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RESEARCH ARTICLE

Variation in Physiological Health of Diademed Sifakas Across Intact and Fragmented Forest at Tsinjoarivo, Eastern Madagascar

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As undisturbed habitat becomes increasingly rare, managers charged with ensuring the survival of endangered primate species must increasingly utilize disturbed and degraded habitats in species survival plans. Yet we have an imperfect understanding of the true long-term viability of primate populations in disturbed habitat, and census data can be misleading because density is not necessarily correlated with habitat quality and population viability in predictable ways. Here we present clinical laboratory data on hematology, serum biochemistry, fat-soluble vitamins, minerals, iron analytes, viral serology, and parasitology of diademed sifaka (*Propithecus diadema*), derived from the capture of 26 individuals spanning eight groups and two habitats (undisturbed vs. disturbed and fragmented) at Tsinjoarivo, Madagascar. Blood from fragment individuals had significantly lower values for several factors: white blood cell counts, bilirubin, total protein, albumin, calcium, sodium, chloride, manganese, zinc, iron and total iron-binding capacity. Several biochemical variables were higher in immature individuals, probably due to active growth. The large number of interhabitat differences suggests that habitat disturbance has an impact on physiological health within this population, perhaps reflecting dietary stress and/or immunosuppression. These results, combined with previous data showing altered diet, slower juvenile growth, and reduced activity in disturbed forest fragments, suggest that fragment sifakas may be less healthy than continuous forest groups. Finally, Tsinjoarivo sifakas have extremely low blood urea nitrogen (perhaps reflecting protein limitation) and selenium levels relative to other lemurs. Despite their survival and reproduction in the short term in fragments, these sifakas may represent a riskier conservation investment than conspecifics in undisturbed forest, and may be more susceptible to environmental stressors. However, more data on the fitness consequences of these biochemical differences are needed for a better interpretation of their impacts on long-term viability prospects. Am. J. Primatol. 72:1013–1025, 2010. © 2010 Wiley-Liss, Inc.

Key words: Madagascar; *Propithecus diadema*; physiology; conservation; habitat fragmentation

INTRODUCTION

Habitat fragmentation and degradation threaten biodiversity and ecosystem integrity worldwide [Chapman & Peres, 2001; Laurance et al., 2000]; indeed, fragmented and degraded habitats constitute a large proportion of many species' remaining habitat. Using satellite technology it is relatively easy to monitor the extent of habitat loss and fragmentation [Harper et al., 2007; Jorge & Garcia, 1997], and to a lesser extent, habitat disturbance [Ingram et al., 2004]. However, relationships between habitat changes and species extinctions are complex and poorly understood, largely due to the confounding effects of fragmentation, degradation, and direct anthropogenic impacts such as hunting [Fahrig, 2003; Redford, 1992]. Given the increasing forest loss, fragmentation, and degradation throughout the world, truly pristine habitat is becoming rare for many species, and planners are increasingly taking advantage of disturbed areas in regional conservation plans. It is therefore critical to know the conservation value of altered habitats: first, how quickly they can regenerate into communities and ecosystems that can function to meet management needs, and second, under what conditions they can sustain viable populations of animal species of interest.

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Biologists have documented differences in population density across habitat disturbance and fragmentation gradients in many study systems [Laurance et al., 2002]. Many authors have demonstrated marked interspecific variation in responses to fragmentation and disturbance (with some species increasing and others decreasing) among primates [Chiarello & de Melo, 2001; Ganzhorn et al., 2003; Lehman et al., 2006a,b; Onderdonk & Chapman, 2000] and other mammals [Goodman & Rakotondravony, 2000; Nupp & Swihart, 2000]. However, densities may be misleading for primates, which are long-lived and relatively mobile. Primate populations in degraded habitats may (1) demonstrate time lags between disturbance or fragmentation and ultimate extinction, and (2) represent sink populations in which groups persist only through immigration from better-quality habitats [North & Ovaskainen, 2007; Pulliam, 1988]. In either case, degraded habitat may contain primates in the short term (sometimes at increased density), but might be of low conservation value for particular species in the long term.

An ideal way of assessing population viability in disturbed habitats is to assess vital demographic statistics (natality, mortality, and migration rates), which can be used in population viability analyses. However, for primates (and other animals with slow life histories), the time required to collect these data is long, which can be problematic when management decisions must be taken quickly. At the other extreme, censuses are rapid and useful in documenting population density differences among habitats [Lehman et al., 2006b], but unless repeated over time they yield few data that can be used to infer population viability. An intermediate solution is to directly compare animals' health and behavior in altered and intact habitats, to identify behavioral and physiological responses to habitat changes. Primatologists have already shown that some species have altered behavior in degraded habitats [Estrada et al., 1999; Irwin, 2008a; Menon & Poirier, 1996; Onderdonk & Chapman, 2000], but the fitness effects of these changes remain relatively unexplored [but see Chapman et al., 2006]. Primates are behaviorally flexible; documenting behavioral shifts is an important first step, but determining how those shifts affect fitness is critical for conservation. It is therefore vital, when possible, to measure fitness and health more directly in primate populations in disturbed habitat.

An increasing number of health assessments have been published for free-ranging primate species, including lemurs [Dutton et al., 2003, 2008; Junge & Garell, 1995; Junge & Louis, 2002, 2005a,b, 2007; Junge et al., 2008]; however, data are limited to relatively few species, often examine a small number of parameters, and typically sample only one habitat. This is unfortunate, since these studies have great potential to provide (1) ''normal'' or expected reference values for a species, (2) qualitative and quantitative data for population viability analysis programs, (3) comparison with the same population at a future date to determine the effects of disturbance (i.e., logging, weather extremes, habitat loss, ecotourism), and (4) comparison among populations, including translocated or captive animals, to understand the relative quality of different habitats, judge various management strategies, help understand the etiologies of captivity-related diseases, and aid in risk assessment of reintroduction programs.

Here we present biomedical health assessment data for 26 diademed sifaka (*Propithecus diadema*) in eight groups, across intact and degraded/fragmented habitat at Tsinjoarivo, Madagascar. We present data on hematology, plasma total protein, serum chemistry, fat-soluble vitamins, trace minerals, measures of iron metabolism, and serological evidence of infectious agents. We ask the following: (1) what are typical baseline values for these parameters for *P. diadema* at Tsinjoarivo? and (2) do values differ between habitats (intact vs. fragmented) and age classes (immature vs. adult)?

METHODS

All animal capture and medical evaluation protocols were approved by McGill University's Animal Care Committee and St. Louis Zoo's IACUC, and adhered to the legal requirements of Madagascar. This research adhered to the American Society of Primatologists' principles for the ethical treatment of nonhuman primates.

Study Site and Study Species

Tsinjoarivo forest (19°41′S, 47°48′E; Fig. 1) is located southeast of Ambatolampy and atop the escarpment dividing Madagascar's central plateau from the eastern coastal lowlands. This region contains a unique, under-explored block of central domain, mid-altitude rainforest; its eastern half remains relatively intact and undisturbed while its western half has been fragmented and degraded by human settlers. Although Tsinjoarivo is being considered for protected status, its current status is ''Classified Forest'' and there is little protection on the ground. Forest loss and disturbance (e.g., selective extraction of hardwood trees) continue in some areas. Tsinjoarivo contains a unique *P. diadema* population that has lower body mass than other *P. diadema* populations. Although previous genetic studies did not assign it to a new subspecies [Mayor et al., 2004], more recent natural history reviews treat it as distinct [Mittermeier et al., 2006]. This sifaka is most likely limited to the small forest block between the Mangoro and Onive rivers, a very small part of *P. diadema*'s overall range ($<2{,}000\,km^2$).

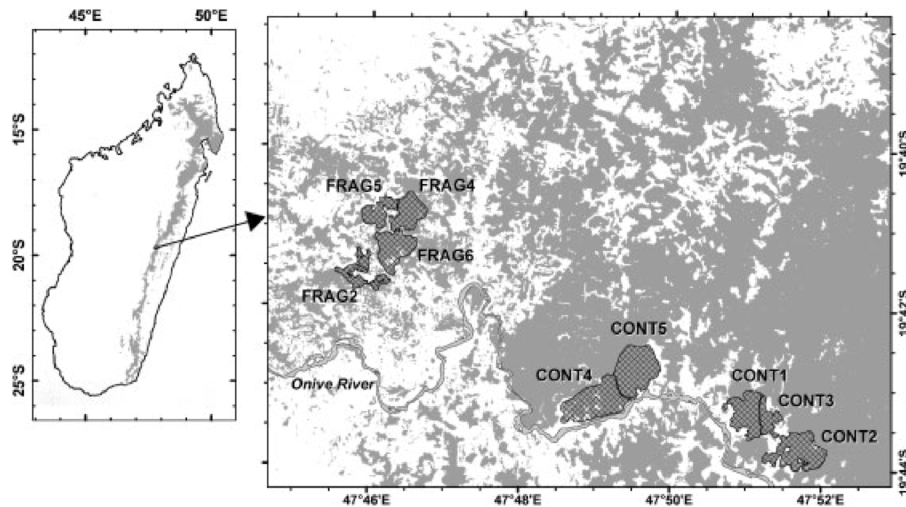

Fig. 1. Location of continuous (CONT) and fragmented (FRAG) forest study groups. Madagascar map shows approximate extent of rainforest; regional map shows rainforest cover based on a 2001 IKONOS satellite image (GeoEye, Dulles, VA). Home ranges are based on ranging data except CONT3 (estimated home range illustrated because this group was not followed systematically). CONT5 was not sampled for this study.

The Tsinjoarivo sifakas have been the subject of behavioral and ecological research since 2002 [Irwin, 2008a,b], centered around three camps. This study examines four ''continuous forest'' groups in intact, undisturbed forest (groups CONT1, CONT2, CONT3 at Vatateza: 19°43.25′S, 47°51.41′E; 1,396 m; group CONT4 at Ankadivory: 19°42.98′S, 47°49.293′E; 1,345 m) and four ''fragment'' groups in disturbed, fragmented forest (groups FRAG2, FRAG4, FRAG5, FRAG6 at Mahatsinjo: 19°40.94′S, 47°45.46′E; 1,590 m). Forest composition differs between sites, largely due to past disturbance; CONT habitats have higher species richness, higher canopy, fewer (but larger) trees, and higher overall basal area per hectare [Irwin, 2006a]. Sifakas' diet composition in terms of plant parts is relatively consistent across the study population [53% of feeding time on foliage, 24% on fruits, 7% on seeds, and 15% on flowers; Irwin, 2008a], but species composition of the diet varies greatly, with FRAG groups relying heavily on mistletoes (a fallback food). Groups are capable of persisting in fragments over the short term (fragment groups have survived and reproduced since at least 2000), but indirect signs of stress are evident: a dietary shift with increased reliance on mistletoe [Irwin, 2008a], reduced body mass, especially for juveniles [Irwin, 2006a; Irwin et al., 2007], and altered activity patterns including reduction of energetically costly activities such as play and ranging [Irwin, 2006a].

Capture and Data Collection Methodology

From 8 to 22 July 2008, we captured 26 sifakas (CONT1: 4, CONT2: 4, CONT3: 1, CONT4: 3, FRAG2: 4, FRAG4: 5, FRAG5: 2, FRAG6: 3). Of these animals, 13 were resident adults (8 female and 5 male) and 13 were aged 1–5 years; 6 of the adult females had infants (<2 months) and 2 were pregnant (near term). Groups were pre-habituated and all captures were performed at close range using a blowgun loaded with Pneu-dart 9 mm disposable nonbarbed darts. Sifakas were immobilized with tiletamine/zolazepam (Telazol®, Fort Dodge Animal Health, Overland Park, Kansas 66225; 25 mg/kg). Once anesthetized, we moved animals to the nearest camp for processing. Each animal was given a complete physical examination and monitored by assessing heart rate, respiratory rate, and body temperature. A balanced electrolyte solution equivalent to the blood volume collected was administered subcutaneously. Animals were allowed to recover in burlap sacks before re-release at the original capture site or near group-mates.

Medical evaluations followed the standard protocol used elsewhere in Madagascar by the Prosimian Biomedical Survey Project (PBSP) Team. This protocol has been used since 2000, on over 570 wild lemurs from 31 species across 16 sites [Dutton et al., 2003, 2008; Junge & Louis, 2002, 2005a,b, 2007; Junge et al., 2008]. Blood samples were collected not exceeding 1% of body weight (1 mL/100 g body weight). Whole blood (0.5 mL) was placed into EDTA anticoagulant and the remaining volume into non-anticoagulant tubes and allowed to clot. Serum tubes were centrifuged within 8 hr of collection, and serum pipetted into plastic tubes and frozen in liquid nitrogen for transport to the Saint Louis Zoological Park (St. Louis, MO) and stored at −70°C until analysis.

Within 2 hr of collection, two blood smear slides were made from each anticoagulant sample; smears were fixed and stained. A total white blood cell (WBC) count was done within 4 hr of collection

(Unopette System, Becton Dickinson & Co., Franklin Lakes, NJ), and stained smears examined microscopically for differential blood cell count and hemoparasite examinations. Serum was submitted to the following laboratories for analysis: serum biochemical profile (AVL Laboratories, St. Louis, MO); 25-hydroxycholecalciferol (vitamin D) and trace mineral analysis (Animal Disease Diagnostic Laboratory); fat soluble vitamin analysis (University of Illinois Nutrition Laboratory); iron metabolism analysis (Kansas State University); viral serology for adenovirus group-specific IgG antibody, herpes virus (SA8), influenza A antibody, rotavirus (SA11) group-specific antibody, reovirus, hepatitis A antibody, and West Nile virus IgG and IgM antibody (for $n = 1$ CONT animals and $n = 11$ FRAG animal except West Nile virus, $n = 6$ FRAG animals; Exoterix, San Antonio TX); viral serology for herpes simplex virus (HSV), simian retroviruses (SRV1, SRV2, SRV5), simian T-lymphotropic virus (STLV), simian immunodeficiency virus (SIV), simian foamy virus (SFV), and measles virus ($n = 5$ CONT animals and $n = 9$ FRAG animals except HSV, $n = 3$ CONT animals and $n = 9$ FRAG animals; Diagnostic Laboratory, Washington National Primate Research Center); serology for *Toxoplasma gondii* IgG ($n = 4$ CONT animals and $n = 12$ FRAG animals; University of Tennessee Comparative Parasitology Service). Pathogens selected are either human pathogens known to exist in Madagascar, therefore having anthropozoonotic potential (adenovirus, herpesviruses, influenza, rotavirus, reovirus, hepatitis, West Nile virus, measles) or primate viruses (SRV, SIV, STLV, SFV) that have not yet been identified in lemurs, but could have significant health implications if present. Fecal samples were collected from freshly voided feces when possible and placed into 10% formalin for endoparasite assessment; results are not presented here as they are part of a longitudinal assessment of known individuals (Raharison and Irwin, unpublished data). Ectoparasites were removed with a cotton swab or forceps and placed in 95% ethanol.

Analysis

Univariate general linear models (GLM) were used to explore interindividual variation. Two fixed factors were investigated: habitat (fragmented forest, at the Mahatsinjo site, vs. continuous forest, incorporating Vatateza and Ankadivory sites), and age class (breeding adults of minimum 5 years old vs. all other immatures, aged 1–4 years and residing in natal groups). We did not explore sex as a fixed factor due to small sample size; this would have reduced the sample within each age/sex/habitat combination to <5. We identified outliers within age/sex combinations using Dixon's test [Sokal & Rohlf, 1995] and repeated some analyses with outliers removed.

We did not apply experiment-wide Bonferroni corrections due to the problems applying these methods to field ecological studies [Moran, 2003]. Instead, we report P-values for all tests, and indicate for each analysis the number of significant relationships expected by chance. Levene's test indicated heterogeneity of variance for several of the ANOVAs. Unfortunately, this was not improved by square root or logarithmic transformations. In each case when a factor was statistically significant within an ANOVA with a positive Levene's test, the difference among habitats or age classes was confirmed using a simple, nonparametric alternative (Mann–Whitney U-test). However, we cannot exclude the possibility that heterogeneity of variances contributed to the non-significant P-values for some factors; thus, significant factors may be under-reported.

RESULTS

All lemurs appeared clinically normal (Table I). One female (CONT1: BR) showed evidence of past eye trauma (left eye with corneal scarring and smaller globe size). All individuals had mites similar to *Liponysella madagascariensis* (H. Klompen, personal communication). Results of hematology, serum biochemistry, serum vitamin, and serum mineral analyses are shown in Tables II–V.

Hematology results were in some cases driven by outliers. For total WBC counts (Table II and Fig. 2), the removal of three unusually high outliers (MAHA4 JUV: 8030 WBC/mcl; MAHA6 PG: 7260 WBC/mcl; and CONT4 RAD: 16960 wbc/mcl; Dixon's test: $P<0.01$) resulted in a significant ANOVA model including a significant effect of site ($P = 0.002$), a near-significant effect of age class

TABLE I. Weight and Vital Signs of Captured Sifakas

	Adults			1–4 yr old		
	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL
N	6	7	13	6	7	13
Mass (g)	5,142±387	5,018±199	5,075±294	4,431±538	3,898±980	4,144±823
Temperature (°C)	35.4±0.4	36.8±1.1	36.1±1.1	35.9±0.7	36.5±1.0	36.2±0.9
Pulse (min^{-1})	108±20	104±13	106±16	111±13	104±14	107±13
Respirations (min^{-1})	41.0±15.8	34.3±13.3	37.4±14.3	47.0±15.4	46.0± 11.7	46.5±12.9

TABLE II. Hematology Results, With Results of GLM Analyses (*P*-Values)

	All animals			Adults			1–4 yr old			Effect of		
	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL	Site	Age	Interaction
N	12	14	26	6	7	13	6	7	13			
Packed cell volume (%)	48.4±4.3 (12)	43.1±3.9 (13)	45.6±4.8 (25)	48.3±5.2 (6)	43.7±3.7 (7)	45.8±4.9 (13)	48.5±3.7 (6)	42.3±4.3 (6)	45.4±5.0 (12)	**0.004**	0.724	0.653
WBC/mcl	5,298±4,294 (10)	3,465±1,824 (14)	4,229±3,154 (24)	5,586±5,624 (6)	3,512±1,732 (7)	4,469±3,979 (13)	4,865±1,480 (4)	3,418±2,050 (7)	3,944±1,926 (11)	0.207	0.766	0.819
Segmented neutrophils (%)	49.3±16.9 (12)	53.4±14.4 (13)	51.4±15.5 (25)	58.8±9.6 (6)	51.7±15.3 (7)	55.0±13.0 (13)	39.7±17.8 (6)	55.3±14.6 (6)	47.5±17.6 (12)	0.475	0.200	0.066
Band neutrophils (%)	0±0 (12)	0±0 (13)	0±0 (25)	0±0 (6)	0±0 (7)	0±0 (13)	0±0 (6)	0±0 (6)	0±0 (12)	–	–	–
Lymphocytes (%)	51.2±15.8 (12)	46.0±14.8 (13)	48.5±15.2 (25)	42.7±5.8 (6)	47.4±16.4 (7)	45.2±12.4 (13)	59.7±18.5 (6)	44.3±14.0 (6)	52.0±17.6 (12)	0.376	0.248	0.100
Eosinophyls (%)	0±0 (12)	0±0 (13)	0±0 (25)	0±0 (6)	0±0 (7)	0±0 (13)	0±0 (6)	0±0 (6)	0±0 (12)	–	–	–
Monophyls (%)	0.42±0.67 (12)	0.23±0.60 (13)	0.32±0.63 (25)	0.17±0.41 (6)	0.14±0.38 (7)	0.15±0.38 (13)	0.67±0.82 (6)	0.33±0.82 (6)	0.50±0.80 (12)	0.488	0.187	0.547
Basophils (%)	0±0 (12)	0±0 (13)	0±0 (25)	0±0 (6)	0±0 (7)	0±0 (13)	0±0 (6)	0±0 (6)	0±0 (12)	–	–	–

Because of missing values for some variables, sample size for these factors is indicated in parentheses. No factors demonstrated heterogeneity of variances (Levene's test, $P>0.05$). Bold indicates a significant factor in the GLM ($P<0.05$).

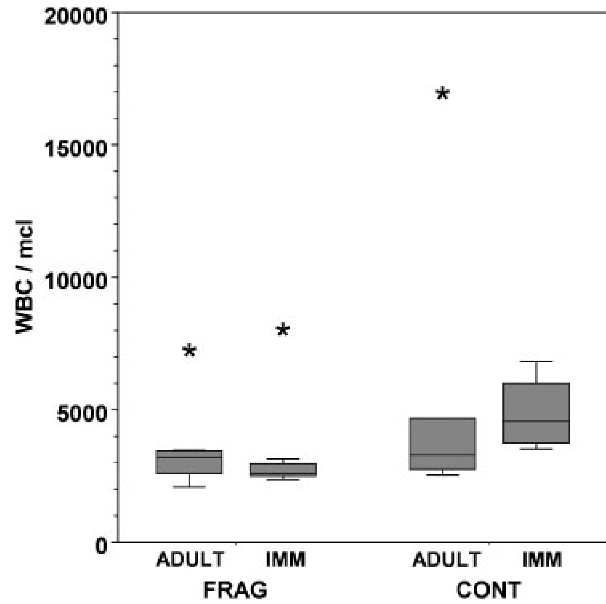

Fig. 2. WBC counts of adult and immature (IMM) sifakas in continuous (CONT) and fragmented (FRAG) habitats.

($P=0.088$), and a significant interaction ($P=0.025$). In general, counts tended to be low for both age classes in FRAG groups, CONT adults were intermediate, and CONT immatures were highest. For this ANOVA, variances were heterogeneous (Levene's test: $P<0.05$), but the site difference persisted in a Mann–Whitney *U*-test either with outliers removed ($Z=-2.596$, $P=0.007$, $N=24$) or included ($Z=-2.021$, $P=0.042$, $N=21$).

No positive results were detected for any of the 15 viral serology assays, and toxoplasmosis titers were negative for all animals tested.

DISCUSSION

Effects of Habitat and Age

For hematology variables, packed cell volume (PCV) was significantly higher in CONT animals, and WBC counts were elevated in CONT animals, especially for immatures (Fig. 2 and Table II). Although we cannot exclude the possibility that differing PCV reflects underlying physiological differences, it is more likely due to the fact that FRAG animals were captured first (when the centrifuge battery was fully charged) and CONT animals were captured second (when the battery was less fully charged and the centrifuge ran more slowly). Thus, values for CONT animals may be more clinically relevant.

For WBC counts, statistically significant effects of site and age class emerged only after outliers were removed; a nonparametric test suggests an effect of site with or without outliers. We believe that the removal of outliers is justified by the complicated time scale on which WBC counts respond to external

TABLE III. Serum Biochemical Profiles, With Results of GLM Analyses (*P*-values)

	All animals			Adults			1–4 yr old			Effect of		
	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL			
N	12	14	26	6	7	13	6	7	13	Site	Age	Interaction
Aspartate transaminase (AST) (IU/L)	29.42±14.21	25.29±14.07	27.19±14.01	25.00±9.86	17.29±13.02	20.85±11.89	33.83±17.33	33.29±10.48	33.54±13.42	0.425	**0.023**	0.488
Alanine aminotransferase (ALT) (IU/L)	43.75±4.27	40.36±13.61	41.92±10.36	43.17±4.36	33.14±14.71	37.77±11.97	44.33±4.50	47.57±7.98	46.08±6.56	0.361	**0.043**[a]	0.082*
Total bilirubin (mg/dL)	0.717±0.248	0.500±0.196	0.600±0.243	0.617±0.117	0.500±0.100	0.554±0.120	0.817±0.313	0.500±0.271	0.646±0.323	**0.020**	0.259	0.259
Alkaline phosphatase (IU/L)	262.3±98.2	257.9±153.2	259.9±128.3	196.0±55.9	157.0±20.4	175.0±43.8	328.5±87.0	358.9±163.3	344.8±129.4	0.913	<**0.001**[b]	0.383*
Gamma glutamyl transferase (GGT) (IU/L)	13.33±4.58	14.64±4.73	14.04±4.62	15.33±2.58	16.14±2.54	15.77±2.49	11.33±5.47	13.14±6.07	12.31±5.63	0.466	0.060	0.779
Total protein (g/dL)	7.44±0.45	7.03±0.47	7.22±0.50	7.58±0.51	7.20±0.48	7.38±0.51	7.30±0.37	6.86±0.43	7.06±0.45	**0.030**	0.093	0.869
Albumin (g/dL)	5.41±0.27	4.97±0.29	5.17±0.36	5.52±0.23	4.94±0.31	5.21±0.40	5.30±0.28	5.00±0.29	5.14±0.32	**0.001**	0.482	0.232
Globulin (g/dL)	2.03±0.28	2.06±0.35	2.05±0.32	2.07±0.36	2.26±0.36	2.17±0.36	2.00±0.21	1.86±0.21	1.92±0.22	0.840	0.058	0.166
Blood urea nitrogen (mg/dL)	3.92±1.44	4.86±1.51	4.42±1.53	4.17±1.17	5.00±1.73	4.62±1.50	3.67±1.75	4.71±1.38	4.23±1.59	0.133	0.521	0.860
Creatinine (mg/dL)	0.558±0.162	0.593±0.114	0.577±0.137	0.617±0.098	0.629±0.076	0.623±0.083	0.500±0.200	0.557±0.140	0.531±0.165	0.522	0.090	0.674*
Phosphorus (mg/dL)	3.70±1.09	4.19±1.18	3.97±1.15	3.30±0.84	3.84±0.75	3.59±0.81	4.10±1.23	4.54±1.48	4.34±1.34	0.276	0.103	0.911
Calcium (mg/dL)	10.38±0.43	9.97±0.58	10.16±0.55	10.18±0.35	9.71±0.52	9.93±0.50	10.58±0.44	10.23±0.56	10.39±0.52	**0.041**	**0.025**	0.766
Glucose (mg/dL)	107.5±14.3	109.6±23.0	108.7±19.2	104.8±17.4	113.1±26.6	109.3±22.4	110.2±11.3	106.1±20.3	108.0±16.2	0.789	0.917	0.444
Sodium (mmol/L)	145.5±2.1	139.9±5.1	142.5±4.9	146.5±2.1	139.4±6.4	142.7±6.0	144.5±1.8	140.3±3.7	142.2±3.6	**0.002**[c]	0.726	0.384*
Potassium (mmol/L)	4.47±0.51	4.08±0.54	4.26±0.55	4.47±0.53	3.93±0.51	4.18±0.57	4.47±0.53	4.23±0.56	4.34±0.54	0.079	0.483	0.483
Chloride (mmol/L)	116.8±2.1	112.9±4.5	114.7±4.1	117.5±1.5	113.0±5.6	115.1±4.7	116.0±2.5	112.9±3.7	114.3±3.5	**0.017**[d]	0.585	0.652*
Creatinine phosphokinase (IU/L)	390.6±301.7	364.6±316.9	376.6±304.0	333.5±234.8	135.3±69.0	226.8±189.6	447.7±370.5	594.0±300.1	526.5±328.6	0.805	**0.011**[e]	0.111*

2.55 significant *P*-values would be expected by chance in this analysis; thus, some of the 11 significant factors reported may be due to chance. *Levene's test is significant ($P<0.05$), indicating heterogeneity of variances.
[a]Significant Levene's test indicates heterogeneity of variances, but significant effect of age confirmed by Mann–Whitney *U*-test directly comparing the two age classes ($Z=-2.057$, $P=0.04$).
[b]Significant Levene's test indicates heterogeneity of variances, but significant effect of age confirmed by Mann–Whitney *U*-test directly comparing the two age classes ($Z=-3.949$, $P<0.001$).
[c]Significant Levene's test indicates heterogeneity of variances, but significant effect of site confirmed by Mann–Whitney *U*-test directly comparing the two habitats ($Z=-3.135$, $P=0.001$).
[d]Significant Levene's test indicates heterogeneity of variances, but significant effect of site confirmed by Mann–Whitney *U*-test directly comparing the two habitats ($Z=-2.303$, $P=0.02$).
[e]Significant Levene's test indicates heterogeneity of variances, but significant effect of age confirmed by Mann–Whitney *U*-test directly comparing the two age classes ($Z=-2.487$, $P=0.01$).
Bold indicates a significant factor in the GLM ($P<0.05$).

TABLE IV. Serum Fat-Soluble Vitamin Analysis, With Results of GLM Analyses (*P*-Values)

	All animals			Adults			1–4 yr old			Effect of Site, Age		
	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL	Site	Age	Interaction
N	12	14	26	6	7	13	6	7	13			
25-Hydroxy Vitamin D (nmol/L)	17.8±4.1	21.6±7.7	19.8±6.5	16.0±4.0	18.9±4.0	17.5±4.1	19.5±3.7	24.4±9.8	22.2±7.7	0.119	0.072	0.671
Retinol (μg/dL)	36.0±13.1	38.1±9.3	37.1±11.0	32.5±15.3	39.1±13.0	36.1±13.9	39.5±10.6	37.2±4.1	38.2±7.5	0.638	0.577	0.328
Retinyl palmitate	1.78±1.18	2.66±1.41	2.25±1.36	1.58±0.66	2.86±0.99	2.27±1.05	1.98±1.59	2.46±1.80	2.24±1.65	0.114	1.000	0.460
Gamma tocopherol (μg/dL)	49.1±21.9	81.8±50.0	66.7±42.3	53.0±26.1	73.0±41.8	63.8±35.6	45.2±18.3	90.6±59.1	69.6±49.4	0.054	0.764	0.437
α-tocopherol (μg/dL)	740±195	837±176	792±188	764±257	870±147	821±203	716±129	804±207	763±174	0.206	0.453	0.903
Lutein+zeaxanthin (μg/dL)	2.01±1.85	1.61±1.01	1.79±1.44	2.00±2.00	2.14±0.97	2.08±1.46	2.02±1.89	1.07±0.79	1.51±1.42	0.493	0.370	0.355
β-carotene (μg/dL)	0.383±0.613	0.329±0.177	0.354±0.427	0.383±0.627	0.357±0.098	0.369±0.411	0.383±0.659	0.300±0.238	0.338±0.459	0.762	0.874	0.874*
Retinyl stearate (μg/dL)	0.208±0.406	0.857±0.627	0.558±0.621	0.083±0.204	0.771±0.594	0.454±0.567	0.333±0.532	0.943±0.695	0.662±0.678	**0.007**	0.340	0.857

1.2 significant *P*-values would be expected by chance in this analysis; thus, the single significant factor reported may be due to chance. *Levene's test is significant ($P<0.05$), indicating heterogeneity of variances. Bold indicates a significant factor in the GLM ($P<0.05$).

stimuli. Long-term changes in baseline values (as well as habitat differences) likely reflect underlying health or infection prevalence, while short-term spikes reflect recent infection. It is possible that the three individuals with high values had had recent infections, and that ANOVA results with these individuals removed more closely reflect underlying health differences. However, the WBC distributions were not altered in a way consistent with recent infection (specifically, increased segmented and band neutrophils). There was a nonsignificant trend toward neutrophilia and lymphopenia in FRAG groups, which on the surface seems consistent with increased stress; however, the neutrophilia and lymphopenia were not associated with WBC counts (data not shown). Choosing which outliers to remove is especially problematic since two of the values (MAHA4 JUV: 8030 wbc/mcl, MAHA6 PG: 7260 wbc/mcl) were statistical outliers, yet within the ''normal'' reference range clinically (for captive lemurs). These two ''borderline'' values may still represent mild elevations due to past illness; conversely, ''normal'' ranges may better represent captive animals but may be too high for stressed wild animals. In summary, the data suggest higher WBC in CONT groups, but more investigation is necessary.

The fact that FRAG animals had the lowest WBC is interesting, as lower body mass and reduced activity also suggest that this population is more stressed [Irwin, 2006a; Irwin et al., 2007]. If infection prevalence is high, one might expect many individuals to have elevated WBC due to recent infections. An alternative hypothesis is that (notwithstanding the outliers) low WBC counts in FRAG groups reflect a weakened immune system due to underlying stress [including dietary differences among populations; Irwin, 2008a]. Several studies have shown that undernutrition causes reduced immune activity in mammals, including calorie-restricted captive rats [Cunha et al., 2003] and fasted captive American mink [Mustonen et al., 2005]. Undernutrition can also lead to increased infection prevalence and intensity [Coop & Kyriazakis, 1999]: for example, increasing protein intake decreases infection intensity of a helminth parasite in lactating ewes [Houdijk et al., 2009], and food supplementation decreases infection intensity of a helminth parasite in wild showshoe hares [Murray et al., 1998].

Several site and age differences were also apparent in serum biochemistry (Table III). FRAG individuals were lower in total bilirubin, total protein, albumin, calcium, sodium, and chloride than CONT individuals. Bilirubin is a breakdown product of heme catabolism. Clinically, elevated bilirubin indicates impairment of normal liver function or an increased breakdown of erythrocytes. Thus, the observed differences may reflect depressed liver function in CONT groups, or slower erythrocyte turnover in FRAG groups. Total

TABLE V. Serum Mineral and Iron Analyte Analysis, With Results of GLM Analyses (*P*-Values)

	All animals			Adults			1–4 yr old			Effect of		
	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL	CONT	FRAG	TOTAL			
N	12	14	26	6	7	13	6	7	13	Site	Age	Interaction
Co (ng/mL)	3.95±2.66	3.00±1.75	3.44±2.22	4.49±3.26	2.49±1.46	3.42±2.56	3.41±2.07	3.51±1.98	3.47±1.93	0.295	0.972	0.245
Cu (μg/mL)	1.03±0.18	1.01±0.20	1.02±0.19	1.09±0.13	0.99±0.26	1.04±0.21	0.96±0.20	1.03±0.14	1.00±0.17	0.871	0.539	0.252
Mn (ng/mL)	5.18±2.08	3.60±1.03	4.33±1.76	4.72±2.02	3.66±1.34	4.15±1.71	5.63±2.21	3.54±0.71	4.51±1.86	**0.023**	0.539	0.431
Mo (ng/mL)	3.15±3.47	1.90±1.76	2.48±2.71	3.03±3.95	1.86±1.50	2.40±2.83	3.27±3.30	1.94±2.11	2.55±2.69	0.269	0.886	0.947
Se (ng/mL)	19.0±9.0	19.8±3.1	19.4±6.4	16.5±4.5	18.9±3.9	17.8±4.2	21.5±11.9	20.7±1.7	21.1±7.8	0.760	0.191	0.543*
Zn (μg/mL)	0.850±0.139 (11)	0.633±0.097	0.728±0.159 (25)	0.814±0.152 (5)	0.619±0.121	0.700±0.163 (12)	0.880±0.134	0.647±0.073	0.755±0.157	<**0.001**	0.340	0.703
Iron (μg/dL)	309±137 (11)	192±69	243±118 (25)	347±191 (5)	154±43	235±155 (12)	277±75	229±73	251±75	**0.008**[a]	0.954	0.092*
TIBC (μg/dL)	589±114 (11)	469±42	522±100 (25)	605±170 (5)	465±48	523±130 (12)	575±49	473±38	520±67	**0.002**[b]	0.760	0.581*
Ferritin (ng/mL)	81.4±43.6 (11)	120.9±79.4	103.5±67.9 (25)	70.2±29.1 (5)	132.9±98.2	106.8±81.3 (12)	90.7±53.9	108.9±60.8	100.5±56.1	0.158	0.950	0.429
Transferrin saturation (%)	51.0±13.3 (11)	41.3±14.6	45.5±14.6 (25)	54.7±16.3 (5)	33.2±8.9	42.2±16.2 (12)	47.8±10.8	49.3±15.2	48.6±12.8	0.069	0.389	**0.038**

1.5 significant *P*-values would be expected by chance in this analysis; thus, some of the three significant factors reported may be due to chance. *Levene's test is significant ($P<0.05$), indicating heterogeneity of variances.
[a]Significant Levene's test indicates heterogeneity of variances, but significant effect of site confirmed by Mann–Whitney *U*-test directly comparing the two habitats ($Z=-2.409$, $P=0.02$).
[b]Significant Levene's test indicates heterogeneity of variances, but significant effect of site confirmed by Mann–Whitney *U*-test directly comparing the two habitats ($Z=-2.985$, $P=0.02$).
Bold indicates a significant factor in the GLM ($P<0.05$).

protein is the sum of albumin (which is produced in the liver and reflects nutritional status) and globulin (which is produced by the immune system and reflects disease exposure). The combination of lower total protein and albumin (but not globulin) in FRAG groups therefore most likely reflects lower protein intake; dietary differences have been documented [Irwin, 2008a] and nutritional differences are currently under investigation. The lower levels of some serum electrolytes (calcium, sodium, and chloride) observed in FRAG groups are also related to intake and may reflect further nutritional differences between the two habitats.

Adults were lower in aspartate transaminase (AST), alanine aminotransferase (ALT), alkaline phosphatase, calcium, and creatine phosphokinase (CPK). This is expected, as these parameters are typically higher in subadult individuals due to active growth (e.g., alkaline phosphatase is a byproduct of osteoblast activity and is elevated when bone growth is occurring).

Among fat-soluble vitamins, only one significant factor was found: fragment individuals had higher levels of retinyl stearate (a form of vitamin A). Again, this is related entirely to diet, as serum concentrations depend on intake. However, the fact that only one of 24 significance tests yielded $P<0.05$ raises the possibility that this significant finding may be due to chance.

Among minerals and iron analytes, FRAG individuals had lower levels of manganese, zinc, and iron, and lower total iron-binding capacity (TIBC). There was a significant age–habitat interaction for transferrin saturation: immatures had similar values across habitats, while CONT adults had higher values and FRAG adults had lower values. The mineral differences (manganese, zinc, and iron) are due to intake differences, and iron analyte differences may also reflect iron intake. Iron is an essential element and involved in many biological systems; its metabolism involves several compartments and movement between the compartments depends on health status, nutritional status, and physiological function. Recent research has clarified the significance of iron in disease of captive lemurs and evaluated monitoring mechanisms [Glenn et al., 2006; Williams et al., 2006, 2008]. Dietary iron is absorbed from the intestinal tract by the transfer molecule transferrin, and is stored in the body as soluble ferritin or insoluble hemosiderin. Under normal physiological conditions serum iron remains stable, but serum ferritin levels vary with iron load (ferritin production increases with iron availability), while transferrin saturation reflects dietary intake (high dietary iron causes increased transferrin saturation) [Smith, 1997]. The combination of lower serum iron, lower TIBC (which reflects fewer transferrin molecules in the blood), and lower transferrin saturation (which approaches significance) in FRAG animals is consistent with lower dietary iron intake. Low serum iron reflects low intake directly, and the magnitude of the difference (38% reduction in FRAG animals) suggests a rather large dietary difference. Low transferrin saturation indicates that the transfer protein (transferrin) is not being utilized to the maximum capacity (transferrin saturation increases when dietary iron increases as more is absorbed and bound, and decreases when dietary iron decreases), and lower TIBC may reflect a reduced need for this transfer protein.

Comparisons With Other Lemur Populations

Utilizing the Prosimian Biomedical Survey Project Database, comparisons to other lemur species can be made. Interspecific comparisons of serum biochemistry reveal large differences in blood urea nitrogen (BUN). Tsinjoarivo *P. diadema* have the lowest mean value of any lemur species sampled, at 4.42 mg/dL (Table VI). Other lemur genera and

TABLE VI. Variance in BUN Across Lemur Species in Madagascar

Species	Site	BUN (mg/dL)			Reference
		Mean	Range	*N*	
Propithecus diadema	Tsinjoarivo	4.42 ± 1.53	2–7	26	This study
Propithecus diadema	Mangerivola	5.0 ± 0	5–5	2	PBSP
Propithecus diadema	Mantadia	6.67 ± 1.86	5–8	6	PBSP
Propithecus tattersalli	Daraina	26.30 ± 19.17	4–85	25	PBSP
Propithecus tattersalli	Daraina	16.2 ± 5.02	n/a	30	Garell and Meyers [1995]
Propithecus deckeni	Tsiombikibo	50.6 ± 18.1	28–90	25	Junge and Louis [2005b]
Indri indri	Various	10.82 ± 3.33	6–17	35	Junge and Louis [2002]; PBSP
Eulemur albifrons	Various	10.63 ± 9.58	0–50	52	Junge et al. [2008]
Eulemur macaco	Lokobe	17.40 ± 5.70	8–29	23	Junge and Louis [2007]
Eulemur rufus	Tsiombikibo	22.80 ± 15.30	7–54	15	Junge and Louis [2005b]
Lemur catta	Tsimanampetsotsa	13.30 ± 4.50	5–20	20	Dutton et al. [2003]
Varecia variegata	Several	9.26 ± 6.40	2–19	60	Junge and Louis [2005a]
Variegata rubra	Masoala	8.58 ± 4.49	3–19	24	Dutton et al. [2008]

PBSP, Prosimian Biomedical Survey Project (R. Junge, unpublished data); BUN, blood urea nitrogen.

species display a wide range of values, up to ten times higher (51 mg/dL). Urea synthesis provides a mechanism for ammonium excretion, and protein is the major source of ammonium for urea synthesis. Changes in BUN can reflect altered renal function (as urea is excreted by the kidneys), altered protein metabolism, or altered state of hydration [Finco, 1997]. Since there is no concurrent evidence of renal compromise, variability in BUN across lemurs most likely reflects protein intake or state of hydration. BUN is therefore a potentially useful indicator of dietary protein (in contrast, total protein reflects physiologic and immunologic proteins and is often not affected by intake).

However, BUN does not sort easily with diet type. Strict frugivores (*Varecia*) have low values, the more omnivorous *Eulemur* and *Lemur* are intermediate, and the mostly folivorous *Propithecus* species range from very low (Tsinjoarivo and other *P. diadema* populations) to intermediate (*P. tattersalli*) to very high (*P. deckeni*). This wide intrageneric variation may be related to habitat: *P. diadema* live in eastern humid rainforests, *P. deckeni* lives in western dry, deciduous forest, and *P. tattersalli* lives in intermediate habitat. Leaves in drier, more seasonally deciduous habitats typically have higher turnover, higher metabolic rates, and are typically of higher quality (more protein, less fiber) than leaves in less seasonal habitats [van Schaik et al., 2005]. Thus, *Propithecus* species in highly deciduous western forest (such as *P. deckeni* at Tsiombikibo) may have much higher available protein intake than do species in evergreen rainforest (such as *P. diadema*), which could converge upon the low-protein diets of strict frugivores. Interestingly, although the sample is small, BUN seems to increase with seasonality. This may also be related to *Propithecus* population density [Irwin, 2006b], which is lowest in rainforest species (1–10 individuals/km^2), highest in dry forest species (37–500 individuals/km^2), and intermediate in *P. tattersalli* (60–70 individuals/km^2). If BUN indeed reflects dietary protein, this suggests that protein intake of *P. diadema* at Tsinjoarivo is relatively low [specifically, the intake of available protein; Rothman et al., 2008], with little difference between continuous and fragmented habitat. Preliminary analyses suggest low dietary protein for Tsinjoarivo sifakas (average available protein content of analyzed foods, dry matter basis = 8.7%; Irwin, unpub. data), as well as for *P. diadema* at Mantadia [6.8%; Powzyk & Mowry, 2003] and a close relative, *P. edwardsi* at Ranomafana [5–6%; Arrigo-Nelson, 2006]. As these levels are close to or below measured protein requirements for similar-sized primates [National Research Council, 2003; Oftedal, 1991], protein should be considered as a potential factor limiting population density and individual growth for rainforest sifakas. However, one alternative explanation worth investigating is that some high BUN values are related to hydration state. Most notably, the *P. deckeni* population was sampled in a dry habitat in the dry season (July); thus, hydration status may have contributed to elevated BUN levels. More research is necessary to determine whether BUN can be a reliable indicator of protein intake.

In terms of iron analytes (iron, ferritin, TIBC, and transferrin saturation), interspecific comparisons suggest that iron may not be a limiting factor for the Tsinjoarivo sifakas (despite the difference between CONT and FRAG individuals). Tsinjoarivo sifakas' average levels of iron, TIBC, and transferrin saturation are higher than those of nine captive lemur species [Williams et al., 2006], and wild *Lemur catta* [Dutton et al., 2003], and their iron and TIBC levels are higher than wild *Propithecus deckeni*, *Eulemur rufus*, *Eulemur albifrons*, *E. macaco*, and *Varecia rubra* [Dutton et al., 2003, 2008; Junge & Louis, 2007; Junge et al., 2008]. Thus, although little is known about species-specific requirements or reference ranges, comparative data suggest that Tsinjoarivo sifakas might not be iron-stressed, and the difference between CONT and FRAG animals may not be clinically relevant.

Finally, Tsinjoarivo sifakas have extremely low serum selenium (19.4 ± 6.4 ng/mL). This is considerably lower than other lemur species sampled to date: *P. diadema* at other sites (56.00 ± 1.41, $n = 2$), *E. albifrons* from various sites (92.05 ± 53.31, $n = 50$), and *Varecia variegata* from various sites (254.57 ± 47.56, $n = 28$) [Junge et al., 2008; R. Junge, Prosimian Biomedical Survey Project, unpublished data]. Selenium values in plants typically reflect soil concentrations, which are known to vary substantially depending on underlying geology [Lenz & Lens, 2009]. In areas with low naturally occurring selenium, deficiencies cause health problems in humans, wild mammals and birds, and livestock; in extreme cases muscle membranes rupture and leak cellular contents, resulting in nonfunctional muscle [''white muscle disease''; Robbins, 2001]. Values for domestic livestock range between 80 and 500 ng/mL [Radostits et al., 2007] and are considered deficient at values less than 25–60 ng/mL. Carnivore values for serum selenium are in the 200–300 ng/mL range, with values less than 120 ng/mL considered deficient. However, minimum requirements in primates have not been well defined. A range of 10–150 ng/mL has been reported for rhesus macaques, and it has been suggested that relatively low levels in primates suggest they may be ''resistant to selenium deficiency'' [Butler et al., 1982].

Management Implications

The data presented here have direct relevance to managing sifaka populations in captivity and in the wild. Previous studies have shown that sifakas persist in small, degraded fragments at Tsinjoarivo over the short term, but various lines of evidence

suggest that fragment-living groups may have a lower-quality diet and reduced energetic status, thus their long-term survival prospects remain uncertain. The data presented here directly reflect physiological health of sifaka groups in habitats with varying degrees of degradation, and suggest that degradation may have impacts on the physiology, and possibly the health, of sifakas. It is especially striking that 12 of 40 variables derived from blood analyses indicated a significant effect of habitat (11 if packed cell volume is removed); this is much more than expected by chance (approximately 2 of 40).

This study also provides important data that will be useful for maintaining sifakas in captivity. This study greatly increases the sample size of ''baseline'' health data for this genus and species, and also reveals a range of variation for unstressed and stressed sifakas in the wild, providing a more meaningful context for judging if captive animals are physiologically stressed. Rainforest sifakas have typically been difficult to keep in captivity, with only one living specimen worldwide; if captive breeding becomes a component of sifaka conservation strategies, understanding their dietary needs will be crucial for success.

Future study is necessary before we can make generalizations about how lemurs and other primates respond to habitat disturbance. This study focuses on a relatively large and mostly folivorous lemur; the proximate pressures of habitat disturbance likely differ among species, and these pressures might be reflected in different baseline physiological parameters. Previous studies relating a species' ecology to its tolerance of disturbance and fragmentation have been lacking since they have relied mainly on observational data, population density, and biomass [Irwin, 2008b; Johns & Skorupa, 1987; van Horne, 1983]; unless data reflect long-term population trends [Chapman et al., 2000], there is a danger of inferring a population is healthy when it is in fact artificially crowded, stressed, and failing to reproduce. Considering physiological data that directly reflect health may: (1) reveal important differences among populations, which might impact population viability, (2) aid in the identification of macro- and micronutrients that may limit growth and reproduction, and (3) contribute to a broader understanding of how interspecific ecological differences cause different likelihoods of extirpation in disturbed habitats.

Finally, it is important to remember that the links between habitat disturbance, physiology, and population health remain poorly known. Demonstrating physiological differences between habitats is an important first step, but a great deal of work will be necessary to determine whether these differences have any consequences for population health. However, if these links can be found, physiological health assessments such as this one could become an important part of a toolkit for rapid assessment of the viability of wild primate populations in degraded habitats.

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