

Association of Anthropogenic Disturbances and Intestinal Parasitism in Ecuadorian Mantled Howler Monkeys, *Alouatta palliata aequatorialis*

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Keywords

Alouatta palliata · Primates · Parasitism · Logging · Anthropogenic disturbance · Ecuador

Abstract

Forest disturbance and human encroachment have the potential to influence intestinal parasite communities in animal hosts by modifying nutritional health, physiological stress, host densities, contact rates, and ranging patterns. Anthropogenic disturbances also have the ability to affect the ecological landscape of parasitic disease, potentially impacting the health of both wildlife and people. Our research investigated the association of forest disturbance and human encroachment on intestinal parasite communities in mantled howler monkeys, *Alouatta palliata aequatorialis*. We found that individual parasite species prevalence was associated with group size and forest disturbance. Proximity to people was not a direct factor influencing intestinal parasitism; rather, several human proximity indices were related to group size, which was in turn related to overall species richness and the presence of specific parasite species. These results, coupled with previous findings, suggest that anthropogenic disturbances are likely influencing intestinal parasite communities. Though no single study has definitively explained all relationships between anthropogenic disturbances and intestinal parasitism, we propose that our models are appropriate for meta-analysis testing across other species and environments.

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Introduction

Studies assessing parasitism in wild primate populations have emanated from an interest in zoonotic pathogen transmission [Muriuki et al., 1998; Howells et al., 2011; Ghai et al., 2014; Cibot et al., 2015], conservation of host species [Wallis and Lee, 1999; Gillespie et al., 2005; Barelli et al., 2015], and ecosystem health [Marcogliese, 2005; Kowalewski et al., 2011]. Modified landscapes due to anthropogenic disturbances have led several researchers to test whether intestinal parasites of host populations vary in response to habitat alteration [e.g., Gillespie et al., 2005; Trejo-Macías et al., 2007; Kowalewski and Gillespie, 2009; Raharivololona and Ganzhorn, 2009; Lane et al., 2011; Junge et al., 2011; Jones et al., 2013]. However, despite various efforts to understand the impact of forest degradation on parasite communities in primates and other wildlife, there still remains the unanswered question of whether specific anthropogenic disturbances affect parasite communities and the degree to which they do so.

Both zoonotic transmission of pathogens and composition of parasite communities in mammals have been linked to human disturbances, as previously outlined [Patz et al., 2000; Chapman et al., 2005; Puttker et al., 2008; Zommers et al., 2013; Helenbrook, 2014; Parsons et al., 2015; reviewed by Han et al., 2016]. In particular, parasite species richness and prevalence of several intestinal parasites have been reported to be higher in selectively logged and forest fragments compared to primary habitat [Gillespie et al., 2005; Salzer et al., 2007; Trejo-Macías et al., 2007; Schwitzer et al., 2010]; however, there are several cases where no effect was found – particularly as it relates to primates [Kowalewski and Gillespie, 2009; Young et al., 2013]. Results of a meta-analysis of howler monkey parasitism research revealed that human proximity and habitat disturbance were not found to have an effect on parasite species richness and the presence of most intestinal parasites [Kowalewski and Gillespie, 2009], though this finding may be due to these studies not assessing habitat disturbance quantitatively. Rather, forest types were characterized dichotomously (e.g., continuous vs. fragmented forest types).

Interactions between the environment, parasites, and hosts are dynamic and complex. Two major anthropogenic factors likely driving intestinal parasitism are forest fragmentation as a result of logging, and human encroachment. In the case of forest fragmentation, there are several hypotheses. First, travel routes can be limited, bringing individuals into greater contact with contaminated foliage or forcing them onto the ground where they are more likely to come into contact with soil-transmitted pathogens [Stoner, 1996; Gillespie et al., 2005; Trejo-Macías et al., 2007; Mbora and McPeck, 2009; Pozo-Montuy et al., 2013]. Groups may also be forced into smaller areas, thereby increasing density, contact rates and subsequent parasite exposure [Stoner, 1996; Nunn et al., 2003; Gillespie et al., 2005; Vitazkova and Wade, 2007; Wells et al., 2007; Mbora and McPeck, 2009; Arroyo-Rodriguez and Dias, 2010]. Edge effects along logged forests can also inhibit or enhance parasitism through altered environmental conditions such as temperature or moisture levels [Chapman et al., 2006a], or increased physiological stress associated with degraded habitats could make individuals more prone to infection [Martinez-Mota et al., 2007]. Fragmentation can also limit individual dispersal between groups or populations which could conceivably force groups into “genetic islands” – limiting their ability to recruit new members and ultimately leading to inbreeding depression [Estrada et al., 2002]. The effect of inbreeding depression on wild primate parasite communities is unknown,

though evidence from captive studies suggests that there is an association between loss of genetic diversity and higher prevalence and abundance of certain parasites [Charpentier et al., 2008; Oklander et al., 2010]. Forest fragmentation has also been shown to affect food availability [Gillespie et al., 2005; Chapman et al., 2006b; Weyher et al., 2006; Dunn et al., 2009]. As a result, host nutrition could be impacted by reduced habitat quality which increases the propensity for infection [Junge et al., 2011]. Likewise, lower density food resources are associated with larger home ranges, forcing individuals to travel further, increasing their encounters with more parasites, and in turn leading to higher parasite abundance and prevalence [Nunn et al., 2003; Gillespie et al., 2005].

The second major factor driving intestinal parasitism in wildlife is human encroachment. As people and communities expand into largely untouched ecosystems, chances for zoonotic transmission are likely to increase. For example, primates living on the forest edge of fragmented habitats see increased contact with agricultural plots and areas with domestic animals – both potential sources of intestinal parasites [Trejo-Macías et al., 2007]. Wildlife may also be more likely to acquire parasites from local human communities due to physical proximity [Graczyk et al., 2002; Goldberg et al., 2007; Davies and Pedersen, 2008; Goldberg et al., 2008; Pedersen and Davies, 2010; Kowalewski et al., 2011]. For example, humans and nonhuman primates interacting in the wild have been shown to share genetically similar *Escherichia coli* [Goldberg et al., 2007, 2008]. Colobus and guenons are also possible reservoirs for zoonotic transmission of *Giardia* and *Cryptosporidium* species [Salzer et al., 2007].

There is evidence that howler monkeys (*Alouatta palliata*), a New World primate species, are negatively affected by anthropogenic disturbances, despite being found in close proximity to people and able to withstand limited habitat degradation [Martínez-Mota et al., 2007; Arroyo-Rodríguez and Dias, 2010]. Howler monkeys provide an excellent example of a primate species that is routinely found near people, providing an opportunity to test hypotheses related to zoonotic transmission. We focused on 2 types of anthropogenic disturbances and their potential impact on parasite communities in mantled howler monkeys, including: (1) forest disturbance as quantified from basal area (cross-sectional area of all trees 1.3 m from the ground within a specified forested area) and percent of trees >40 cm diameter at breast height (DBH; indicative of forest quality [Cottam and Curtis, 1956; Arroyo-Rodríguez et al., 2007]), and (2) human encroachment as defined by proximity to the research station, roads, agricultural plots, and local communities. Our aim was to help unravel the relationship between identified intestinal parasites in a New World primate and indices of anthropogenic disturbance. Understanding how anthropogenic disturbances influence intestinal parasite communities in primate populations is important for management and conservation planning purposes. Further, there is a clear benefit to the people who live near these tropical forests if we can better understand how environmental changes may impact parasitic disease dynamics and zoonotic transmission.

Methods

The Bilsa Biological Station (00°21'33" N, 79°42'02" W; 300–750 m; Fig. 1) is located in northwestern Ecuador, roughly 60 km from the Pacific Ocean. The reserve spans 3,300 ha and is surrounded on 3 sides by the Mache Chindul Ecological Reserve which is adjacent to 2 local

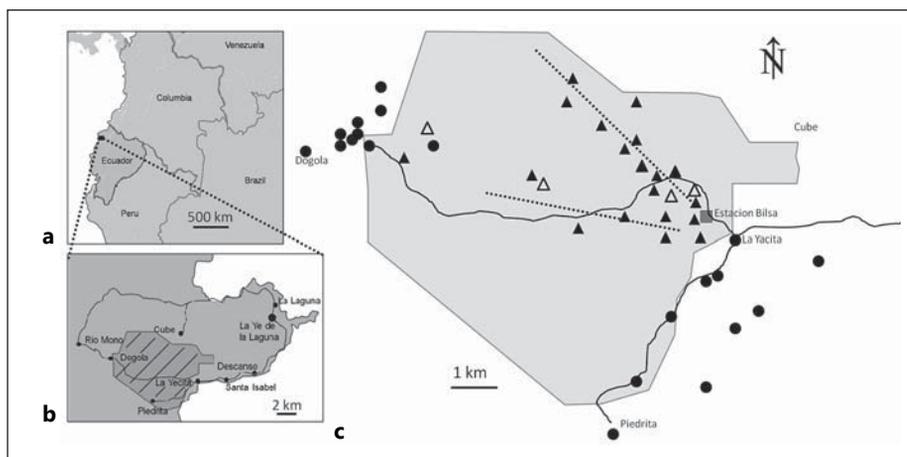


Fig. 1. Field research took place in northwestern Ecuador (a) at the Bilsa Ecological Station – highlighted with diagonal lines (b). Two 5-km transects were used, running through both secondary and primary forest, along which mantled howler monkey groups were systematically sampled (triangles). Groups with more than 1 individual are shown as solid triangles. There are numerous homes, represented by circles, in 3 communities surrounding the reserve (c).

communities. This field site is ideal for our study because varying levels of forest disturbance are present, including 20% secondary forest, 2 nearby communities of less than 200 people in total, and a single road that splits the reserve into 2 similarly sized areas [Ortega-Andrade et al., 2010; Helenbrook, 2014]. The reserve is also devoid of recent hunting pressures, which means that wildlife – particularly monkeys – are often found living in close proximity to people.

To sample primate groups, teams of 2–5 people systematically traversed two 5-km transects collecting 96 fresh fecal samples from 23 primate groups ranging in size from solitary to 10 individuals from June until August 2010. Five sampled groups had 2–3 individuals, 7 groups had 4–5 individuals, 6 groups had 6–7 individuals, and 1 group had 10 individuals. Four solitary individuals were found and were not included in our modeling analysis because a minimum of 2 individuals is needed to account for sampling effort, as described below. Primate groups were systematically sampled along each transect. We sampled the entire group, then moved to the next group further along the transect. Towards evening, the location of the last sampled group would be noted, and sampling would begin the following morning prior to howler movement, with the next group encountered further along the transect from the previous group. It is possible that groups were missed, but our main purpose was to avoid repeat sampling of either individuals or groups [Helenbrook et al., 2015b]. Every effort was made to sample all individuals within a group, though some individuals may have escaped detection or did not defecate during the collection period.

The location of each monkey was recorded using a global positioning system. This information, coupled with satellite imagery, was used to define 4 indicators of human encroachment (proximity), distance from each monkey to the nearest road, distance to the nearest agricultural field, distance to the Bilsa Biological Station, and distance to the nearest human settlement. A forest disturbance estimate was calculated based on where each howler group was located. The first individual howler sampled within a group was used as the central location of the forest plot for that group. Two methods were used to assess forest disturbance: basal area was estimated using data from point-centered quarter methods in 10-m circular plots and percentage of trees

greater than 40 cm DBH was calculated for each plot – both of these measures are inversely associated with forest disturbance [Cottam and Curtis, 1956; Arroyo-Rodriguez et al., 2007]. All trees with a DBH ≥ 10 cm were measured. We were not able to set up a forest plot for 1 howler group because of steep terrain so there were 22 forest plot locations in total.

Fecal samples were collected using disposable gloves and sterile tongue depressors to manipulate a portion of the sample into 50-mL tubes containing 10 mL RNAlater[®] (Qiagen Inc., Valencia, CA, USA), and some into zinc polyvinyl alcohol fixative [Helenbrook et al., 2015b]. These samples were then examined for intestinal parasites using trichrome stain on fecal smears, centrifugal flotations, and sedimentations at the Fish and Wildlife Disease Laboratory at SUNY-ESF, Syracuse, New York. Slides were scanned at 20 \times objective lens using a Nikon 80i compound microscope with Nomarski and phase objectives. Images were captured at 40 \times objective lens with a 3MP IDEA digital camera and analyzed with photomicrography software (Diagnostic Instruments Inc. Spot RT Software 4.6, Sterling Heights, MI, USA). Samples preserved in RNAlater[®] were used for PCR-based detection of *Blastocystis* spp. because they are cryptic but common intestinal parasites found in primates [Stensvold et al., 2009]. DNA was extracted from approximately 200 mg of feces using the QIAamp DNA Stool Mini Kit (Qiagen, Hilden, Germany). *Blastocystis* spp. individuals were confirmed using protocols and PCR primers BH1F/BHRDr, and BLF/BLR and protocols described in Helenbrook et al. [2015a]. Results from fecal smears, flotations, and sedimentation were combined to confirm presence or absence, and are subsequently reported as a single value for all calculations. Thirteen intestinal parasite taxa were recovered as previously described in Helenbrook et al. [2015b].

Statistical Analysis

Parasite species richness within each group of howler monkeys was adjusted for sampling effort by controlling for group size using 3 nonparametric species richness estimators (Jackknife, ICE, and Chao 2 in EstimateS 9.1.0) [Chao, 1987; Colwell, 2013; Helenbrook et al., 2015b]. To be included in this analysis, groups needed 2 or more individuals, resulting in a sample size of $n = 19$ groups. All 3 richness estimates were examined in the group analyses because of their precision, minimally biased predictions, and relative performance compared to other methods [Walter and Morand, 1998]. A significant positive relationship was previously reported between group size and nonparametric species richness estimates [Helenbrook et al., 2015b]. Additionally, the Spearman rank correlation was used to quantify the association between parasite species richness (at the group level) with each measurement of human proximity and forest structure. Multiple regression was then used to assess relative contributions of the anthropogenic factors on group level parasite species richness. Multiple regression models evaluated all explanatory variables regardless of statistical significance in single variable models. Analyses were also conducted focusing on the response variable presence/absence of individual parasite species within each group. A Mann-Whitney U test was used to evaluate whether differences between the medians of sites with parasite species present and sites with the species absent for each of the measures of human proximity and forest disturbance were significant. The Spearman correlation was used to quantify the association between the proportion of monkeys in a group infected with a particular parasite with each of the individual human proximity and forest disturbance measurements.

Finally, structural equation modeling was used to test various relationships between factors using linked regression equations [Mбора and McPeck, 2009; Grace et al., 2010]. Some advantages of structural equation models (SEM) include the ability to use both continuous and binary data, greater statistical power than conventional multiple regression analyses, and the use of latent variables – multiple measurements for a single conceptual variable which allows for estimates and removal of measurement error [Beran and Violato, 2010]. Two sets of SEMs were created based on univariate results. One set of models was created to test the relationship between ecological disturbances and parasite species richness in monkey groups, while the other was developed to test the relationship of these same ecological indices with the presence of specific parasite species. Empirical data were tested against hypothesized path models to determine path coefficients and their standard errors using generalized least squares, a method particularly conducive to smaller sample sizes [Beran and Violato, 2010]. A χ^2 statistic, Akaike information criterion

Table 1. Parasite prevalence among 96 individuals and 23 groups using presence/absence data from pooled results of fecal smears, flotations, and sedimentations

	Parasite species	Percentage of individuals positive	Percentage of groups positive
Apicomplexa	<i>Cyclospora</i> sp.	18	46
	<i>Isospora</i> sp.	3	9
Other protozoa	<i>Balantidium</i> sp.	9	26
	<i>Blastocystis</i> spp.	60 ¹	100 ¹
	<i>Chilomastix</i> sp.	4	17
	<i>Dientamoeba</i> sp.	3	13
	<i>Entamoeba</i> spp.	56	87
	<i>Iodamoeba</i> sp.	5	22
Nematoda	<i>Enterobius</i> sp.	3	9
	<i>Capillaria</i> sp.	78	100
	<i>Strongyloides</i> spp.	88	100
	<i>Trypanoxyuris</i> sp.	12	13
Platyhelminthes	<i>Controrchis</i> sp.	15	39

¹ *Blastocystis* confirmation using PCR-based detection.

(AIC) and root mean square error of approximation (RMSEA) were used to assess model fit. Lower AIC values are associated with best fit models, and RMSEA values <0.05 are generally considered to be indicative of good fit. AIC penalizes model complexity, weighting those most parsimonious models the highest. Residual plots were examined to evaluate whether model assumptions were satisfied. All statistical analyses were done with STATISTICA 10 for Windows (Stat-Soft Inc., Tulsa, OK, USA).

Ethical Note

All methods reported in this manuscript were noninvasive and adhered to guidelines set forth by the Institutional Animal Care and Use Committee at SUNY-ESF in New York, and this research adhered to *Folia Primatologica* principles for the ethical treatment of primates. Permission to import and transfer biological samples was approved by the Center for Disease Control (Permit No. 2009-06-089), and research was approved in country according to guidelines and permit No. 033-FAU-DPE-MA approved by the Director Provincial de Esmeraldas, Lic. Guillermo Oleas Zabala from the Ministerio del Ambiente in Quito, Ecuador.

Results

Thirteen intestinal parasite genera were recovered across the 23 mantled howler monkey groups sampled (Table 1). Nineteen groups had more than 1 individual and were included in the subsequent analyses. Neither actual parasite species richness nor any of the 3 species richness estimators were strongly correlated with human proximity or forest structure variables at the group level (Appendix 1) as the highest Spearman correlation was 0.42 between Jackknife species richness and percentage of trees

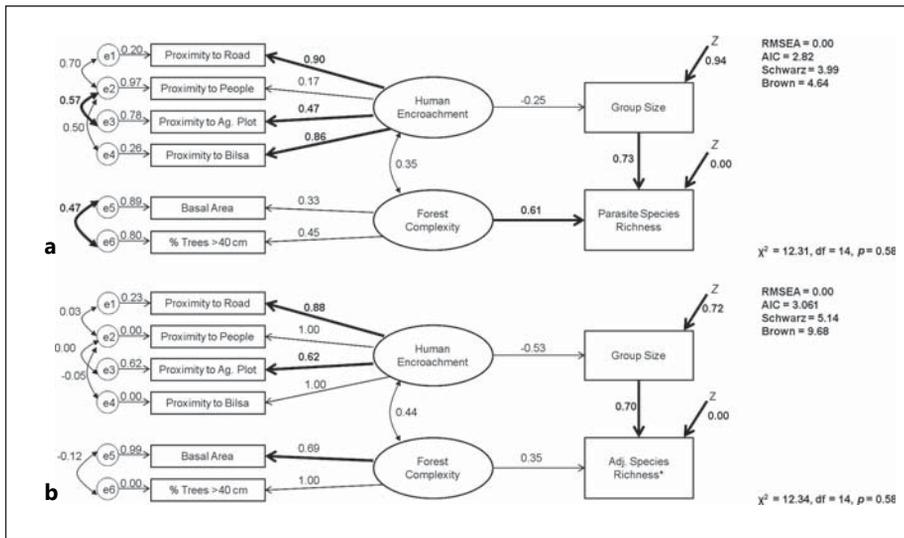


Fig. 2. Best-fit SEMs for overall species richness (a) and adjusted species richness (b) taking into account group sample size. Similar to species richness models, *Controrchis* sp. infections were dependent on a combination of group size, human proximity (indirectly), and forest disturbance measurements (directly). Forest disturbance was not significantly associated with the presence of individuals infected with *Entamoeba* spp.; rather, group size was a driving force which was influenced by human proximity measurements.

>40 cm DBH. Species richness was not significantly associated with the suite of human proximity and forest disturbance variables when evaluated via a multiple regression model. All explanatory variables together yielded an R^2 of only 0.21, and the full model with all explanatory variables was not statistically significant (i.e., $p = 0.70$ for the test of the null hypothesis that all regression coefficients were simultaneously equal to 0, F statistic with 6 and 14 degrees of freedom).

Presence of specific parasite species was associated with several of the human proximity and forest disturbance measurements. Although none of the associations were statistically significant at $\alpha = 0.05$, we examined effect sizes (i.e., magnitude of differences in medians or size of Spearman correlation) to describe trends observed from the data. The strongest associations observed were that monkey groups with individuals infected with *Chilomastix* sp. tended to be found closer to human settlements than those that showed no infection (Appendix 2 and 3), and a higher prevalence of *Blastocystis* spp. infections was found in groups nearest to the research station (Appendix 3). This same trend was found in these 2 species across all anthropogenic measurements – *Chilomastix* sp. and *Blastocystis* spp. were much more common in disturbed areas. Conversely, groups with *Controrchis* spp. were more likely to be found in forest that had lower levels of disturbance. None of the Spearman correlations between proportion of infections within a group and a human proximity or forest disturbance variable exceeded 0.40 in absolute value (Appendix 3).

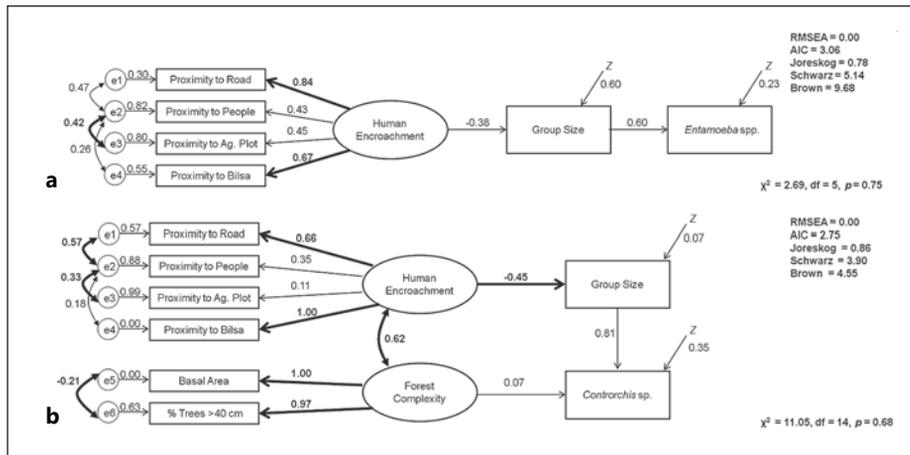


Fig. 3. Best-fit SEMs for a specific intestinal parasite, including the presence of *Entamoeba* spp. (a) and the presence of *Contrororchis* sp. (b). In both models, group size plays a significant role in predicting both measured species richness and estimated species richness which takes into account sampling effort. A χ^2 statistic, AIC and RMSEA were used to assess model fit. Lower AIC values are associated with best-fit models, and RMSEA values <0.05 are generally considered to be indicative of good fit. AIC penalizes model complexity, weighting those most parsimonious models the highest. Bold arrows signify significant contributions to the model, though nonsignificant (not bold) arrows are still instrumental to the overall model design.

Structural Equation Models

Though there were no significant *direct* relationships between risk factors and specific parasites or species richness, there were several SEMs that were created and tested using univariate and multivariate results ($p < 0.10$) from individual monkey analyses. The first set of SEMs focused on predicting species richness (Fig. 2). Best-fit SEMs for overall species richness were similar to individual parasite species models in that anthropogenic disturbances were not directly related to the number of parasite species found in a group. Rather, group size was an intermediate factor associated with both measured parasite species richness (Fig. 2a) and estimated parasite species richness which takes into account sampling effort (Fig. 2b). Likewise, those groups with both lower basal area and lower percentage of trees >40 cm DBH tended to have more parasite species. Forest structure and human proximity were not significantly correlated with one another – though groups further from people tended to be found in areas with slightly higher forest structure. A goodness of fit test for the fitted SEM indicated that the data matched the model well. Further, model fit was deemed acceptable based on RMSEA and AIC values, while Schwarz and Brown measurements suggest that observed parasite species richness was a better model fit than any of the adjusted species richness estimates.

The second set of SEMs focused on predicting the presence of specific parasite species (Fig. 3, *Entamoeba* spp. and *Contrororchis* sp.). Groups found with *Entamoeba* spp. were significantly larger, and these larger groups were more likely to be found closer to people than uninfected groups. Alternatively, the presence of *Contrororchis* sp.

was dependent upon a combination of both human proximity and forest disturbance variables. Groups infected with *Controrchis* sp. tended to be larger than uninfected groups and tended to be found in areas with higher forest complexity (e.g., basal area and percentage of trees >40 cm DBH). Every group was infected with *Strongyloides* spp., *Capillaria* sp., and *Blastocystis* spp., making this approach uninformative for predicting these parasite species.

Discussion

Anthropogenic disturbances such as increasing human encroachment of tropical forests, logging (and subsequent fragmentation, habitat degradation, and habitat loss), ecotourism, livestock introduction, and agriculture are all likely drivers of changing intestinal parasite communities and emerging infectious diseases. We found that parasite species richness was not solely related to any one environmental risk factor, but was rather dependent on the interplay of forest complexity (e.g., basal area and percentage of trees >40 cm DBH), group size, and human proximity measurements. Similarly, the presence of *Controrchis* sp. was dependent on these same interactions, while *Entamoeba* spp. were more likely to be found in larger groups which were closer to people.

Species Richness Models

No direct association was found between overall species richness and environmental risk factors, nor was the presence of specific parasite species found to be directly dependent on any measured risk factors. Proximity to people was not a direct factor influencing intestinal parasitism; rather, several human proximity indices were related to group size which was in turn related to overall species richness and the presence of specific parasite species.

With a sample size of 19 groups, low statistical power is a possible contributing factor to such associations not being detected as statistically significant. The SEM results support a causal chain in which we found that larger groups saw an increase in parasite species richness, previously attributed to higher contact rates and subsequent transmission rates of directly transmitted parasites [Freeland, 1976; Côté and Poulin, 1995; Arneberg et al., 1998; Nunn et al., 2003; Cross et al., 2009]. This trend is also relevant for the vast majority of intestinal parasite species found in this study: groups infected with 10 different parasite species were larger than those groups that were uninfected, though this relationship was not statistically significant at the group level. The other 3 parasite species in this study were found in all monkey groups and as a result could not be compared.

Finding larger groups near human settlements is at first unexpected, because these areas are often degraded. And as previously reported, secondary forests and disturbed habitat are largely associated with reduced primate group size [Gonzalez-Kirchner, 1998; McCann et al., 2003]. Primary forest normally harbors larger groups because of increased carrying capacity associated with more available food types and better quality food sources, whereas in disturbed forests primates are limited to a lower quality and quantity of food [Clarke et al., 2002; Gillespie et al., 2005; Marshall et al., 2005; Cristóbal-Azkarate and Arroyo-Rodriguez, 2007; Martinez-Mota et al., 2007; Gillespie and Chapman, 2008]. However, in this case, primate groups living on

the edge of agricultural plots or near fruit-bearing tree plantations may actually be larger due to higher-density food sources, which results in increased reproductive rates and carrying capacity [Chapman et al., 1995].

The forests in our sampled area have had nearly 15 years to recover from previous anthropogenic disturbance such as logging. These secondary forests may actually provide an ideal heterogeneous habitat for howlers [Ortega-Andrade et al., 2010], thus explaining why those groups near people were larger. The fact that degraded habitats might not necessarily mean poor-quality food sources might explain why changes in forest structure were not as strongly related to parasite species richness as expected. Mantled howler monkeys are also well adapted to changing food sources as a result of past deforestation [Peres, 1997; Pinto et al., 2003]. Perhaps more recent effects of logging would have resulted in a larger impact on ranging patterns, diet, and stress, subsequently providing a more distinct contrast with undisturbed primary forest. Pinto et al. [2003] outline other possibilities why howler behavior might not change with modified forest structure, including low logging intensity, untouched forest adjacent to harvested areas, and extended time period between last harvest and sampling.

Individual Parasite Species Models

Forest complexity was not significantly associated with overall species richness at the group level in univariate analysis. However, when using structural equation modeling, which controls for group membership at the individual level, forest structure measurements were found to be a contributing factor – along with group size – to overall species richness. Specifically, groups with *Cyclospora* sp., *Enterobius* sp., *Trypanoxyuris* sp., *Dientamoeba* sp., *Chilomastix* sp., *Entamoeba* spp., and *Iodamoeba* sp. were all found in areas with lower basal area and a lower percentage of trees >40 cm DBH, though none were statistically significant. Additional testing is needed to confirm this pattern; however, if several parasite species are more prevalent in degraded habitat, this pattern could be explained by associated changes in group size, edge effects, or nutritional stress. The exceptions to this rule included *Controrchis* sp., *Blastocystis* spp., and *Balantidium* sp. which tended to be found in areas with higher basal area and a higher percentage of trees >40 cm DBH (though again, no significant statistical differences were found).

Two SEMs were developed for specific parasite species: *Entamoeba* spp. and *Controrchis* sp. (Fig. 3). The presence of *Entamoeba* spp. was dependent solely on group size, which was in turn related to human encroachment indices. Previous studies have suggested that *Entamoeba* spp. infections were more common in degraded habitat [Gillespie et al., 2005; Chapman et al., 2006a, b]. However, though this was a trend in our study as well, it was not a statistically significant contributing factor in any of our models. Like many of the described species found in this study, *Entamoeba* spp. are transmitted through the fecal-oral route. Thus, one would expect higher transmission rates of contagious parasite species in larger groups [Côté and Poulin, 1995; Patterson and Ruckstuhl, 2013]. The *Controrchis* sp. SEM was similar to overall species richness design: group size was a driving force and forest disturbance secondarily. Other studies have found a greater prevalence of *Controrchis* sp. in fragmented habitat. *Cecropia* trees, which harbor ants infected with *Controrchis* sp., are primarily found in disturbed habitat and are often eaten by howler monkeys. The monkeys may be ingesting the ants and thereby becoming infected

with the parasites [Kowalzik et al., 2010]. One explanation for this apparent contradiction is that *Cecropia* trees are still found in open gaps in primary forest [Sanford et al., 1986], suggesting that gaps could create a higher prevalence of *Controrchis* sp. in monkeys living in these areas. Further investigation of tree species utilized by monkeys and testing of ants in areas of varying habitat disturbance could likely confirm or refute this hypothesis.

Implications

Expanding human populations, particularly in tropical countries where many of the world's primate species live, will likely lead to greater interspecies interactions and subsequent expansion of infectious diseases throughout both primate and human populations as reported in previous studies [Wolfe et al., 2007; Vitazkova, 2009; Pedersen and Davies, 2010]. Likewise, anthropogenic disturbances such as logging have the potential to modify intestinal parasite communities in primate populations, subsequently impacting host health. These results are likely to be applicable to other disturbed systems, suggesting a potential threat to other wildlife populations.

Additional studies looking at more recent and more severe levels of anthropogenic disturbance would prove insightful, and increased sampling of individuals across populations would expand the breadth of the inference achievable from the sample data. We are cautious in suggesting a universal model applicable to all primates. Rather, we anticipate our models can be tested using empirical data from other primate studies or through meta-analysis.

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Statement of Ethics

All methods reported in this manuscript were noninvasive and adhered to guidelines set forth by the Institutional Animal Care and Use Committee at SUNY-ESF in New York, and were approved in country according to guidelines and permission from the Ministerio del Ambiente in Quito, Ecuador.

Disclosure Statement

None of the authors have any conflicts of interest to declare.

Appendix 1

Matrix of Spearman Correlations between Disturbance Variables, Measured Species Richness Using Fecal Smear, Flotation and Sedimentation Techniques, and Three Species Richness Estimators (Chao2, Incidence Coverage-Based Estimator, and Jackknife), Which Take into Account Sampling Effort

	Measured species richness	Chao2	ICE	Jack
Roads	0.08	-0.06	-0.04	0.06
People	0.03	-0.10	-0.18	-0.16
Agriculture	0.29***	0.06	0.11	0.08
Bilsa	-0.20	0.15	0.04	0.08
Basal area	0.04	0.20	0.16	0.23
Percent trees >40 cm DBH	0.00	0.35*	0.36*	0.42*

*** $p < 0.05$, * $p < 0.20$.

Appendix 2

Summary Table of Anthropogenic Disturbances Associated with Gastrointestinal Parasites

	Roads, m	People, m	Agriculture, m	Bilsa, m	Basal area, m ² /ha	Trees >40 cm DBH, %
<i>Cyclospora</i> sp.	369, 681	1,253, 1,524	1,038, 805	1,003, 2,513*	7.2, 9.8	0.1, 0.1
<i>Isoospora</i> sp.	465, 569	1,015, 1,281	903, 885	837, 1,697	8.7, 8.1	0.1, 0.1
<i>Balantidium</i> sp.	681, 393	1,496, 1,281	755, 885	1,233, 1,697	11.1, 8.0	0.1, 0.1
<i>Chilomastix</i> sp.	269, 681	456, 1,506**	423, 921	442, 1,860*	7.2, 9.8	0.1, 0.1
<i>Dientamoeba</i> sp.	884, 332	1,597, 1,147	1,356, 859	988, 1,449	5.4, 10.4*	0.0, 0.1
<i>Entamoeba</i> spp.	453, 1,236	1,415, 1,075	910, 750	1,124, 3,103*	8.0, 9.8	0.1, 0.1
<i>Iodamoeba</i> sp.	884, 453	2,305, 1,147	859, 910	2,271, 1,124	9.7, 7.8	0.1, 0.1
<i>Enterobius</i> sp.	1,152, 565	1,657, 1,281	982, 885	1,394, 1,287	3.6, 9.8*	0.1, 0.1
<i>Trypanoxyuris</i> sp.	691, 332	1,901, 1,147	910, 859	1,449, 1,124	11.1, 7.2	0.1, 0.1
<i>Controrchis</i> sp.	453, 677	2,305, 988*	1,145, 733*	2,271, 966	11.7, 7.8	0.12, 0.07*

Values shown are medians for 2 groups defined by whether the howler monkey group is infected (value on the left for each pair) or not infected with each parasite. p values are provided from Mann-Whitney U tests testing the null hypothesis of no difference in medians. *Strongyloides* spp., *Capillaria* sp., and *Blastocystis* spp. were found in every group. ** $p < 0.10$, * $p < 0.20$.

Appendix 3

Proportion of Individuals Infected within a Group versus Disturbance Variables

	Roads	People	Agriculture	Bilsa	Basal area	Trees >40 cm DBH
<i>Cyclospora</i> sp.	-0.05	-0.13	0.20	-0.28	-0.28	-0.07
<i>Isospora</i> sp.	-0.07	-0.14	-0.01	-0.14	0.01	0.01
<i>Balantidium</i> sp.	-0.02	0.00	0.09	-0.09	0.18	0.00
<i>Blastocystis</i> spp.	-0.12	-0.23	-0.06	-0.39**	-0.16	-0.03
<i>Chilomastix</i> sp.	-0.19	-0.38**	-0.23	-0.35*	-0.07	-0.04
<i>Dientamoeba</i> sp.	0.23	0.09	0.21	-0.08	-0.35*	-0.19
<i>Entamoeba</i> spp.	0.20	-0.11	-0.15	-0.39	-0.31*	-0.30*
<i>Iodamoeba</i> sp.	0.26	0.13	0.16	0.02	0.20	-0.02
<i>Enterobius</i> sp.	0.11	-0.03	0.02	-0.09	-0.33*	-0.23
<i>Trypanoxyuris</i> sp.	0.29*	0.16	0.06	0.01	0.16	-0.09
<i>Controrchis</i> sp.	0.01	0.27	0.26	0.06	0.17	0.26

** $p < 0.10$, * $p < 0.20$.

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