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<http://dx.doi.org/10.4314/mcd.v10i2.4>

Comparison of parasitic infections and body condition in rufous mouse lemurs (*Microcebus rufus*) at Ranomafana National Park, southeast Madagascar

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ABSTRACT

Body condition may be an important indicator for many infectious diseases and parasites, and may ultimately affect an individual's fitness. Although some research has correlated body condition and parasite loads in other nonhuman primates, little information has been investigated in prosimian primates. In this study we compare parasitic infections and body condition in a member of the Cheirogaleidae family (*Microcebus rufus*: rufous mouse lemur) at Ranomafana National Park, southeast Madagascar. This species is characterized by seasonal fattening in preparation for the dry season followed by torpor, and it is important to understand the fluctuation between parasites and infections according to seasonal body condition. We trapped 72 individuals of the species inside Ranomafana National Park (RNP) after the dry season. These individuals were brought to the Centre Valbio Laboratory (CVB) and were subcutaneously micro-chipped with subdermal transponders for permanent identification. We recorded morphometric data, body condition, species richness and prevalence of ectoparasites and gastrointestinal parasites. We found that individuals that had both high number of parasite species as well as high prevalence of ectoparasites and gastrointestinal parasites had better body condition. There is some indication that being in good condition is important in controlling infections.

RÉSUMÉ

La condition physique peut être un indicateur important pour de nombreuses maladies infectieuses et pour les parasites, et peut finalement affecter l'aptitude d'un individu. Si certaines études ont montré la relation entre condition physique et charges parasitaires chez des primates non humains, peu d'informations étaient disponibles en ce qui concerne les prosimiens. Dans cette étude, les infections parasitaires et l'état de santé du microcèbe roux *Microcebus rufus* de la famille des Cheirogaleidae ont été étudiées dans le Parc National de Ranomafana, Sud-est de Madagascar. Cette espèce est caractérisée par sa capacité à accumuler des matières grasses à la base de la queue afin de se préparer à la

saison sèche au cours de laquelle elle rentre en torpeur ; il est donc important de comprendre la fluctuation saisonnière entre les parasites et les infections selon l'état de santé des individus. Soixante-douze animaux de cette espèce ont été capturés à l'intérieur du Parc National de Ranomafana après la saison sèche. Les individus capturés ont été rapportés au Centre Valbio où ils ont été marqués avec une puce électronique sous-cutanée servant de transpondeur pour l'identification permanente. Nous avons collecté des données morphométriques pour documenter la condition physique, la richesse spécifique et la prévalence des ectoparasites et des parasites gastro-intestinaux. Nous avons constaté que les individus présentant à la fois un grand nombre d'espèces de parasites ainsi qu'une forte prévalence d'ectoparasites et de parasites gastro-intestinaux avaient une meilleure condition physique. Les résultats semblent indiquer qu'un bon état est important dans le contrôle des infections.

INTRODUCTION

Knowledge of animal body condition is of considerable importance in many ecological studies, as well as in disease research (i.e., Coop and Holmes 1996, Alzaga et al. 2008, Munyeme et al. 2010), and as a wildlife management tool (Ezenwa et al. 2010). It may be an important indicator for many infectious diseases and parasites, and may ultimately affect an individual's fitness (Sheldon and Verhulst 1996). Animals in poorer condition often are more heavily parasitized than individuals in better condition (Wilford 1986, Chapman et al. 2006, Tompkins et al. 2011), as poor body condition can lead to susceptibility to parasites and furthermore lead to "vicious circle" of continuing parasite infections and deterioration of the host (Beldomenico and Begon 2010).

Although studies relating body condition, diet and parasites/disease have been conducted on wild and captive monkeys and apes (e.g., Chapman et al. 2006, Altizer et al. 2007), little is known about the relationships between body condition, and parasites in prosimians primates, especially in the nocturnal prosimi-

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mians. Collecting descriptive and analytic baseline data on body condition and parasitic infections is important in determining patterns of health status and will assist in effective disease management and conservation planning.

In this study we compare the extent of parasitic infections and body condition in a member of the Cheirogaleidae family (*Microcebus rufus*: rufous mouse lemur) at Ranomafana National Park (RNP), southeast Madagascar. This species is characterized by seasonal fattening (though not for all individuals at this site) in preparation for the dry season followed by torpor, and it is important to understand the relationship between parasites, disease, and body condition (Wright and Martin 1995). The specific objectives in this paper are to investigate relationships between body condition and multiple measures of parasitism. We hypothesized that i) there is a positive association among body condition indices, ii) there are differences in the measures of body condition index between individuals, and iii) there are differences in measures of parasitic infection between individuals on the basis of sex, site and period of study. Also iv) animals in poorer condition will exhibit higher parasite richness, abundance, and prevalence.

METHODS

STUDY SITE. Mouse lemurs were trapped at two sites from

August until December 2012 in RNP, E047°20', S21°16' (Wright 1992, Wright and Andriamihaja 2002). The first is within RNP at the Talatakely trail system (centroid at E047°25'17.0", S21°15'43.5"), which was clear cut in small areas before 1947 and selectively logged from 1986–1990 before the creation of the national park in 1991 (Wright et al. 2009). The second site was at the research station Centre Valbio (near Campsite location, centroid at E047°25'10.7", S21°15'12.1"), which was clear-cut in 2001.

TRAPPING METHOD. Trapping methods were based on methods used by Wright and Martin (1995) and Atsalis (1999). In each site, aluminum live traps (XLR, Sherman traps inc., Florida, USA, 22.2 x 6.6 x 6.6 cm) baited with banana were set in pairs at 25 m intervals, no more than 3 m from the ground along two transects (Talatakely transect is 1.5 km and Campsite 1 km long).

DATA COLLECTION. Data were collected at three selected periods: Period 1 (beginning of reproductive season: mid-August until the beginning of October), Period 2 (mating season: defined as the dates between which the first and the last vaginal opening was observed, from the beginning to the end of October), and Period 3 (gestation period: from November to December). Traps that contained *Microcebus rufus* were taken to the research cabin at RNP or to the laboratory at Centre Valbio. *M. rufus* brought back to the CVB field station or RNP research cabin were put into separate small linen bags to prevent escape, and were sexed, weighed with a digital scale (Fisher Scientific 200GXO), measured for tail circumference at the widest point with a thread (this thread after measured with an electronic caliper), and scanned for microchips. All new captures were microchipped with subdermal transponders (Fecava Eurochips, Vetcare, Finland) for permanent identification. All animals were released on the same night they were trapped at the site of their capture.

We recorded from non-anaesthetized rufous mouse lemur, body weight (BW), crown rump length (CR, from cranium arch to the base of tail), tail length (TL, from the base to the tip of tail), head length (HL, from tip of nose to the prominent point of occipi-

tal), head width (HW, between two temporal), circumference of the base of tail (CRT) and circumference of mid tail (CRMT). We measured CR, TL, HL, HW, CRT, CRMT to the nearest of mm using an electronic caliper, and BW to the nearest value in grams using a digital scale. We determine sex on the basis of external morphology.

Individual fecal samples of this species were collected from traps, handling bags or directly from anus for the gastrointestinal parasite analysis. We did a direct analysis without preserved fecal samples and ≈0.3 g of feces were used. Two versions of a modified method, outlined by Gillespie (2006), for the gastrointestinal parasite analysis were used. We performed fecal flotation using MgSO₄ solution and quantified the parasite eggs or larvae in McMaster Chamber (Weber Scientific International United Kingdom). We obtained egg count per gram (EPG) by dividing the count by the weight of feces used. Nematodes and flukes are too heavy to float up in the flotation liquid, so the fecal sedimentation method is necessary to identify these helminthes. We used a modified Baermann method (Zohdy 2012). Fresh fecal samples were weighed and folded in tissue paper. Each sample is put in a funnel that has a rubber hose into a glass test-tube containing water, so that the water level reaches the feces. Three days later the larvae are concentrated in the water and we centrifuged the sample and decanted the water. We examined the sediment and made a diagnosis of the nematode larvae, and counted them under microscope. We divided the count by the weight and quantified larvae per gram of feces. Each individual was checked for the ectoparasites. Ectoparasites were counted and scored according to the abundance: 1= no ectoparasites, 2= some (between 1 and 20), score 3= many (over 20).

STATISTICAL ANALYSIS. We calculated three different body condition estimates: body weight (BW), residuals of the linear regression of body weight against total body length (OLS) and tail circumference index (CRT). To estimate the OLS index of *Microcebus rufus*, we performed linear regression of log₁₀ BW against log₁₀ of total body length (TBL=HL + CR + TL) in SPSS 21.0 program. The residual of this analysis were used as the index of body condition and individuals with positive residuals are considered to be in better condition than predicted for their size, while individuals with negative residual are considered to be in relatively poorer condition (Green 2001, Blackwell 2002, Schulte-Hostedde et al. 2005). A log transform data was used to meet the assumption of linearity between body weights against total body length. As *M. rufus* store fat at the base of tail during the period of resource abundance, we computed a transformed index of circumference of tail (CRT index= CRMT/CRT) which reflected an individual's fattening level. We calculated mean ± standard error (SE) for all parameters which indicated body condition index. A bivariate two-tailed Pearson's correlation were used to examine the association between the measures of condition index and a Generalized Linear Model (GzLM) to estimate the difference between individuals on the basis of sex, site and period of study. For model selection, Akaike's information criterion AIC with adjustment for small size AICc (Sugiura 1978) was used for ranking the quality of each model. The AICc value for each model is compared to the lowest AICc value to generate (Δ_i) and to compute the Akaike weights (w_i). As a rule of thumb $\Delta_i < 2$ indicate that there is substantial support for the model, while value greater than $\Delta_i > 10$ indicates that there is no support for the model (Burnham and Anderson 2002).

We analyzed parasite prevalence, richness and abundance as a measurement of the parasite infection. Parasite prevalence is the proportion of a population infected by a particular parasite. The parasite richness was defined as the total number of species of parasites found in one individual, and the parasite abundance was defined as the total number of eggs and larvae (for helminth parasites) per gram of feces or score for the ectoparasite count that we have quantified. We used Chi-square tests of independence to compare the prevalence of infection between sex, site, and period of study, and a nonparametric test *H* of Kruskal-Wallis for analysis of variance, was used to compare the variation of the parasite abundance and parasite richness through sex, site and period of study. We also used Spearman correlation to assess whether different parasites were independently correlated and to measure the association between parasite infection and body condition index. For all analyses when individuals were sampled more than once for each period, we used the mean mass, mean circumference of tail, and mean parasite load. All statistical analyses were two tailed and $P < 0.05$ was considered statistically significant.

RESULTS

Seventy-two individual *Microcebus rufus* were captured between August and December 2012 (32 females and 40 males) summarized in Table 1. There were 43 captures during the beginning of the reproductive season (Period 1), 37 during the mating season (Period 2) and 40 during the period of gestation (Period 3).

CALCULATION AND COMPARISON OF THE BODY CONDITION MEASUREMENTS. The OLS regression equation of \log_{10} BW against \log_{10} TBL was: $y = 0.62x + 0.20$ ($r=0.31$, $t=3.41$, $p<0.05$). We have found a negative relation between BW and OLS index ($r=-0.948$, $n =107$, $p<0.05$) and between CRT index and OLS index ($r=-0.28$, $n =107$, $p< 0.05$). The individuals with heavier weight have lower OLS index and those with fat tails have higher OLS index.

To analyze the difference between individuals, we performed an analysis of model selection including the three factors (sex, site and period of study). For OLS and BW indices, model with three factors had the lowest AICc value and had the highest model weight (Supplementary Material Table S1). Model based only on sex were the best model for Log10 CRT index with 92% of probability, Figure 1 shows the variation of the measure of body condition index between sexes, site through the period of study. Female individuals from Talatakely site were heavier than females in Campsite at the beginning of the reproductive season. Both lost

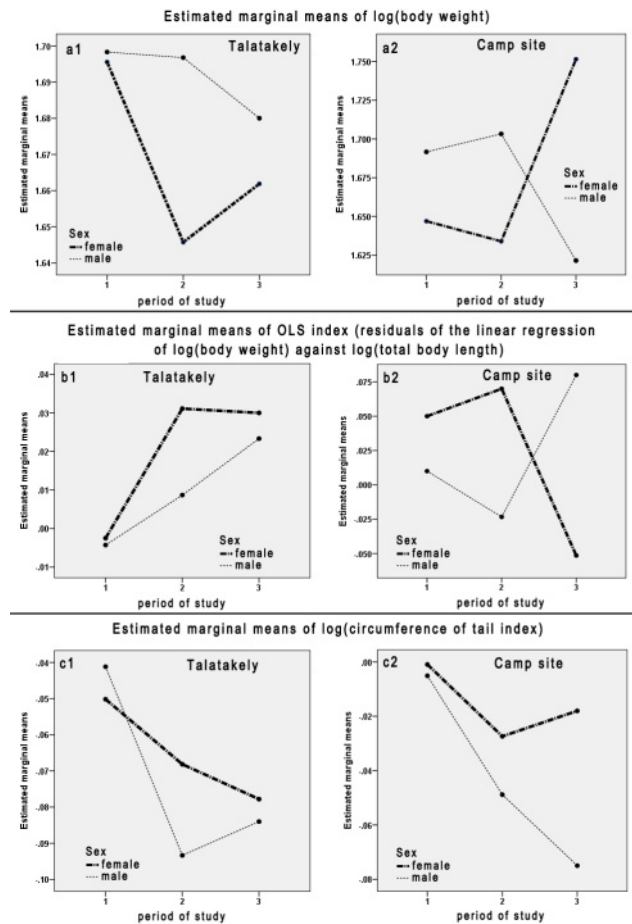


Figure 1. Variation of body condition index between sexes and site through the period of study. Variation based on mean marginal estimated from variable, dark dotted line represent variation in female and light dotted line for the variation in male.

weight during the mating season and later their weight increased. Males in both sites lost weight through the period of study.

According to OLS index, female from campsite were in better condition until mating season, but beyond this period, their condition became worse. However females in Talatakely had increasing index through these periods as the males in both sites (Figure 1, b1 and b2). Based on the CRT index, males were in better condition than females. During the mating season, individual *Microcebus rufus* were in better condition according to the CRT index (Figure2, c1 and c2). This seasonal difference may be due to increased fat in their tails, as source of energy during the mating period.

Table 1. Capture success of *Microcebus rufus* according to season.

Period	Sex	Campsite		Talatakely		Total	
		captured	recaptured	captured	recaptured	captured	recaptured
1	Male	12	0	22	0	34	0
	Female	5	0	4	0	9	0
	Combined	17	0	26	0	43	0
2	Male	2	4	4	13	6	17
	Female	2	3	6	3	8	6
	Combined	4	7	10	16	14	23
3	Male	0	6	0	12	0	18
	Female	12	3	3	4	15	7
	Combined	12	9	3	16	15	25
Total		33	16	39	32	72	48

COMPARISON OF PARASITIC INFECTIONS AND PARASITE RICHNESS. From the fecal analysis, we identified two gastrointestinal parasite species: one cestode (Platyhelminthes, Hymenolepis sp.) (Figure 2a), and one nematode (Nematoda, Strongyloides sp.) (Figure 2b). We also found the ectoparasite Lemurpediculus verruculosus (Durdin et al. 2010) (Figure 2c). Prevalence of infection with Strongyloides sp. and Lemurpediculus verruculosus differed significantly by host sex and site. Male rufous mouse lemur had higher prevalence of nematodes and ectoparasites, individuals in campsite were more parasitized by Strongyloides sp., while those in Talatakely were more parasitized by ectoparasites. In Hymenolepis sp. prevalence, a significant diffe

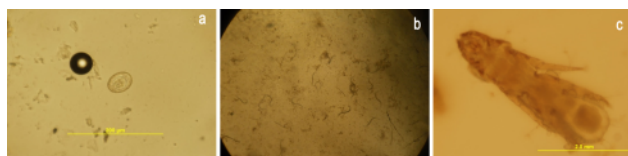


Figure 2. Gastrointestinal parasites (a = *Hymenolepis* sp., Platyhelminthes; b = *Strongyloides* sp., Nematoda), and ectoparasite (c = *Lemurpediculus verruculosus*) identified in *Microcebus rufus* fecal analysis.

rence was found only between sexes, males were more parasitized than females (Supplementary Material Table S2).

The Kruskal-Wallis test reveals that there were effects of sex and site on parasites abundance of *Hymenolepis* sp., and *Lemurpediculus verruculosus*, inspection of the group median suggests that males were an important reservoir for both gastrointestinal parasites and ectoparasites species and individuals in Talatakey were more affected. The difference between two sites concerning the parasite richness wasn't statistically significant (Supplementary Material Table S3). Both sex and site were reservoir of *Strongyloides* sp., and the abundance of this parasite increased by period of study (Supplementary Material Table S3). There was a positive statistically significant association between the abundance of *Hymenolepis* sp. and *Strongyloides* sp. ($r=0.25$, $n=119$, $P<0.05$), and between the abundance of *Hymenolepis* sp. and *Lemurpediculus verruculosus* ($r=0.33$, $n=120$, $P<0.05$).

ASSOCIATION BETWEEN PARASITES INFECTIONS AND BODY CONDITION INDEX. We found a weak significant negative correlation between the CRT index and abundance of *Lemurpediculus verruculosus* ($r=-0.192$, $n=120$, $P<0.05$). Parasite richness was statistically significantly negatively correlated with CRT index ($r=-0.193$, $n=120$, $P<0.05$). We did not find a significant association between body weight, OLS residual index and the parasite abundance and richness (Table 2).

DISCUSSION

COMPARISON OF BODY CONDITION INDEX IN *MICROCEBUS RUFUS*. The calculation and comparison of body condition indices of *Microcebus rufus* were studied for only five months. However, this period encompassed a variety of phases in the biological cycle of *M. rufus*: beginning of reproductive season, mating season, and gestation period. Furthermore, the representation of a wide range of individual condition indices was provided by the large sample. We assessed body condition using standard and non-destructive measures for small mammals (Blackwell 2002), like body weight (Jakob et al. 1996) and the OLS residual in-

Table 2. Correlation between measures of body condition index and the parasite abundance and richness (n = sample size, r = correlation coefficient of spearman, p = probability, BW = body weight, CRT = crown rump length, OLS =residuals of the linear regression of body weight VS. total body length)

Model	n	r	p
BW VS. <i>Lemurpediculus verruculosus</i> score	120	-0.08	0.38
BW VS. nematode abundance	119	0.13	0.17
BW VS. cestode abundance	120	0.04	0.69
BW VS. parasite richness	120	0.02	0.80
CRT index VS. <i>L. verruculosus</i> score	120	-0.19	0.04
CRT index VS. nematode abundance	119	-0.00	0.98
CRT index VS. cestode abundance	120	-0.14	0.13
CRT index VS. parasite richness	120	-0.19	0.03
OLS residual index VS. <i>L. verruculosus</i> score	107	0.13	0.19
OLS residual index VS. nematode abundance	106	-0.02	0.86
OLS residual index VS. cestode abundance	107	0.04	0.66
OLS residual index parasite richness	107	0.08	0.44

dex (Green 2001). However, our species experiences seasonal fluctuation in body fat and accumulates fat at the base of tail (Wright and Martin 1995, Atsalis 1999a), therefore we introduced the tail circumference index.

The two indices OLS and CRT correlated so that those lemurs with higher fat reserves had a higher OLS index. Males at both sites exhibited increased body weight and were in better condition based on indices prior to the onset of the mating season, but lost it soon afterwards possibly as a result of their mate searching strategies which usually involve high activity and defense of habitat already occupied by females (Martin 1972). Individuals in Talatakey had more fat reserve at the base of tail than those in Campsite and females from Talatakey site were also heavier and had higher OLS index than those in Campsite. This could be due to Talatakey being a higher quality habitat than Campsite.

We have found a problem using the OLS residual index, the fit regression was not very high ($r=0.31$), and the scaling theory about the cubic relationships between mass and length was not met in the study. The scaling exponent which equal to the slope in the regression equation ($b=0.62$) was different to the cubic relationship ($b=3.0$) (LaBarbera 1989, Blackwell 2002). Thus we found the residual index from regression of body weight on body length is not appropriate to predict the body condition for this lemur species. It seems that the condition predicted by the circumference of tail index was the best indicator of energy state of this species.

The main limitation of this study was the lack of an absolute measure of body condition. An animal in good condition is assumed to have more energy reserves than an animal in poor condition (Schulte-Hostedde 2005). It is important to estimate the nutritional condition of the habitat, to measure an animal's fitness, and to examine the effect of potential parasites on the host animal (Wilson et al. 2002). The benefit of better body condition for males is that they may be better able to monopolize access to reproductive females during mating season, while also being better able to defend against other males. Males in poorer condition are therefore likely to experience lower reproductive success (Rasoazanabary 2006). Ultimately, body condition is an ability of the animal to successfully reproduce and thus the reproductive success should be assessed (Lewis and Kappeler 2005) which we were not able to do. More parameters are needed to validate the measure of body condition in this species. Specifically, examining the impact of circumference of tail index on reproductive success in this species is likely to reveal important information.

COMPARISON OF PARASITES INFECTIONS IN *MICROCEBUS RUFUS*. *Microcebus rufus* at Ranomafana National Park were found to be co-infected with a combination of two intestinal helminthes (*Strongyloides* sp. and *Hymenolepis* sp.) and one louse species (*Lemurpediculus verruculosus*) through the beginning reproductive season, mating season and gestation period. We detected that males were more infected by parasites than females and had higher parasite diversity. These patterns have been observed in variety of mammal taxa (i.e., Schalk and Forbes 1997, Moore and Wilson 2002, Schulte-Hostedde et al. 2005). Males carrying more parasites than females has often been attributed to the immunosuppressive effect of androgens such as testosterone (Folstad and Karter 1992). However, Zohdy (2012) has reported no significant difference in testosterone level between the sexes in *M. rufus*, the same author showed that this factor is unlikely to be responsible for the high parasite loads seen in males. Other pro-

posed explanations relate to body size dimorphism (Zuk and McKean 1996), and mating system that puts one sex at a disadvantage with regard to transmission of parasites (Moore and Wilson 2002). *M. rufus* is not dimorphic with respect to size. So the best explanation for the sex differences in parasite load and richness may be likely due to the mating system. *M. rufus* have polygandrous mating system (Atsalis 2008) so the competition and interaction between males could be higher, that pattern leading to increase parasite infestation in males (Nunn et al. 2003).

We have found a difference in parasite infection between sites, individuals in Campsite were more infected by *Strongyloides* sp. However individuals in Talatakely were more infected by *Lemurpediculus verruculosus* and *Hymenolepis* sp. These results could be related to the differences in life cycles of parasites, in host-parasite system, host body condition and ecological factors (i.e., Eley et al. 1989, Coe 1993, Friedman and Lawrence 2002, Gillespie et al. 2005, Mborá and McPeck 2009). *Strongyloides* sp. characterized by its direct life cycles (the parasites is transmitted directly from one host to the next without an intermediate host or vector of another species), so individual *Microcebus rufus* in campsite could be more in contact with the infective larvae of that parasites (Radespiel et al. 2015) because of their sleeping mode and possible territory overlap. And the sleeping site could be contaminated by feces from infected individuals. In addition, Campsite also exhibits higher habitat disturbance with villagers living very near this buffer zone than Talatakely inside Ranomafana National Park (Wright 1992). This might reduce the amount of *M. rufus* habitat and increase possibility of interplay between animals, humans and parasite infection. In contrast, according to Zohdy (2012) the dependence of sucking lice on direct host-host interaction suggests that individuals in Talatakely engage in more physical social interaction and will therefore be the causes of the spread of ectoparasites infestations. Concerning the infestation of *Hymenolepis* sp. this could be related to the diet of the Talatakely *M. rufus* population which may include the intermediate host of the parasites like an arthropod, which we were not able to investigate in this study.

Statistically significant differences in *Strongyloides* sp. larvae abundance in feces were found across periods of study. Abundance varied significantly over the three periods. A lower larvae count was detected at the first period (beginning of reproductive season), while higher abundance was found during the third period (gestation period). This could be indicative of lower intensity of nematode infection at the beginning of reproductive season, which is the end of dry season, and higher intensity infection when gestation period is approaching, which is the beginning of raining season at RNP. This pattern could be related to the life history of this nematode, the seasonal variation of the intensity of infection within individuals and individual vulnerability toward infection under some sort of environmental challenge (Radespiel et al. 2015). The climate during breeding season favors hatching of nematode eggs, which increases the abundance of larvae ready to infect individual *Microcebus* spp. (Ganzhorn and Raharivololona 2010). In this study we used fecal samples from different individuals. However, according to Ganzhorn and Raharivololona (2010), a within-individual analysis across seasons is often desirable.

ASSOCIATION BETWEEN PARASITES INFECTIONS AND BODY CONDITION INDEX. Parasites, by their very nature, derive resources from the host and may affect host survival and re-

production directly, but also indirectly by reducing host body condition (Coop and Holmes 1996, Neuhaus 2003, Gillespie and Chapman 2005). Thus, parasite infections are considered to be a critical component in conservation biology (May 1988). Individuals in poor condition might be unable to resist parasitic infection because of the energetic expense of mounting an immune defense (Martin et al. 2003).

In contrast, our results indicate that individuals who had more parasites species and high prevalence on louse infections and gastrointestinal parasites had more fat in their tail (lower CRT index). This suggests that a good quality host might be able to sustain higher parasite loads (Bize et al. 2008, Seppälä et al. 2008). Furthermore, parasitic infections might not always lead to immediate energetic costs for the host. Rather, the costs might be manifested in longer-term reductions in fitness (Willis and Poulin 1999). For our animal study we could not detect signs of clinical significance, and some parasite-host relationships might be initially commensalistic, but affect animals more severely when intrinsic or ecological stress increases. A long term study examining body condition and parasite infections in this species is needed to support our findings.

CONCLUSION

More parameters are needed to validate measurements of body condition in *Microcebus rufus*, although body condition, predicted by the circumference of tail index, was the best indicator of energy state in this species. Sex and site differences in parasitic infection were found. Male *M. rufus* were more infected by parasites than females and had higher parasite diversity. Individuals in Campsite were more infected by *Strongyloides* sp. However individuals in Talatakely were more infected by *Lemurpediculus verruculosus* and *Hymenolepis* sp. These results could be related to the differences in parasite life cycle, in host-parasite system, or ecological factors. The relationship between body condition and parasitic infection, in this study, reveals that animals in better condition were more infected by parasites because of relatively better quality resources available in that host.

ACKNOWLEDGEMENTS

We would like to thank the University of Antananarivo, University of Helsinki, Stony Brook University, Department of Paleontology and Biological Anthropology Tananarive, Madagascar National Park, Primate Conservation Inc. for funding and MICET with Centre Valbio for logistics and technical support. We are grateful to three anonymous reviewers who allowed us to improve an original version of this contribution.

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SUPPLEMENTARY MATERIAL.

Available online only.

Table S1. Seven candidate models for each measures of body condition index in *Microcebus rufus* at RNP. Model selection based on Akaike's information criterion (AIC).

Table S2. Comparison of the prevalence of infection of *Microcebus rufus* between sex, site, and period of study.

Table S3. Comparison of parasite abundance and parasite richness of *Microcebus rufus* between sex, site, and period of study.