5 Captive Feeding Recommendations

The results from our three quantified variables: nutrient intake, food passage, and digestibility were all consistent with Diet 3 (and 2) promoting physiological values for *Nycticebus* spp. more consistent with free-ranging animals than results obtained on the typical captive Diet 1. The data gathered here

also help us to determine that *Nycticebus* are well adapted to utilize the nutrients and energy within fermentable fibres, which can greatly benefit both oral and gastrointestinal health in this group of species. If dietary nutrient recommendations suggested in Table 8.2 cannot be duplicated, at the very least every effort to decrease dietary WSC and increase fibre fractions should be made in the feeding management of captive lorises. This can be easily achievable by removing domestic fruits, reducing or removing grain-based concentrate feeds, and/or focusing on vegetables and gum Arabic as primary source of dietary carbohydrates instead. Positive differences were observed at the CWRC in this study; other facilities have reported similar improvements with reduced dietary WSC and increased overall fibre in primate diets, targeting gorillas (Lukas et al. 2014), lemurs (Britt et al. 2015), pygmy slow lorises (Cabana and Plowman 2014) and slender lorises (Williams et al. 2015).

8.5 CONCLUSION

The diet created based on the nutrient framework of wild slow loris intakes led to similar, and we assume, normal digestive physiological responses duplicating those of healthy free ranging wild slow loris. Altered dietary nutrient profiles, notably higher in fibre and lower in soluble carbohydrates, compared to current typical captive diets resulted in longer food mean retention times, and and higher fermentation capacity for fibre fractions and calcium. Further, the modified captive diet (based on wild diet nutrient ranges) emulates wild feeding responses, and has led to stabilisation of slow loris weights and reduction in heath issues. Our nutrient recommendations have been validated using multiple physiological techniques. Future studies should focus on dental health issue progression on lower sugar diets, as well as changes to the gut microbiome and impacts on overall immune function with diet improvements.

CHAPTER IX

SYNTHESIS

This thesis aimed to create and validate nutrient recommendation for slow lorises under human care, such as in zoos or rescue centres. We also aimed to show why this research was necessary. The overwhelming majority of zoos do not feed their slow lorises like they eat in the wild, which has been linked with illnesses and poor breeding performance. Strikingly, very few institutions fed gum to their slow lorises and instead provided lots of fruits, dairy and grains (Chapter 3). For species whose main diet is composed of gum and very little fruit (Chapter 4, Das et al. 2014; Rode-Margono et al. 2015; Starr and Nekaris 2012; Streicher 2004; Wiens et al. 2006), this does not lead to a diet that is appropriate for the behavior, morphology or physiology of the slow lorises. The statistical link between the high soluble carbohydrate diets and dental disease provides as evidence with the high abundance of fruit in the diet and the lack of gum with the development of dental disease for these animals. Our results provide further evidence that captive diets are causing health issues, as first mentioned by Debyser (1995) and Fuller et al. (2014). This may also negatively affect the chances of released slow lorises. With such a small percentage of translocations being succesfull (Moore 2012), the diet they are fed for a prolonged time at their rescue centres may leave a footprint, such as lowered immunity, atrophied masseter muscles (reduced ability to gouge for gum), overhabituation to sweet foods (which are not abundant in released habitats) and lack/loss of insect hunting skills. The potential impact of successfully creating an appropriate diet for slow lorises not only affects the captive population worldwide, but also may affect the success rates of translocation efforts.

The diet of the wild Javan slow loris agreed with our hypothesis and was consistent with the results of Chapter 3, they had a diet very high in gum and insects with little to no fruit. Our methods allowed us to result in quantifiable results instead of relying on the proportion of time spent feeding which was an accurate proxy for only half of the food items (Knott 2001; Felton et al. 2009). Indeed, the slow lorises at our field site fed on gum, insects and nectar and a minor amount of plant parts such as flowers, fruits and leaves. We calculated nutrient intake due to the

quantitative data. Lorises maintained a very specific protein to carbohydrates to fat ratio, which means they were able to meticulously control their nutrient intakes. Even with seasonal variation in food abundance, there was strict control, similar to observations reported with leaf eating monkeys (Johnson et al. 2015). The austral winter period's dryer environment led to less abundant food resources, forcing slow lorises to ingest more gum. During this time, they had to prioritise insects, their only source of protein, to ensure they ingested their minimum intake (Rothman et al. 2011). On average, their dietary intake of fibre was quite high, and the soluble carbohydrates were very low in natural diets, which is the opposite of the situation seen for captive diets. If slow lorises are indeed physiologically adapted for a high fibre, low sugar diet, then feeding them the opposite for a long time period would likely have negative health consequences (Cabana 2014).

Slow lorises have a physiology which is reactive to the presence of gum and chitin, supporting our theory that they are adapted to a high fibre diet. Using a standard food mean retention time experiment with the captive slow lorises (and using the data based on wild diet composition), we observed a similar response in slow lorises as previously reported for the exudativorous marmosets (Power and Oftedal 1996). Slow lorises were able to physiologically slow down their food passage rate when gum was included in the diet. The gum filled diet was higher in fibre which has the greatest impact on gut motility (Lambert and Fellner 2011). Pygmy slow lorises with gum in their diets appear to be the primate with the longest mean retention time relative to body size. This is surprising due to its small size of between 350 and 450 g. Such a small primate that spends 40 % of its foraging time on insects (Star et al. 2013) should not have such an emphasis on fermentation according to the Jarman-Bell Theory (Gaulin 1979). The very slow digestion time may have originated from their low metabolism that has allowed their gut microbiome to become more adapted to fibre and chitin fermentation. This would also serve the purpose of the slow lorises metabolism by providing free energy

(Amato 2016). Gum has to be fermented by gut microbes, and slowing the food passage rate down gives the microbes more chances to digest the plant fibres (Lambert and Rothman 2015). High fibre dietary constituents would in turn be conducive to an optimal gut lumen pH to cultivate a community of microbes beneficial for their immune system, nutrient acquisition and disease prevention (Amato 2016; Gomez et al. 2016). Some of their gut microbes also possess the ability to digest chitin, which previously has only been demonstrated in Goeldi's monkeys (Macdonald et al. 2013). Opting for vertebrate protein instead of feeding insects in captivity may have some health repercussions not previously discussed within the literature. Insects have different amino acid proportions compared with chicken, beef or mice (Finke 2015), possibly leading to kidney health issues (Debyser 1995). Chitin may provide a source of energy as well as a substrate for beneficial gut microbes. Ensuring captive animals have an optimal gut flora is essential, not only for good health and metabolic function, but also for translocation success. With this new evidence, we now posit that the majority of captive slow lorises suffer from severe dysbiosis which should be the focus of future studies. Diets high in sugar and protein and low in fermentable substrates (such as fibre and/or chitin) are the main cause of gut microbiome dysbiosis (Clayton et al. 2016).

A zoo or rescue centre diet based on our nutrient recommendations could replicate the physiological responses of wild slow lorises eating a wild diet, which should be more conducive to good health than current diets. Our trial diets led to a slower food passage rate, higher apparent digestion coefficients, and a more balanced and natural nutrient intake for the slow lorises. Protein was digested in the same efficiency even as it increased in concentration. Providing more evidence of their physiolgocial adaptations to diets high in fibre, increasing this nutrient in the diet did not lead to a decreased digestive efficiency. As fibre increases, the gut passage rate also increases, providing more opportunities for the gut microbiome to digest

the fibre, gaining a peak in digestive efficiency. This diet has been in use for more than one year now at the rescue centre and has since been adopted in many zoos, all with great success.

The theory originally put foreward by Debyser (1995) and followed up by Fuller et al. (2014) and Cabana and Plowman (2014) observing a link between diet and health issues of captive slow lorises has been supported by the evidence put foreward in this thesis. Gut microbiome dysbiosis is one possible mechanism for the many health issues we observe, as well as high sugar content leading to dental disease which further complicates feeding. This general rule can be extrapolated for any hindgut fermenting primate and also perhaps the marsupial Petaurids who share a close ecological niche (Smith 1984). Marmosets, gibbons, howler monkeys, cercopithecines, gorillas, orangutans etc. would all benefit from a lower sugar, higher fibre diet, assuming they would receive the same health benefits of an optimum gut microbiome. Microbiome research in primates has unanimously shown a significant different community and overall reduction in diversity in captive congeners compared to wild (Amato 2016, Clayton et al. 2016; Gomez et al. 2015; 2016). We would expect the same results in exudativorous mammals which require a healthy gut microbiome to digest their main dietary staple. Released slow lorises had diets much higher in fruits and nectar and lower in gums than free ranging individuals (Moore 2012). This may have occurred due to not recognizing gum as food or having atrophied masseter muscles after not being fed gum for more than three years. Alternatively, it may be due to them not being able to digest it efficiently because of their gut dysbiosis. They instead focus on soluble carbohydrates which don't rely on gut microbe fermentation (Hall 2003). Microbiome analyses should be conducted and compared withint he exudativorous mammal niche, especially in regards to captive versus wild.

CHAPTER X

NUTRITION GUIDELINES FOR SLOW LORISES (*NYCTICEBUS* SPP.)

The research of this PhD thesis led to the validation of nutrient recommendations for slow lorises. The importance of certain food items to their physiology has also been shown, and should be reflected within their captive diets. Below is the guide to feeding slow lorises in captivity.

Nutrient recommendations to be followed:

Nutrient	Concentration (DM basis)	Nutrient	Concentration (DM basis)
Crude Protein (%)	23.50	Cu (mg/kg)	11.22
Crude Fat (%)	2.37	Fe (mg/kg)	69.16
Soluble Fiber (%)	10.50	Mg (%)	0.37
ADF (%)	11.00	Na (%)	0.38
NDF (%)	19.00	Vit A (IU A/g)	2.06
Ca (%)	0.45	Vit D (IU A/g)	0.53*
P (%)	0.16	Vit E (mg/kg)	0.97*

Foods to be fed:

Gum: This is the most important food for slow lorises and should be given daily. You can provide either the raw crystals or the refined white gum Arabic. The texture is ideal to include supplements or medication if need be.

Insects: This should be the main source of protein and fat for the slow lorises. A variety is suggested, as well as varying daily between the domestic available species (crickets, locusts, mealworms, superworms, waxworms etc.).

Pellets: Primate or insectivore pellets can be part of a balanced diet for captive slow lorises, although they are not necessary to attain a balanced diet. If used, they must only be provided in sufficient amounts to fulfill the above nutrient requirements and must be relatively low in soluble carbohydrates. A leaf-eater type pellet is generally successful or five grams of an insectivore pellet. No more than 20% of the daily metabolic needs of the slow lorises should be fulfilled by pelleted feed. The feeds should not be wetted down but instead fed dry to increase abrasive contact with teeth.

Vegetables: The rest of the energy requirements should be provided as vegetables. This is not necessarily to provide nutrients (as the bulk will come from insects and gum) but mostly to provide energy and to spread feeding behavior throughout the night, all the while keeping fibre proportions high and sugar proportions low.

Foods to be avoided:

Fruits: these food items are high in sugar and low in fibre and other nutrients. For this reason they are useless and not recommeneded to be used as feed items for slow lorises. Their high sugar load can actually hinder the beneficial species of gut microbes and reduce digestive efficiency as well as immunity.

Animal Products (meat, dairy): the ingestion of vertebrate prey is sporadic in the wild and not at all necessary. Eggs may be used as occasional enrirchment items but the use of dairy or meat is completely useless and provides fat and protein contents that would unbalance the entire diet. The protein is better to come from insects which also contain chitin which may feed unique gut microbes in the process.

How much to be fed:

Using the below equation to calculate the amount of energy required by your slow loris which is known as the basal metabolic rate (BMR). Simply enter the weight (W) of the slow lorises target weight in kg:

$BMR=42W^{0.75}$

BMR is given in Kcal/day and only informs you on the base amount of energy that is necessary for slow lorises. Therefore, you must multiply your BMR by 2.5. Lactating females must be provided with 1.5X the amount of energy for the first six months of the juveniles life.

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APPENDIX I – ZOO QUESTIONNAIRE

Pygmy Slow Loris Diet Survey

Francis Cabana M.Sc.
Nutrition Researcher
PhD Candidate
francis.cabana@paigntonzoo.org.uk
Whitley Wildlife Conservation Trust
Oxford Brooked University

Thank you for taking the time to answer this survey. Please fill out to the best of your abilities, and return to me as soon as possible, either this file via e-mail or by completing the online survey:

http://www.surveymonkey.com/s/GN7MRHD

1. **How many pygmy slow lorises do you currently care for? Please include sex, life stage and latest weight if available (Baby, juvenile, adult).**

2. **Are your individuals in a nocturnal or diurnal enclosure?**

3. **Please list the feed items given in their daily diets. Please list, to the best of your knowledge, the amounts (in grams) or standard amounts (1 carrot, 2 apples etc.) and brands if commercial feeds used.**

DAY	DIET	Extras
Monday		
Tuesday		
Wednesday		
Thursday		
Friday		
Saturday		
Sunday		

4. **When and how often is food prepared and presented?**

Once a day ☐

Twice a day ☐

Three times a day or more? ☐

Times:

Are the feedings different in composition? If yes please describe:

5. **How is the food presented?**

Plate or bowl on floor/surface ☐

Plate or bowl fixed at branches ☐

Other:

1. **Are there any seasonal changes in diets?**

2. **What food enrichment items are they provided with?**

3. **How long has the current diet and feeding routine been in practice at your zoo?**

4. **Do any of your lorises have any recurring health problems that may be diet related? If so, please give details:**

Teeth
Digestion
Skeletal (deformation, arthrosis)
Internal organs (liver, kidney)
Obesity
Pelage/fur condition

Your Name:
Your Contact Information:

APPENDIX II - ETHICS APPROVAL LETTER

Professor Anna Nekaris
Director of Studies
Department of Social Sciences
Faculty of Humanities and Social Sciences
Oxford Brookes University
Headington Campus

9 March 2015

Dear Professor Nekaris

UREC Registration No: 150900
Using feeding ecology and food chemistry (nutrients and secondary metabolites) to influence captive husbandry of the Javan slow loris

Thank you for the email of 9 March 2015 outlining the response to the points raised in my previous letter about the PhD study of your research student Francis Cabana and attaching the revised documents. I am pleased to inform you that, on this basis, I have given Chair's Approval for the study to begin.

The UREC approval period for this study is two years from the date of this letter, so 9 March 2017. If you need the approval to be extended please do contact me nearer the time of expiry.

Should the recruitment, methodology or data storage change from your original plans, or should any study participants experience adverse physical, psychological, social, legal or economic effects from the research, please inform me with full details as soon as possible.

Yours sincerely

Hazel Abbott
Chair of the University Research Ethics Committee

cc Giuseppe Donati, Second Supervisor
Francis Cabana, Research Student
Maggie Wilson, Research Ethics Officer
Jill Organ, Research Degrees Team
Louise Wood, UREC Administrator

UNIVERSITY RESEARCH ETHICS COMMITTEE, FACULTY OF HEALTH AND LIFE SCIENCES

Headington Campus Gipsy Lane

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Tel: 01865 482639

APPENDIX III – PUBLICATIONS

CHAPTER III – PUBLISHED

Cabana F, Nekaris KAI (2015) Diets high in fruits and low in gum exudates promote the occurrence and development of dental disease in pygmy slow loris (*Nycticebus pygmaeus*). *Zoo Biology 34*:547-553.

CHAPTER IV – PUBLISHED

Cabana F, Dierenfeld E, Wirdateti W, Donati G, Nekaris KAI (2017) The seasonal feeding ecology of the Javan slow loris (*Nycticebus javanicus*). *American Journal of Physical Anthropology 01: 1-12.*

CHAPTER I – ACCEPTED WITH MINOR REVISIONS

Journal of Integrated Zoology

CHAPTER VI – ACCEPTED WITH MAJOR REVISIONS

International Journal of Primatology

CHAPTER VIII – ACCEPTED WITH MINOR REVISIONS

Journal of Animal Physiology and Animal Nutrition

COLLABORATIONS PUBLISHED DURING PhD

Moore RS, Cabana F, Nekaris KAI (2015) Factors influencing stereotypic behaviours of animals rescued from Asian animal markets: A slow loris case study. *Applied Animal Behaviour Science 166*:131-136.

Williams E, Cabana F, Nekaris KAI (2015) Improving diet and activity of insectivorous primates in captivity: Naturalizing the diet of Northern Ceylon gray slender loris, Loris lydekkerianus nordicus. *Zoo Biology 34*:473-482.

Clayton JB, Vangay P, Huang H, Ward T, Hillmann BM, Al-Ghalith GA, Travis DA, Long HT, Van Tuan B, Van Minh V, Cabana F, Nadler T, Toddes B, Murphy T, Glander KE, Johnson TJ (2016) Captivity humanizes the primate microbiome. *Proceedings of the National Academy of Sciences* 36:201521835

APPENDIX IV – JAVAN SLOW LORIS DIET TRIALS RAW DATA (n=15)

Legend: mg = magnesium, p = phosphorous, na = sodium, sol fibre = soluble fibre, adf = acid detergent fibre, ndf = neutral detergent fibre, WSC = water soluble carbohydrates.

All nutrients are presented on a dry matter concentration basis.

			Javan Slow Loris Enclosure Number								
Diet	**Day**	**Nutrient**	**e3**	**e5**	**i2**	**i10**	**s9**	**s12**	**t3**	**t4**	**e7**
1	1	ash	6.65	8.48	9.16	6.19	5.98	9.00	6.96	7.53	16.91
1	1	calcium	0.14	0.14	0.15	0.20	0.14	0.17	0.13	0.15	0.24
1	1	copper	14.78	13.94	13.36	14.44	14.32	13.34	13.91	14.49	16.72
1	1	crude fat	6.71	5.99	6.72	10.29	6.59	7.98	6.51	6.97	6.03
1	1	crude protein	19.18	17.15	17.59	24.65	17.18	19.80	16.79	19.66	28.85
1	1	gross energy	3.97	3.96	3.95	3.99	3.94	3.97	3.95	3.99	4.08
1	1	iron	58.32	54.86	49.45	51.28	49.36	47.58	46.75	56.84	122.00
1	1	mg	0.44	0.43	0.47	0.59	0.48	0.49	0.44	0.41	0.41
1	1	p	0.25	0.22	0.22	0.34	0.23	0.26	0.22	0.25	0.33
1	1	na	0.13	0.10	0.10	0.17	0.12	0.13	0.13	0.14	0.01
1	1	sol fibre	1.61	1.91	2.01	1.49	1.59	1.94	1.71	1.70	2.82
1	1	adf	3.89	3.95	3.99	4.15	3.37	4.11	3.44	4.05	8.22
1	1	ndf	14.47	14.66	14.03	12.07	14.69	13.19	14.58	14.14	13.02
1	1	WSC	52.99	53.72	52.50	46.80	55.56	50.03	55.16	51.70	35.19
1	2	ash	1.73	2.28	2.11	1.99	1.72	1.64	1.80	1.72	2.05
1	2	calcium	0.06	0.07	0.06	0.06	0.05	0.05	0.05	0.05	0.05
1	2	copper	6.37	6.40	5.16	5.08	4.48	4.23	6.48	4.92	6.20
1	2	crude fat	2.16	6.15	6.14	6.06	4.00	3.06	2.36	3.46	3.21
1	2	crude protein	9.88	13.88	11.86	12.17	8.71	6.96	10.16	8.85	9.49
1	2	gross energy	1.77	2.25	1.97	1.79	1.61	1.67	1.95	1.70	2.36
1	2	iron	68.97	76.57	62.57	61.65	52.08	48.74	71.96	56.80	70.84
1	2	mg	0.12	0.17	0.16	0.14	0.13	0.15	0.13	0.13	0.19
1	2	p	0.20	0.15	0.13	0.14	0.10	0.07	0.11	0.10	0.10
1	2	na	0.01	0.05	0.05	0.05	0.03	0.02	0.01	0.03	0.01
1	2	sol fibre	4.38	5.26	5.18	4.44	4.49	4.82	4.46	4.46	5.90
1	2	adf	11.60	15.59	14.10	12.55	11.55	12.19	12.99	11.95	16.84
1	2	ndf	16.08	21.70	19.68	17.48	16.12	17.03	18.03	16.65	23.49
1	2	WSC	70.15	55.99	60.21	62.30	69.45	71.31	67.65	69.32	61.76
1	3	ash	1.78	1.77	2.10	2.83	1.83	1.81	1.86	1.61	1.84

1	3	calcium	0.13	0.11	0.13	0.23	0.12	0.11	0.12	0.08	0.12
1	3	copper	8.44	9.50	11.21	10.98	9.77	10.67	8.68	11.63	10.28
1	3	crude fat	6.76	6.28	7.69	12.35	6.63	6.18	7.07	4.60	6.48
1	3	crude protein	11.61	10.45	12.84	20.80	11.45	10.88	11.29	8.14	11.03
1	3	gross energy	2.44	2.99	3.53	3.19	2.66	2.85	2.99	3.41	3.00
1	3	iron	40.20	38.15	42.72	69.00	51.40	50.61	38.81	33.59	46.17
1	3	mg	0.46	0.48	0.60	0.68	0.42	0.44	0.47	0.51	0.47
1	3	p	0.18	0.16	0.19	0.32	0.17	0.17	0.17	0.12	0.17
1	3	na	0.02	0.02	0.02	0.04	0.03	0.03	0.02	0.02	0.03
1	3	sol fibre	1.44	1.75	1.98	2.09	1.53	1.48	2.01	1.57	1.69
1	3	adf	2.55	2.99	3.47	4.39	2.58	2.34	3.68	2.17	2.82
1	3	ndf	12.76	16.48	18.82	17.43	15.16	15.73	17.55	17.42	16.74
1	3	WSC	67.09	65.02	58.55	46.59	64.93	65.40	62.23	68.23	63.91
1	4	ash	3.73	4.59	4.90	4.04	3.49	4.57	3.89	3.98	7.63
1	4	calcium	0.12	0.12	0.12	0.18	0.11	0.12	0.11	0.10	0.15
1	4	copper	10.85	10.94	10.90	11.18	10.48	10.35	10.66	11.38	12.17
1	4	crude fat	5.73	6.75	7.54	10.52	6.31	6.31	5.84	5.51	5.76
1	4	crude protein	14.91	15.21	15.51	21.13	13.69	13.80	14.02	13.44	18.10
1	4	gross energy	3.00	3.37	3.47	3.29	3.01	3.11	3.26	3.34	3.46
1	4	iron	61.41	62.18	56.74	66.71	56.04	53.87	57.76	53.98	87.64
1	4	mg	0.37	0.40	0.45	0.52	0.38	0.40	0.38	0.39	0.39
1	4	p	0.23	0.19	0.20	0.29	0.18	0.18	0.18	0.17	0.22
1	4	na	0.06	0.06	0.06	0.10	0.07	0.07	0.06	0.07	0.02
1	4	sol fibre	2.72	3.27	3.36	2.94	2.79	3.02	3.00	2.83	3.82
1	4	adf	6.61	8.26	7.91	7.73	6.42	6.83	7.37	6.66	10.22
1	4	ndf	15.88	19.37	19.26	17.23	16.86	16.85	18.39	17.68	19.53
1	4	WSC	59.75	54.07	52.80	47.09	59.64	58.47	57.85	59.39	48.98
1	5	ash	3.22	3.97	4.23	3.49	3.02	3.94	3.36	3.44	6.59
1	5	calcium	0.10	0.10	0.11	0.16	0.10	0.10	0.10	0.09	0.13
1	5	copper	9.37	9.45	9.41	9.66	9.05	8.94	9.21	9.83	10.51
1	5	crude fat	4.95	5.83	6.51	9.09	5.45	5.45	5.05	4.76	4.98
1	5	crude protein	12.88	13.14	13.39	18.25	11.82	11.92	12.11	11.61	15.63
1	5	gross energy	2.59	2.91	2.99	2.84	2.60	2.69	2.82	2.88	2.99
1	5	iron	53.04	53.70	49.00	57.61	48.40	46.53	49.88	46.62	75.69
1	5	mg	0.32	0.34	0.39	0.45	0.33	0.34	0.33	0.33	0.34

1	5	p	0.20	0.17	0.17	0.25	0.16	0.16	0.16	0.15	0.19
1	5	na	0.05	0.05	0.05	0.08	0.06	0.06	0.05	0.06	0.02
1	5	sol fibre	2.35	2.82	2.90	2.54	2.41	2.61	2.59	2.45	3.30
1	5	adf	5.71	7.13	6.83	6.68	5.54	5.90	6.37	5.75	8.83
1	5	ndf	13.71	16.73	16.63	14.88	14.56	14.55	15.88	15.27	16.86
1	5	WSC	65.24	60.33	59.23	54.30	65.15	64.13	63.60	64.93	55.94
2	1	ash	1.42	1.70	2.14	2.20	1.18	2.20	1.14	0.98	1.81
2	1	calcium	0.13	0.21	0.20	0.17	0.13	0.22	0.14	0.12	0.22
2	1	copper	3.63	5.08	5.73	5.70	3.64	6.32	3.74	3.14	5.44
2	1	crude fat	7.55	9.62	11.96	12.35	6.81	12.50	6.66	5.66	10.33
2	1	crude protein	13.84	17.68	21.79	22.56	12.88	23.06	12.83	10.87	19.51
2	1	gross energy	3.39	2.83	3.05	3.49	3.41	3.00	3.27	3.35	2.76
2	1	iron	38.68	56.53	60.83	47.41	29.75	57.43	32.18	29.63	61.54
2	1	mg	0.04	0.22	0.14	0.06	0.13	0.19	0.16	0.14	0.21
2	1	p	0.20	0.24	0.31	0.32	0.17	0.31	0.17	0.15	0.27
2	1	na	0.13	0.16	0.21	0.25	0.15	0.24	0.14	0.11	0.18
2	1	sol fibre	1.55	4.12	5.56	1.24	2.42	3.63	2.90	2.39	3.65
2	1	adf	2.48	3.49	4.08	4.30	2.36	2.15	2.16	1.76	3.03
2	1	ndf	16.45	11.88	10.80	19.39	17.07	17.03	17.05	15.73	19.34
2	1	WSC	60.74	59.12	53.31	43.50	62.06	45.21	62.32	66.76	49.01
2	2	ash	2.41	2.52	2.60	2.94	2.68	3.31	2.84	3.22	2.89
2	2	calcium	0.16	0.23	0.22	0.16	0.28	0.24	0.34	0.26	0.28
2	2	copper	4.51	6.28	6.12	5.79	7.96	7.12	9.00	7.73	7.72
2	2	crude fat	7.41	9.48	8.97	8.81	12.16	10.07	15.52	11.68	12.06
2	2	crude protein	15.23	20.23	19.48	19.21	26.30	22.32	32.28	25.32	25.57
2	2	gross energy	1.28	2.18	2.23	2.13	2.84	2.86	2.40	2.85	2.61
2	2	iron	51.34	48.51	45.99	35.38	43.77	48.58	75.50	50.10	59.02
2	2	mg	0.10	0.25	0.24	0.11	0.32	0.26	0.32	0.27	0.31
2	2	p	0.22	0.27	0.26	0.27	0.34	0.30	0.44	0.34	0.34
2	2	na	0.15	0.25	0.24	0.26	0.36	0.29	0.36	0.33	0.31
2	2	sol fibre	1.11	3.60	3.40	1.12	4.77	3.61	4.70	3.76	4.52
2	2	adf	2.70	3.00	3.10	3.46	3.33	4.01	3.46	3.93	3.52
2	2	ndf	14.03	16.38	16.27	18.85	17.15	17.31	17.74	17.09	17.76
2	2	WSC	60.92	51.39	52.68	50.19	41.71	46.99	31.62	42.69	41.72
2	3	ash	2.51	2.79	1.80	2.65	2.77	2.73	2.65	2.33	2.33
2	3	calcium	0.09	0.27	0.17	0.22	0.22	0.21	0.25	0.19	0.19
2	3	copper	10.2	9.43	11.3	7.54	7.64	7.17	8.44	8.80	8.80

			8		9						
2	3	crude fat	7.78	15.81	8.72	12.32	12.68	11.80	13.89	10.37	10.37
2	3	crude protein	16.52	33.72	19.24	26.25	26.91	25.05	29.67	22.31	22.31
2	3	gross energy	3.95	3.45	3.42	3.43	3.50	3.49	3.40	3.46	3.46
2	3	iron	23.83	23.09	28.87	23.54	22.77	22.91	23.74	25.45	25.45
2	3	mg	0.21	0.26	0.35	0.25	0.23	0.23	0.27	0.28	0.28
2	3	p	0.21	0.44	0.24	0.33	0.34	0.31	0.38	0.28	0.28
2	3	na	0.25	0.52	0.30	0.39	0.40	0.37	0.45	0.33	0.33
2	3	sol fibre	9.68	6.66	3.55	10.98	11.24	12.29	8.65	8.89	8.89
2	3	adf	12.18	6.65	2.03	11.20	11.98	13.06	8.45	8.70	8.70
2	3	ndf	20.91	18.34	11.79	14.33	14.76	16.21	11.05	14.62	14.62
2	3	WSC	52.28	29.34	58.45	44.45	42.88	44.21	42.74	50.37	50.37
2	4	ash	2.66	2.56	2.57	2.94	2.90	2.59	2.55	3.02	2.41
2	4	calcium	0.18	0.18	0.19	0.21	0.21	0.20	0.19	0.20	0.20
2	4	copper	5.93	6.21	6.33	6.70	7.50	7.20	6.54	7.94	5.73
2	4	crude fat	9.57	9.23	8.72	11.38	10.35	9.50	9.17	10.41	8.74
2	4	crude protein	14.53	14.63	14.34	18.18	17.84	16.42	15.01	18.34	13.90
2	4	gross energy	3.41	3.35	3.41	3.47	3.54	3.35	3.35	3.64	3.26
2	4	iron	59.75	63.07	69.24	59.27	75.65	71.78	66.72	82.52	58.93
2	4	mg	0.77	0.76	0.73	0.89	0.81	0.77	0.75	0.79	0.77
2	4	p	0.15	0.15	0.14	0.21	0.19	0.16	0.14	0.19	0.15
2	4	na	0.00	0.00	0.01	0.00	0.01	0.01	0.05	0.01	0.01
2	4	sol fibre	8.27	8.27	8.01	7.57	7.39	7.95	8.20	7.18	8.25
2	4	adf	19.45	18.47	18.60	18.22	18.89	17.57	18.29	19.98	16.82
2	4	ndf	28.26	27.38	28.12	56.61	27.40	26.16	27.21	28.58	25.95
2	4	WSC	44.98	46.20	46.25	10.89	41.51	45.33	46.06	39.65	49.00
2	5	ash	2.48	2.63	2.51	2.95	2.62	2.98	2.52	2.63	2.60
2	5	calcium	0.15	0.24	0.21	0.21	0.23	0.24	0.25	0.21	0.24
2	5	copper	6.70	7.43	8.13	7.08	7.35	7.65	7.62	7.59	7.61
2	5	crude fat	8.89	12.14	10.55	12.34	11.55	12.06	12.44	10.48	11.41
2	5	crude protein	16.53	23.72	20.58	23.71	23.08	23.88	24.69	21.13	22.35
2	5	gross energy	3.31	3.25	3.33	3.44	3.65	3.49	3.42	3.66	3.32
2	5	iron	47.74	52.58	56.36	45.54	47.28	55.19	54.49	51.62	56.36

2	5	mg	0.31	0.41	0.40	0.36	0.41	0.40	0.41	0.41	0.43
2	5	p	0.21	0.30	0.26	0.31	0.29	0.30	0.31	0.26	0.29
2	5	na	0.15	0.26	0.21	0.25	0.25	0.25	0.28	0.21	0.23
2	5	sol fibre	5.67	6.23	5.64	5.75	7.10	7.56	6.72	6.11	6.96
2	5	adf	10.12	8.69	7.65	10.22	10.05	10.12	8.90	9.45	8.82
2	5	ndf	21.90	20.34	18.42	30.02	21.00	21.10	20.09	20.91	21.36
2	5	WSC	50.20	41.16	47.94	30.98	41.74	39.98	40.25	44.85	42.28
3	1	ash	4.88	4.64	4.37	4.59	4.67	4.84	4.63	4.74	4.64
3	1	calcium	0.38	0.60	0.44	0.54	0.23	0.31	0.53	0.28	0.57
3	1	copper	13.68	8.45	7.00	8.21	9.11	10.97	7.76	11.15	8.41
3	1	crude fat	26.91	13.16	8.87	13.11	14.00	19.82	11.02	19.20	13.01
3	1	crude protein	53.95	28.48	20.57	26.61	28.88	39.62	23.26	39.48	27.97
3	1	gross energy	3.25	3.47	3.43	3.21	3.27	3.00	3.55	3.48	3.45
3	1	iron	85.54	57.34	70.48	73.27	103.97	111.53	60.12	85.07	61.26
3	1	mg	0.12	0.19	0.20	0.12	0.10	0.12	0.11	0.10	0.17
3	1	p	0.77	0.39	0.47	0.49	0.72	0.74	0.44	0.74	0.42
3	1	na	0.69	0.37	0.22	0.31	0.31	0.43	0.31	0.50	0.36
3	1	sol fibre	0.45	4.92	3.99	4.18	0.78	0.72	4.36	0.56	4.61
3	1	adf	6.15	3.62	3.78	3.69	5.86	6.07	3.78	6.03	3.77
3	1	ndf	13.74	18.59	19.91	18.61	17.85	16.74	18.55	19.61	18.57
3	1	WSC	0.52	35.13	46.28	37.08	34.60	18.98	42.54	16.97	35.81
3	2	ash	5.95	5.32	7.03	5.83	7.52	6.11	6.47	6.40	5.82
3	2	calcium	0.55	0.53	0.53	0.66	0.55	0.59	0.58	0.51	0.60
3	2	copper	8.78	6.84	8.14	7.18	9.03	8.87	8.78	8.35	8.64
3	2	crude fat	13.12	18.37	13.42	8.89	10.69	12.78	11.54	13.32	12.53
3	2	crude protein	27.91	30.82	24.84	19.24	22.59	26.46	24.56	27.32	26.12
3	2	gross energy	3.17	2.61	2.99	3.17	3.34	3.23	3.38	3.04	3.26
3	2	iron	67.34	56.37	57.72	62.47	55.02	62.25	53.04	66.71	60.41
3	2	mg	0.24	0.15	0.17	0.21	0.19	0.18	0.19	0.26	0.18
3	2	p	0.44	0.46	0.46	0.32	0.45	0.44	0.43	0.45	0.42
3	2	na	0.30	0.35	0.26	0.19	0.25	0.30	0.29	0.28	0.30
3	2	sol fibre	5.01	4.38	5.35	6.62	5.79	5.36	5.61	4.91	5.46
3	2	adf	4.61	3.14	4.80	3.61	5.50	4.46	4.67	4.88	4.16
3	2	ndf	19.76	18.08	20.28	18.13	18.42	20.50	11.03	18.37	15.33
3	2	WSC	33.26	27.41	34.43	47.91	40.78	34.15	46.40	34.59	40.20

3	3	ash	4.46	4.29	4.33	4.43	4.40	4.28	4.49	4.46	4.52
3	3	calcium	0.50	0.36	0.38	0.46	0.43	0.33	0.43	0.38	0.54
3	3	copper	7.46	6.74	7.13	7.65	7.16	6.56	9.26	8.66	8.08
3	3	crude fat	10.79	5.57	8.78	10.71	9.39	6.99	14.99	13.40	12.64
3	3	crude protein	24.49	19.70	21.49	22.69	20.04	16.85	32.45	28.49	27.09
3	3	gross energy	3.11	3.44	3.44	3.55	3.51	3.62	3.37	3.34	3.19
3	3	iron	84.71	79.15	76.59	58.92	64.73	68.96	72.88	78.24	74.65
3	3	mg	0.26	0.27	0.24	0.10	0.10	0.15	0.20	0.15	0.20
3	3	p	0.44	0.48	0.50	0.51	0.52	0.54	0.55	0.59	0.45
3	3	na	0.23	0.18	0.21	0.29	0.24	0.18	0.38	0.32	0.30
3	3	sol fibre	4.31	3.40	3.37	3.80	3.66	3.06	2.97	2.64	4.39
3	3	adf	3.83	4.24	4.21	3.66	3.71	4.09	4.44	4.47	3.67
3	3	ndf	19.50	18.73	10.15	18.36	18.87	19.64	17.98	18.19	19.32
3	3	WSC	40.76	51.71	55.25	43.81	47.30	52.24	30.09	35.46	36.43
3	4	ash	5.45	10.30	7.05	5.42	5.60	5.65	5.65	5.71	5.87
3	4	calcium	0.63	0.23	0.56	0.65	0.67	0.66	0.63	0.62	0.63
3	4	copper	7.56	8.65	7.83	7.32	7.47	7.52	7.55	7.34	7.54
3	4	crude fat	12.82	1.25	9.75	12.10	12.78	12.84	12.43	11.12	11.88
3	4	crude protein	25.96	15.95	21.49	24.55	24.47	24.59	25.06	23.75	24.11
3	4	gross energy	2.92	3.63	3.18	3.05	3.01	3.00	2.99	3.14	3.04
3	4	iron	82.01	72.52	68.62	72.84	71.00	72.04	76.84	70.22	73.55
3	4	mg	0.79	0.77	0.73	0.80	0.76	0.76	0.78	0.82	0.77
3	4	p	0.40	0.36	0.40	0.38	0.41	0.41	0.40	0.38	0.40
3	4	na	0.05	0.00	0.03	0.04	0.05	0.05	0.04	0.04	0.04
3	4	sol fibre	5.41	5.62	5.87	5.67	5.75	5.72	5.55	5.70	5.68
3	4	adf	4.20	9.73	5.46	4.00	3.94	4.01	4.27	4.36	4.39
3	4	ndf	20.83	21.73	13.19	17.65	18.21	19.29	18.91	21.38	17.20
3	4	WSC	34.94	50.77	48.52	40.28	38.94	37.63	37.95	38.04	40.94
3	5	ash	5.70	6.75	6.26	5.57	6.10	5.74	5.84	5.86	5.73
3	5	calcium	0.57	0.47	0.53	0.64	0.52	0.52	0.60	0.49	0.64
3	5	copper	10.31	8.44	8.28	8.35	9.01	9.33	9.17	9.76	8.98
3	5	crude fat	17.50	10.55	11.23	12.32	12.89	14.42	13.74	15.69	13.77
3	5	crude protein	36.39	26.11	24.31	25.60	26.39	29.57	28.97	32.74	28.95
3	5	gross energy	3.42	3.62	3.59	3.57	3.61	3.53	3.65	3.58	3.56
3	5	iron	87.8	72.9	75.1	73.5	81.05	86.56	72.2	82.5	74.21

			9	8	9	6			9	7	
3	5	mg	0.39	0.38	0.37	0.34	0.32	0.33	0.35	0.37	0.36
3	5	p	0.56	0.46	0.50	0.47	0.58	0.59	0.50	0.59	0.46
3	5	na	0.35	0.25	0.20	0.23	0.23	0.26	0.28	0.31	0.28
3	5	sol fibre	4.17	5.04	5.11	5.57	4.39	4.09	5.08	3.80	5.54
3	5	adf	5.17	5.70	5.02	4.11	5.23	5.12	4.72	5.43	4.40
3	5	ndf	20.30	21.21	17.47	20.01	20.17	20.95	18.28	21.33	19.37
3	5	WSC	20.11	35.38	40.73	36.50	34.45	29.33	33.17	24.39	32.18

APPENDIX V GREATER SLOW LORIS DIET TRIAL RAW DATA (n=15)

Legend: mg = magnesium, p = phosphorous, na = sodium, sol fibre = soluble fibre, adf = acid detergent fibre, ndf = neutral detergent fibre, WSC = water soluble carbohydrates.

All nutrients are presented on a dry matter concentration basis.

			Greater Slow Loris Enclosure Number											
Diet	**Day**	**Nutrient**	e4	e8	s4	s6	d6	d7	t5	s5	a3	i4	i7	t7	d1
1	1	ash	11.83	5.97	9.18	10.52	5.36	4.97	4.99	8.84	8.69	6.87	7.35	6.10	7.18
1	1	calcium	0.14	0.14	0.15	0.15	0.13	0.16	0.13	0.14	0.16	0.15	0.14	0.26	0.15
1	1	copper	11.87	14.79	14.03	13.74	14.36	15.00	14.47	13.82	14.19	14.37	14.21	14.31	14.28
1	1	crude fat	5.14	6.71	6.05	5.59	6.45	8.12	6.52	5.52	6.80	6.74	6.40	19.96	6.53
1	1	crude protein	12.97	18.63	18.10	17.66	16.48	20.97	16.60	16.29	19.49	18.34	17.68	42.77	17.98
1	1	gross energy	3.93	3.96	3.97	3.97	3.93	3.97	3.94	3.95	3.87	3.93	3.92	4.24	3.94
1	1	iron	38.98	56.83	59.41	61.09	48.07	54.85	47.23	55.34	60.14	54.39	54.28	26.70	54.44
1	1	mg	0.40	0.49	0.42	0.40	0.51	0.53	0.48	0.43	0.48	0.48	0.47	0.26	0.47
1	1	p	0.15	0.24	0.22	0.21	0.22	0.28	0.22	0.20	0.25	0.24	0.23	0.58	0.23
1	1	na	0.08	0.11	0.10	0.09	0.11	0.14	0.12	0.09	0.10	0.11	0.11	0.61	0.11
1	1	sol fibre	2.44	1.56	1.98	2.17	1.54	1.39	1.47	1.98	1.91	1.69	1.76	0.76	1.74
1	1	adf	3.78	3.69	4.28	4.51	3.17	3.67	3.09	3.95	4.34	3.76	3.79	5.04	3.78
1	1	ndf	14.68	14.59	14.48	14.56	14.90	13.80	14.92	14.91	14.05	14.42	14.58	5.38	14.51
1	1	WSC	55.38	54.10	52.19	51.67	56.81	52.14	56.97	54.44	50.97	53.63	54.00	25.79	53.80
1	2	ash	2.12	1.91	1.97	2.12	2.35	1.81	1.94	2.14	2.10	2.35	1.53	1.90	2.12
1	2	calcium	0.07	0.05	0.05	0.05	0.06	0.07	0.05	0.06	0.10	0.05	0.04	0.05	0.06
1	2	copper	6.65	2.90	4.55	4.90	4.33	6.78	6.47	5.73	8.75	6.75	3.29	5.65	6.24
1	2	crude fat	5.04	5.52	4.24	4.51	7.77	2.48	2.77	5.52	5.11	4.91	2.98	3.30	4.72
1	2	crude protein	13.34	6.70	8.05	8.58	11.54	10.90	10.18	11.99	18.18	12.41	5.27	9.40	11.97
1	2	gross energy	2.03	1.93	2.12	2.35	2.32	1.75	2.20	2.09	1.49	2.68	1.58	2.10	2.13
1	2	iron	76.11	39.61	55.18	59.90	58.28	72.39	73.78	67.97	91.93	81.60	39.39	65.95	72.26
1	2	mg	0.14	0.20	0.19	0.21	0.22	0.11	0.16	0.17	0.05	0.02	0.05	0.16	0.13
1	2	p	0.15	0.07	0.08	0.09	0.13	0.12	0.10	0.13	0.21	0.13	0.05	0.10	0.13
1	2	na	0.04	0.04	0.03	0.03	0.06	0.02	0.01	0.04	0.06	0.02	0.02	0.02	0.03
1	2	sol fibre	4.77	6.08	5.96	6.48	6.32	4.32	5.08	5.32	3.06	6.11	4.87	5.11	5.24
1	2	adf	13.62	15.40	15.83	17.59	17.61	11.28	15.08	14.74	7.86	18.97	12.06	14.83	14.73
1	2	ndf	18.92	21.62	22.15	24.60	24.67	15.63	20.97	20.55	10.65	26.45	16.90	20.67	20.50
1	2	WSC	60.58	64.25	63.59	60.19	53.67	69.18	64.14	59.80	63.96	53.88	73.32	64.73	60.69

1	3	ash	1.89	1.83	1.90	1.75	2.05	1.74	1.87	1.87	1.70	1.76	1.84	1.67	1.82
1	3	calcium	0.12	0.11	0.12	0.10	0.14	0.10	0.13	0.11	0.10	0.10	0.12	0.11	0.11
1	3	copper	10.18	9.42	10.08	10.89	9.71	10.27	8.51	9.91	10.00	10.25	10.54	7.92	9.93
1	3	crude fat	6.78	6.64	6.87	5.66	8.00	5.82	7.24	6.74	5.68	5.94	6.43	6.22	6.44
1	3	crude protein	11.37	10.66	11.45	9.66	13.30	9.46	12.02	10.74	9.46	9.90	11.35	10.30	10.65
1	3	gross energy	3.08	3.22	3.26	3.30	2.78	3.33	2.64	3.31	3.14	3.11	2.86	2.54	3.09
1	3	iron	44.67	35.52	34.87	37.30	61.37	37.66	43.11	42.59	36.36	44.09	47.35	35.18	43.21
1	3	mg	0.49	0.50	0.57	0.51	0.41	0.48	0.46	0.47	0.48	0.44	0.48	0.43	0.46
1	3	p	0.17	0.16	0.17	0.14	0.20	0.14	0.18	0.16	0.14	0.15	0.17	0.15	0.16
1	3	na	0.03	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.03	0.02	0.02
1	3	sol fibre	1.79	2.00	1.83	1.70	1.87	1.94	1.72	2.11	1.76	1.80	1.46	1.62	1.84
1	3	adf	3.06	3.53	3.17	2.67	3.46	3.20	3.15	3.72	2.86	2.96	2.35	2.87	3.15
1	3	ndf	17.21	18.16	16.94	17.46	17.57	18.94	14.98	19.76	17.28	17.99	15.08	14.06	17.71
1	3	WSC	62.75	62.71	62.84	65.47	59.08	64.04	63.89	60.89	65.88	64.41	65.30	67.75	63.38
1	4	ash	5.81	3.56	4.79	5.28	3.58	3.12	3.23	4.71	4.58	4.03	3.93	3.55	4.07
1	4	calcium	0.12	0.11	0.12	0.11	0.12	0.12	0.11	0.11	0.13	0.11	0.11	0.15	0.12
1	4	copper	10.52	9.94	10.51	10.83	10.41	11.75	10.80	10.80	12.08	11.50	10.28	10.22	11.17
1	4	crude fat	6.22	6.92	6.29	5.78	8.15	6.02	6.06	6.52	6.45	6.45	5.80	10.81	6.49
1	4	crude protein	13.82	13.20	13.79	13.16	15.15	15.15	14.23	14.31	17.28	14.90	12.58	22.91	14.88
1	4	gross energy	3.31	3.34	3.43	3.53	3.31	3.32	3.22	3.43	3.12	3.56	3.07	3.26	3.36
1	4	iron	58.58	48.39	54.80	58.04	61.50	60.46	60.18	60.83	69.09	66.03	51.71	46.87	62.30
1	4	mg	0.38	0.44	0.43	0.41	0.42	0.41	0.40	0.39	0.37	0.35	0.37	0.31	0.39
1	4	p	0.17	0.17	0.17	0.16	0.20	0.20	0.18	0.18	0.22	0.19	0.16	0.30	0.19
1	4	na	0.06	0.06	0.06	0.05	0.07	0.07	0.06	0.06	0.07	0.06	0.06	0.24	0.06
1	4	sol fibre	3.30	3.53	3.58	3.80	3.57	2.81	3.03	3.45	2.47	3.52	2.97	2.75	3.23
1	4	adf	7.50	8.29	8.54	9.08	8.89	6.66	7.82	8.22	5.52	9.42	6.67	8.34	7.94
1	4	ndf	18.63	19.94	19.64	20.76	20.95	17.74	18.65	20.25	15.39	21.58	17.07	14.71	19.33
1	4	WSC	55.53	56.39	55.49	55.02	52.17	57.97	57.83	54.21	56.30	53.04	60.63	48.03	55.22
1	5	ash	5.02	3.07	4.13	4.56	3.09	2.70	2.79	4.07	3.96	3.48	3.39	3.06	3.52
1	5	calcium	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.11	0.09	0.10	0.13	0.10
1	5	copper	9.09	8.58	9.08	9.35	8.99	10.15	9.33	9.33	10.43	9.93	8.88	8.83	9.64
1	5	crude fat	5.37	5.98	5.43	4.99	7.04	5.20	5.23	5.63	5.57	5.57	5.00	9.34	5.60
1	5	crude protein	11.93	11.40	11.91	11.37	13.08	13.09	12.29	12.36	14.92	12.87	10.86	19.78	12.85
1	5	gross energy	2.86	2.88	2.96	3.05	2.86	2.87	2.78	2.96	2.69	3.08	2.65	2.81	2.90

1	5	iron	50.59	41.79	47.33	50.13	53.11	52.22	51.97	52.54	59.67	57.03	44.65	40.48	53.81
1	5	mg	0.33	0.38	0.37	0.35	0.36	0.35	0.35	0.34	0.32	0.30	0.32	0.27	0.34
1	5	p	0.15	0.15	0.15	0.14	0.17	0.17	0.16	0.16	0.19	0.16	0.14	0.26	0.16
1	5	na	0.05	0.05	0.05	0.04	0.06	0.06	0.05	0.05	0.06	0.05	0.05	0.21	0.05
1	5	sol fibre	2.85	3.05	3.09	3.28	3.08	2.42	2.62	2.98	2.13	3.04	2.56	2.37	2.79
1	5	adf	6.48	7.16	7.37	7.84	7.68	5.75	6.75	7.10	4.77	8.14	5.76	7.20	6.86
1	5	ndf	16.09	17.22	16.96	17.93	18.09	15.32	16.11	17.49	13.29	18.64	14.74	12.70	16.70
1	5	WSC	61.59	62.34	61.56	61.15	58.69	63.70	63.58	60.46	62.26	59.44	66.00	55.12	61.33
2	1	ash	2.44	2.68	2.95	3.25	2.07	2.28	2.51	2.56	2.82	3.10	3.41	2.18	2.66
2	1	calcium	0.35	0.39	0.42	0.47	0.30	0.33	0.36	0.37	0.40	0.44	0.49	0.31	0.38
2	1	copper	6.79	7.47	8.22	9.04	5.77	6.35	6.98	7.13	7.84	8.63	9.49	6.06	7.39
2	1	crude fat	11.15	12.27	13.49	14.84	9.48	10.43	11.47	11.71	12.88	14.17	15.58	9.95	12.14
2	1	crude protein	25.60	28.16	30.98	34.07	21.76	23.94	26.33	26.88	29.57	32.52	35.78	22.85	27.87
2	1	gross energy	2.89	3.18	3.50	3.85	2.46	2.70	2.97	3.03	3.34	3.67	4.04	2.58	3.15
2	1	iron	13.45	14.80	16.27	17.90	11.43	12.58	13.83	14.12	15.53	17.09	18.80	12.00	14.64
2	1	mg	0.45	0.50	0.54	0.60	0.38	0.42	0.46	0.47	0.52	0.57	0.63	0.40	0.49
2	1	p	0.32	0.35	0.39	0.43	0.27	0.30	0.33	0.34	0.37	0.41	0.45	0.29	0.35
2	1	na	0.18	0.20	0.22	0.24	0.15	0.17	0.19	0.19	0.21	0.23	0.25	0.16	0.20
2	1	sol fibre	3.09	3.40	3.74	4.11	2.63	2.89	3.18	3.24	3.57	3.93	4.32	2.76	3.36
2	1	adf	16.13	17.74	19.52	21.47	13.71	15.08	16.59	16.94	18.63	20.49	22.54	14.40	17.56
2	1	ndf	17.72	19.49	21.44	23.59	15.06	16.57	18.23	18.61	20.47	22.51	24.76	15.82	19.29
2	1	WSC	43.09	37.40	31.14	24.25	51.63	46.79	41.47	40.24	34.27	27.70	20.47	49.21	38.05
2	2	ash	2.68	2.95	3.25	3.57	2.28	2.51	2.76	2.82	3.10	3.41	3.75	2.40	2.92
2	2	calcium	0.39	0.42	0.47	0.51	0.33	0.36	0.40	0.40	0.44	0.49	0.54	0.34	0.42
2	2	copper	7.47	8.22	9.04	9.94	6.35	6.98	7.68	7.84	8.63	9.49	10.44	6.67	8.13
2	2	crude fat	12.27	13.49	14.84	16.32	10.43	11.47	12.61	12.88	14.17	15.58	17.14	10.95	13.35
2	2	crude protein	28.16	30.98	34.07	37.48	23.94	26.33	28.96	29.57	32.52	35.78	39.36	25.13	30.65
2	2	gross energy	3.18	3.50	3.85	4.23	2.70	2.97	3.27	3.34	3.67	4.04	4.44	2.84	3.46
2	2	iron	14.80	16.27	17.90	19.69	12.58	13.83	15.22	15.53	17.09	18.80	20.68	13.20	16.11
2	2	mg	0.50	0.54	0.60	0.66	0.42	0.46	0.51	0.52	0.57	0.63	0.69	0.44	0.54
2	2	p	0.35	0.39	0.43	0.47	0.30	0.33	0.36	0.37	0.41	0.45	0.49	0.31	0.38
2	2	na	0.20	0.22	0.24	0.26	0.17	0.19	0.20	0.21	0.23	0.25	0.28	0.18	0.22
2	2	sol fibre	3.40	3.74	4.11	4.52	2.89	3.18	3.50	3.57	3.93	4.32	4.75	3.03	3.70
2	2	adf	17.74	19.52	21.47	23.62	15.08	16.59	18.25	18.63	20.49	22.54	24.80	15.84	19.31

2	2	ndf	19.49	21.44	23.59	25.94	16.57	18.23	20.05	20.47	22.51	24.76	27.24	17.40	21.22
2	2	WSC	37.40	31.14	24.25	16.68	46.79	41.47	35.61	34.27	27.70	20.47	12.51	44.13	31.85
2	3	ash	2.32	2.55	2.80	3.09	1.97	2.17	2.38	2.43	2.68	2.95	3.24	2.07	2.52
2	3	calcium	0.33	0.37	0.40	0.44	0.28	0.31	0.34	0.35	0.38	0.42	0.46	0.30	0.36
2	3	copper	6.45	7.10	7.81	8.59	5.48	6.03	6.63	6.77	7.45	8.20	9.01	5.76	7.02
2	3	crude fat	10.59	11.65	12.82	14.10	9.00	9.90	10.89	11.12	12.23	13.46	14.80	9.45	11.53
2	3	crude protein	24.32	26.75	29.43	32.37	20.67	22.74	25.01	25.54	28.09	30.90	33.99	21.71	26.47
2	3	gross energy	2.75	3.02	3.32	3.65	2.33	2.57	2.82	2.88	3.17	3.49	3.84	2.45	2.99
2	3	iron	12.78	14.06	15.46	17.01	10.86	11.95	13.14	13.42	14.76	16.23	17.86	11.40	13.91
2	3	mg	0.43	0.47	0.52	0.57	0.36	0.40	0.44	0.45	0.49	0.54	0.60	0.38	0.47
2	3	p	0.30	0.33	0.37	0.40	0.26	0.28	0.31	0.32	0.35	0.39	0.42	0.27	0.33
2	3	na	0.17	0.19	0.21	0.23	0.15	0.16	0.18	0.18	0.20	0.22	0.24	0.15	0.19
2	3	sol fibre	2.94	3.23	3.55	3.91	2.50	2.74	3.02	3.08	3.39	3.73	4.10	2.62	3.20
2	3	adf	15.32	16.86	18.54	20.40	13.02	14.33	15.76	16.09	17.70	19.47	21.42	13.68	16.68
2	3	ndf	16.83	18.52	20.37	22.41	14.31	15.74	17.31	17.68	19.44	21.39	23.53	15.02	18.33
2	3	WSC	45.94	40.53	34.58	28.04	54.05	49.45	44.39	43.23	37.56	31.31	24.44	51.75	41.15
2	4	ash	2.96	2.98	3.06	3.18	3.21	3.39	3.48	3.59	3.70	3.82	3.95	4.08	3.48
2	4	calcium	0.22	0.22	0.23	0.24	0.24	0.25	0.26	0.27	0.28	0.29	0.30	0.30	0.26
2	4	copper	7.68	7.72	7.83	8.12	8.16	8.69	8.92	9.18	9.47	9.77	10.13	10.44	8.90
2	4	crude fat	10.60	10.65	10.91	11.29	11.48	12.08	12.41	12.80	13.21	13.64	14.11	14.56	12.41
2	4	crude protein	17.93	17.95	18.29	19.01	19.16	20.32	20.84	21.48	22.18	22.87	23.69	24.43	20.84
2	4	gross energy	3.77	3.82	3.93	4.05	4.14	4.34	4.46	4.60	4.75	4.90	5.07	5.23	4.46
2	4	iron	78.23	78.80	80.34	83.34	83.52	88.93	91.29	94.03	97.05	100.06	103.70	106.95	91.18
2	4	mg	0.86	0.87	0.89	0.92	0.95	0.98	1.01	1.04	1.08	1.11	1.15	1.19	1.01
2	4	p	0.18	0.18	0.19	0.20	0.20	0.21	0.21	0.22	0.23	0.23	0.24	0.25	0.21
2	4	na	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.03	0.03	0.03	0.02
2	4	sol fibre	8.57	8.83	9.03	9.21	9.66	9.97	10.27	10.59	10.93	11.31	11.68	12.05	10.28
2	4	adf	20.14	20.42	21.04	21.65	22.01	23.16	23.82	24.57	25.35	26.16	27.07	27.93	23.82
2	4	ndf	29.77	30.29	31.19	32.07	32.84	34.35	35.36	36.48	37.64	38.87	40.20	41.48	35.37
2	4	WSC	38.74	38.13	36.55	34.46	33.31	29.86	27.91	25.66	23.26	20.80	18.05	15.45	27.89
2	5	ash	2.80	2.84	2.81	2.87	2.92	2.99	3.03	3.07	3.13	3.18	3.24	3.29	3.03
2	5	calcium	0.25	0.25	0.25	0.25	0.26	0.27	0.27	0.27	0.28	0.28	0.29	0.29	0.27

2	5	copper	7.94	8.07	8.16	8.27	8.41	8.58	8.71	8.85	8.99	9.14	9.30	9.45	8.71
2	5	crude fat	12.17	12.30	12.35	12.33	12.72	12.99	13.16	13.35	13.56	13.81	14.04	14.26	13.13
2	5	crude protein	24.18	24.41	24.52	24.49	25.19	25.79	26.12	26.48	26.89	27.40	27.86	28.30	26.05
2	5	gross energy	3.68	3.69	3.73	3.80	3.83	3.93	3.99	4.05	4.12	4.18	4.26	4.32	3.98
2	5	iron	55.64	57.39	57.85	58.56	60.02	60.79	61.87	62.81	63.85	64.96	66.00	67.09	61.84
2	5	mg	0.43	0.44	0.45	0.45	0.46	0.47	0.48	0.48	0.49	0.50	0.51	0.52	0.48
2	5	p	0.30	0.31	0.31	0.31	0.32	0.32	0.33	0.33	0.34	0.35	0.35	0.36	0.33
2	5	na	0.26	0.26	0.26	0.26	0.26	0.27	0.27	0.28	0.28	0.29	0.29	0.30	0.27
2	5	sol fibre	7.23	7.26	7.20	7.30	7.55	7.68	7.77	7.88	8.02	8.17	8.30	8.43	7.77
2	5	adf	9.94	9.92	9.88	10.08	10.21	10.51	10.63	10.77	10.96	11.15	11.34	11.52	10.62
2	5	ndf	21.94	22.13	22.35	22.82	23.23	23.62	23.97	24.36	24.78	25.19	25.60	26.02	23.99
2	5	WSC	43.91	44.37	45.29	46.35	46.66	47.58	48.35	49.19	50.01	50.78	51.64	52.49	48.42
3	1	ash	4.98	4.66	4.68	4.74	4.57	4.48	4.68	4.62	4.75	4.42	4.83	4.67	4.70
3	1	calcium	0.54	0.60	0.35	0.52	0.54	0.45	0.33	0.46	0.44	0.41	0.36	0.26	0.23
3	1	copper	8.76	8.49	9.71	8.84	7.92	7.33	9.81	8.48	11.23	8.73	12.60	11.02	9.72
3	1	crude fat	14.57	13.30	15.55	14.11	11.50	9.55	15.82	12.74	20.03	13.15	24.60	18.60	14.91
3	1	crude protein	30.72	28.84	32.34	29.97	24.27	20.61	32.81	26.91	42.03	29.16	48.95	38.58	31.34
3	1	gross energy	2.81	3.42	3.54	3.31	3.60	3.62	3.50	3.51	3.31	3.56	2.93	3.62	3.58
3	1	iron	113.58	62.01	76.35	78.30	52.92	59.28	79.80	67.58	82.25	63.85	104.04	77.29	88.25
3	1	mg	0.24	0.20	0.10	0.18	0.11	0.10	0.10	0.12	0.24	0.19	0.12	0.10	0.09
3	1	p	0.45	0.40	0.64	0.48	0.44	0.49	0.66	0.52	0.59	0.54	0.77	0.74	0.71
3	1	na	0.29	0.37	0.42	0.36	0.34	0.27	0.42	0.35	0.52	0.36	0.57	0.51	0.39
3	1	sol fibre	3.83	4.81	1.84	3.77	4.49	3.83	1.62	3.43	2.20	3.05	0.64	0.53	0.61
3	1	adf	5.02	3.75	5.26	4.45	3.57	3.81	5.37	4.30	5.43	4.31	5.94	5.93	6.04
3	1	ndf	19.27	18.75	16.81	18.35	18.21	18.63	16.78	17.99	16.85	18.04	15.24	15.41	16.80
3	1	WSC	30.46	34.45	30.62	32.84	41.45	46.73	29.91	37.73	16.34	35.23	6.38	22.74	32.25
3	2	ash	5.56	5.56	7.51	6.11	5.67	7.51	6.92	6.55	5.33	5.68	5.84	5.56	5.98
3	2	calcium	0.57	0.56	0.41	0.54	0.60	0.55	0.57	0.56	0.52	0.60	0.62	0.46	0.53
3	2	copper	7.81	5.99	9.65	8.02	8.83	9.05	9.08	8.75	7.99	8.73	8.42	9.35	10.27
3	2	crude fat	16.19	16.15	17.97	15.71	12.73	10.87	12.20	12.88	16.47	13.01	12.09	15.86	17.12
3	2	crude protein	29.30	26.06	33.33	28.70	27.88	22.85	24.75	26.05	31.56	27.19	25.00	35.45	34.46
3	2	gross energy	2.77	2.47	2.83	2.83	3.09	3.30	3.23	3.11	2.83	3.22	3.23	2.09	2.97
3	2	iron	67.0	60.9	67.9	64.0	70.2	57.4	60.3	63.0	69.5	63.3	60.7	149.	82.1

			7	7	2	9	9	2	1	3	7	4	8	24	9
3	2	mg	0.16	0.16	0.21	0.18	0.17	0.19	0.16	0.17	0.24	0.18	0.17	0.46	0.18
3	2	p	0.46	0.41	0.59	0.47	0.44	0.45	0.46	0.46	0.46	0.43	0.41	0.51	0.55
3	2	na	0.32	0.26	0.35	0.31	0.31	0.25	0.28	0.29	0.33	0.31	0.29	0.18	0.37
3	2	sol fibre	4.79	5.14	3.67	4.77	5.20	5.75	5.56	5.32	4.30	5.28	5.66	3.49	3.98
3	2	adf	3.67	3.02	6.07	4.23	4.13	5.51	5.00	4.72	3.95	4.15	4.04	5.82	5.10
3	2	ndf	19.25	19.61	18.44	18.16	18.07	19.45	21.36	19.26	19.31	20.15	20.53	19.53	19.42
3	2	WSC	29.70	32.62	22.75	31.32	35.65	39.32	34.47	35.26	27.33	33.97	36.54	23.60	23.02
3	3	ash	4.46	4.52	4.38	4.47	4.48	4.28	4.43	4.42	4.51	4.51	4.45	4.49	4.29
3	3	calcium	0.52	0.45	0.38	0.47	0.55	0.40	0.48	0.48	0.53	0.55	0.39	0.31	0.34
3	3	copper	7.81	9.67	8.24	8.45	7.94	6.74	7.53	7.67	7.95	8.12	8.53	10.31	6.63
3	3	crude fat	11.49	16.22	11.85	13.05	11.92	7.25	10.48	10.68	12.26	12.59	13.02	17.43	7.28
3	3	crude protein	25.66	35.56	28.60	29.23	25.72	17.84	22.70	23.87	25.96	27.02	27.08	37.16	17.45
3	3	gross energy	3.31	3.26	3.26	3.26	3.41	3.84	3.49	3.50	3.23	3.30	3.45	3.32	3.57
3	3	iron	70.64	79.37	88.97	78.41	60.53	49.73	61.42	62.52	72.61	67.07	70.47	85.78	70.37
3	3	mg	0.22	0.26	0.33	0.25	0.17	0.16	0.14	0.18	0.17	0.18	0.10	0.19	0.16
3	3	p	0.44	0.54	0.51	0.49	0.44	0.48	0.48	0.47	0.46	0.44	0.60	0.69	0.53
3	3	na	0.28	0.40	0.27	0.31	0.32	0.24	0.28	0.29	0.29	0.32	0.33	0.43	0.19
3	3	sol fibre	4.34	2.98	2.95	3.67	4.56	3.77	4.08	4.02	4.31	4.48	2.72	1.29	3.18
3	3	adf	3.71	4.61	4.73	4.18	3.47	3.70	3.60	3.74	3.63	3.57	4.28	5.34	4.07
3	3	ndf	19.55	18.25	20.15	19.32	18.84	19.12	18.85	19.03	19.15	18.99	17.74	17.03	19.74
3	3	WSC	38.84	25.45	35.02	33.94	39.04	51.51	43.54	42.01	38.12	36.89	37.71	23.89	51.24
3	4	ash	6.51	5.59	7.49	6.37	5.31	6.91	5.44	6.01	6.06	5.61	7.00	7.94	7.79
3	4	calcium	0.61	0.58	0.54	0.59	0.68	0.60	0.66	0.63	0.50	0.57	0.55	0.48	0.59
3	4	copper	7.66	8.53	8.80	8.13	7.45	7.82	7.46	7.72	10.87	8.43	8.32	8.95	8.26
3	4	crude fat	10.87	15.94	12.69	12.85	13.48	10.55	12.90	12.44	23.73	15.18	11.80	11.51	10.69
3	4	crude protein	22.32	31.50	25.50	25.86	25.46	21.65	25.20	24.54	43.80	30.93	24.57	24.96	20.51
3	4	gross energy	3.16	2.91	3.02	3.03	3.05	3.15	3.08	3.08	2.50	2.91	3.14	3.11	3.15
3	4	iron	66.09	85.52	76.51	75.42	68.35	66.10	69.11	69.74	112.45	87.79	70.95	76.57	61.87
3	4	mg	0.75	1.00	0.80	0.83	0.82	0.71	0.83	0.80	1.26	0.99	0.83	0.82	0.64
3	4	p	0.40	0.49	0.49	0.45	0.41	0.41	0.40	0.42	0.71	0.48	0.45	0.49	0.45
3	4	na	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.04	0.03	0.03	0.04
3	4	sol fibre	5.89	4.40	5.17	5.29	5.62	5.95	5.61	5.62	2.35	4.44	5.37	5.08	6.10
3	4	adf	4.83	4.92	6.11	5.06	3.71	5.13	3.95	4.46	6.42	5.02	5.65	6.77	5.63
3	4	ndf	19.88	19.62	16.41	18.28	19.44	22.38	19.99	20.02	17.48	20.23	22.20	18.60	17.70

3	4	WSC	40.42	27.35	37.91	36.66	36.31	38.51	36.47	36.99	8.93	28.05	34.43	36.99	43.31
3	5	ash	5.92	5.59	6.62	5.96	5.51	6.37	5.90	5.94	5.68	5.56	6.08	6.23	6.26
3	5	calcium	0.62	0.60	0.46	0.58	0.65	0.55	0.56	0.59	0.55	0.59	0.53	0.42	0.46
3	5	copper	8.81	8.99	10.01	9.20	8.84	8.51	9.32	8.97	10.46	9.35	10.41	10.90	9.59
3	5	crude fat	14.61	16.94	15.97	15.32	13.65	10.51	14.14	13.40	19.93	14.83	16.92	17.44	13.75
3	5	crude protein	29.70	33.54	32.94	31.28	28.42	22.81	29.00	27.88	39.42	31.43	34.54	37.44	28.53
3	5	gross energy	3.31	3.32	3.48	3.42	3.62	3.83	3.66	3.63	3.26	3.57	3.51	3.34	3.65
3	5	iron	87.28	79.16	85.18	81.46	69.32	63.95	74.43	72.29	92.64	77.56	84.22	106.94	83.24
3	5	mg	0.38	0.45	0.40	0.40	0.35	0.32	0.34	0.35	0.53	0.42	0.34	0.43	0.29
3	5	p	0.48	0.51	0.61	0.52	0.48	0.50	0.55	0.51	0.61	0.52	0.61	0.67	0.62
3	5	na	0.26	0.29	0.30	0.28	0.28	0.22	0.28	0.26	0.33	0.28	0.34	0.32	0.27
3	5	sol fibre	5.18	4.77	3.75	4.81	5.46	5.31	4.64	5.06	3.62	4.74	3.96	2.86	3.81
3	5	adf	4.74	4.48	6.10	4.93	4.09	4.99	4.93	4.73	5.34	4.69	5.48	6.56	5.73
3	5	ndf	21.44	20.96	19.75	20.38	20.50	21.88	21.17	20.98	20.02	21.29	20.82	19.41	20.26
3	5	WSC	28.34	22.96	24.73	27.05	31.92	38.42	29.79	31.80	14.95	26.89	21.64	19.49	31.20

APPENDIX VI PHILLIPINE SLOW LORIS DIET TRIAL RAW DATA (n=4)

Legend: mg = magnesium, p = phosphorous, na = sodium, sol fibre = soluble fibre, adf = acid detergent fibre, ndf = neutral detergent fibre, WSC = water soluble carbohydrates.

All nutrients are presented on a dry matter concentration basis.

			Phillipine Slow Loris Enclosures		
Diet	Day	Nutrient	e1	e2	t8
1	1	ash	4.63	6.75	8.33
1	1	calcium	0.12	0.12	0.14
1	1	copper	15.44	14.29	12.82
1	1	crude fat	5.17	4.77	6.85
1	1	crude protein	16.24	14.31	15.75
1	1	gross energy	3.93	3.93	3.93
1	1	iron	62.19	53.78	37.26
1	1	mg	0.48	0.44	0.48
1	1	p	0.21	0.18	0.21
1	1	na	0.08	0.08	0.12
1	1	sol fibre	1.44	1.75	1.94
1	1	adf	3.37	3.36	3.37
1	1	ndf	15.90	15.82	14.11
1	1	WSC	58.06	58.35	54.96
1	2	ash	1.97	1.73	1.90
1	2	calcium	0.07	0.08	0.04
1	2	copper	7.63	8.05	5.84
1	2	crude fat	3.32	2.92	3.48
1	2	crude protein	13.43	14.86	10.20
1	2	gross energy	1.87	1.34	2.16
1	2	iron	82.59	83.61	69.69
1	2	mg	0.11	0.04	0.16
1	2	p	0.15	0.17	0.11
1	2	na	0.03	0.04	0.01
1	2	sol fibre	4.15	2.49	4.75
1	2	adf	11.70	6.86	15.06
1	2	ndf	16.17	9.31	20.96
1	2	WSC	65.11	71.18	63.46
1	3	ash	1.88	1.59	1.54
1	3	calcium	0.13	0.09	0.08
1	3	copper	9.23	8.66	7.78
1	3	crude fat	7.06	5.11	5.33
1	3	crude protein	11.78	8.59	7.66
1	3	gross energy	2.97	3.02	3.07

1	3	iron	35.85	32.61	32.61
1	3	mg	0.54	0.46	0.35
1	3	p	0.18	0.13	0.11
1	3	na	0.02	0.02	0.02
1	3	sol fibre	1.75	1.65	2.28
1	3	adf	3.12	2.60	4.04
1	3	ndf	15.55	16.29	20.22
1	3	WSC	63.73	68.42	65.25
1	4	ash	3.11	3.69	4.32
1	4	calcium	0.12	0.11	0.10
1	4	copper	11.84	11.37	9.69
1	4	crude fat	5.70	4.69	5.74
1	4	crude protein	15.20	13.85	12.32
1	4	gross energy	3.22	3.04	3.36
1	4	iron	66.23	62.33	51.17
1	4	mg	0.41	0.34	0.36
1	4	p	0.20	0.18	0.16
1	4	na	0.05	0.05	0.06
1	4	sol fibre	2.69	2.16	3.29
1	4	adf	6.67	4.70	8.24
1	4	ndf	17.46	15.19	20.27
1	4	WSC	58.53	62.58	57.35
1	5	ash	2.69	3.19	3.73
1	5	calcium	0.10	0.09	0.08
1	5	copper	10.23	9.82	8.37
1	5	crude fat	4.92	4.05	4.96
1	5	crude protein	13.13	11.96	10.64
1	5	gross energy	2.78	2.63	2.90
1	5	iron	57.20	53.83	44.19
1	5	mg	0.36	0.30	0.31
1	5	p	0.17	0.15	0.14
1	5	na	0.04	0.04	0.05
1	5	sol fibre	2.32	1.87	2.84
1	5	adf	5.76	4.06	7.12
1	5	ndf	15.08	13.12	17.51
1	5	WSC	64.19	67.68	63.16
2	1	ash	2.76	3.04	3.34
2	1	calcium	0.31	0.34	0.38
2	1	copper	6.41	7.05	7.76
2	1	crude fat	9.62	10.58	11.64
2	1	crude protein	24.56	27.02	29.72
2	1	gross	2.98	3.28	3.61

		energy			
2	1	iron	119.00	130.90	143.99
2	1	mg	0.50	0.55	0.61
2	1	p	0.28	0.31	0.34
2	1	na	0.11	0.12	0.13
2	1	sol fibre	4.00	4.40	4.84
2	1	adf	17.56	19.32	21.25
2	1	ndf	19.01	20.91	23.00
2	1	WSC	44.05	38.46	32.30
2	2	ash	3.04	3.34	3.67
2	2	calcium	0.34	0.38	0.41
2	2	copper	7.05	7.76	8.53
2	2	crude fat	10.58	11.64	12.80
2	2	crude protein	27.02	29.72	32.69
2	2	gross energy	3.28	3.61	3.97
2	2	iron	130.90	143.99	158.39
2	2	mg	0.55	0.61	0.67
2	2	p	0.31	0.34	0.37
2	2	na	0.12	0.13	0.15
2	2	sol fibre	4.40	4.84	5.32
2	2	adf	19.32	21.25	23.37
2	2	ndf	20.91	23.00	25.30
2	2	WSC	48.46	42.30	35.53
2	3	ash	2.48	2.73	3.01
2	3	calcium	0.28	0.31	0.34
2	3	copper	5.77	6.35	6.98
2	3	crude fat	8.66	9.52	10.48
2	3	crude protein	22.10	24.31	26.75
2	3	gross energy	2.68	2.95	3.25
2	3	iron	107.10	117.81	129.59
2	3	mg	0.45	0.50	0.54
2	3	p	0.25	0.28	0.30
2	3	na	0.10	0.11	0.12
2	3	sol fibre	3.60	3.96	4.36
2	3	adf	15.80	17.38	19.12
2	3	ndf	17.11	18.82	20.70
2	3	WSC	39.65	34.61	29.07
2	4	ash	2.61	2.87	3.16
2	4	calcium	0.29	0.32	0.35
2	4	copper	6.06	6.66	7.33
2	4	crude fat	9.09	10.00	11.00
2	4	crude protein	23.21	25.53	28.08

2	4	gross energy	2.82	3.10	3.41
2	4	iron	112.46	123.70	136.07
2	4	mg	0.47	0.52	0.57
2	4	p	0.26	0.29	0.32
2	4	na	0.10	0.11	0.13
2	4	sol fibre	3.78	4.16	4.57
2	4	adf	16.59	18.25	20.08
2	4	ndf	17.96	19.76	21.74
2	4	WSC	41.63	36.34	30.52
2	5	ash	2.74	3.01	3.31
2	5	calcium	0.31	0.34	0.37
2	5	copper	6.36	7.00	7.70
2	5	crude fat	9.55	10.50	11.55
2	5	crude protein	24.37	26.81	29.49
2	5	gross energy	2.96	3.25	3.58
2	5	iron	118.08	129.89	142.87
2	5	mg	0.50	0.55	0.60
2	5	p	0.28	0.31	0.34
2	5	na	0.11	0.12	0.13
2	5	sol fibre	3.97	4.37	4.80
2	5	adf	17.42	19.17	21.08
2	5	ndf	18.86	20.75	22.82
2	5	WSC	43.71	38.16	32.05
3	1	ash	3.93	3.54	4.32
3	1	calcium	0.59	0.53	0.65
3	1	copper	9.23	8.31	10.15
3	1	crude fat	5.87	5.28	6.46
3	1	crude protein	25.94	23.35	28.53
3	1	gross energy	3.60	3.24	3.96
3	1	iron	55.61	50.05	61.17
3	1	mg	0.15	0.14	0.17
3	1	p	0.40	0.36	0.44
3	1	na	0.19	0.17	0.21
3	1	sol fibre	7.60	6.84	8.36
3	1	adf	11.30	10.17	12.43
3	1	ndf	16.50	14.85	18.15
3	1	WSC	47.76	52.98	42.54
3	2	ash	5.79	5.21	6.37
3	2	calcium	0.54	0.48	0.59
3	2	copper	9.35	8.41	10.28
3	2	crude fat	15.02	13.52	16.53
3	2	crude	31.64	28.47	34.80

		protein			
3	2	gross energy	2.76	2.49	3.04
3	2	iron	97.40	87.66	107.14
3	2	mg	0.27	0.24	0.30
3	2	p	0.49	0.44	0.54
3	2	na	0.28	0.25	0.31
3	2	sol fibre	4.38	3.94	4.81
3	2	adf	4.99	4.49	5.49
3	2	ndf	19.83	17.84	21.81
3	2	WSC	27.72	34.95	20.49
3	3	ash	4.41	3.97	4.85
3	3	calcium	0.35	0.31	0.38
3	3	copper	8.49	7.64	9.34
3	3	crude fat	12.58	11.32	13.83
3	3	crude protein	27.23	24.51	29.95
3	3	gross energy	3.45	3.10	3.79
3	3	iron	75.54	67.99	83.09
3	3	mg	0.15	0.14	0.17
3	3	p	0.61	0.55	0.67
3	3	na	0.32	0.29	0.35
3	3	sol fibre	2.40	2.16	2.64
3	3	adf	4.56	4.11	5.02
3	3	ndf	18.17	16.35	19.99
3	3	WSC	37.61	43.85	31.37
3	4	ash	7.58	6.82	8.33
3	4	calcium	0.54	0.49	0.59
3	4	copper	8.51	7.66	9.36
3	4	crude fat	11.33	10.20	12.47
3	4	crude protein	23.35	21.01	25.68
3	4	gross energy	3.13	2.82	3.45
3	4	iron	69.80	62.82	76.78
3	4	mg	0.76	0.69	0.84
3	4	p	0.46	0.42	0.51
3	4	na	0.03	0.03	0.04
3	4	sol fibre	5.52	4.97	6.07
3	4	adf	6.02	5.42	6.62
3	4	ndf	19.50	17.55	21.45
3	4	WSC	38.24	44.42	32.07
3	5	ash	6.19	5.57	6.81
3	5	calcium	0.47	0.42	0.52
3	5	copper	10.30	9.27	11.33
3	5	crude fat	16.03	14.43	17.64

3	5	crude protein	33.51	30.15	36.86
3	5	gross energy	3.50	3.15	3.85
3	5	iron	91.47	82.32	100.61
3	5	mg	0.35	0.32	0.39
3	5	p	0.63	0.57	0.70
3	5	na	0.31	0.28	0.34
3	5	sol fibre	3.54	3.19	3.90
3	5	adf	5.92	5.33	6.51
3	5	ndf	20.16	18.15	22.18
3	5	WSC	24.11	31.70	16.52

Two published papers at the end of the thesis have been removed from the electronic version for copyright reasons

These are:

Francis Cabana and K A I Nekaris, **Diets High in Fruits and Low in Gum Exudates Promote the Occurrence and Development of Dental Disease in Pygmy Slow Loris (Nycticebus pygmaeus)**, *Zoo Biology* 34: 547-553 (2015)

Francis Cabana, Ellen Dierenfeld, Wirdateti Wirdateti, Giuseppe Donati, K A I Nekaris, **The seasonal feeding ecology of the Javan Slow Loris ,(Nycticebus javanicus),** *Am J Phys Anthropol* (2017): 1-15