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Ecological Factors Associated with Habitat Use of Baird's Tapirs (*Tapirus Bairdii*)

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ECOLOGICAL FACTORS ASSOCIATED WITH HABITAT USE OF BAIRD'S TAPIRS

(*TAPIRUS BAIRDII*)

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ABSTRACT

The Baird's tapir, the largest terrestrial mammal in Central America and a crucial seed disperser and ecosystem engineer, has experienced a 50% loss of habitat in its geographic range during the past 30 years. Efforts to conserve this species need to consider factors that have contributed to its endangerment, such as how human presence and human-mediated habitat change may influence tapir behavior, body condition, and disease susceptibility. In this thesis I had two goals: 1) understand how human disturbance affects tapir activity patterns, and 2) noninvasively determine the association of disturbance with tapir health. I first compared the activity patterns of tapirs, humans, and jaguars between sites with and without timber extraction and between camera stations varying in human activity. Second, I investigated the association of human activity with putative parasite counts found in field-collected feces and the relationship of putative parasite counts to tapir body condition. As part of the parasitological study I compared parasites from tapirs with those found in domestic animals, and I compared the effectiveness of ethanol and formalin for long-term preservation of fecal samples. Tapir activity did not significantly differ relative to timber extraction or human activity. Tapirs were nocturnal in all sites with >80% of all tapir captures occurring between 1900 and 0500 hours; however, the occasional occurrence of daytime activity at all study sites suggests the potential for tapir habituation to, or tolerance of humans. While human activity was moderately correlated with a reduction in body condition, the associations between human activity and parasite load, and parasite load and body condition were not significant. There are similarities in parasite eggs and

worms from tapir fecal samples to those typically found in horses, but not those of cattle. Formalin-stored samples exhibited higher parasite richness and averaged more total eggs than ethanol-stored samples; however, the total worms found did not significantly differ between the chemicals. Given the connection between human activity and negative health outcomes for tapirs in protected areas of Northwestern Belize, research needs to expand to encompass the more fragmented habitat that tapirs may utilize across their range.

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me focused on the things I could control, and for doing an outstanding job teaching me how to run PCR and to troubleshoot with primers and PCR settings.

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The field aspect of my study was incredibly intimidating, and there were several times when completing the analysis of my data seemed impossible. Lucky for me, I have Sean Houlihan, my wonderful fiancé who finds the silver lining in any situation while somehow managing to not drive me crazy with his optimism. In the past three years, he has been my field assistant, my motivator, my shoulder to cry on, and the guiding hand that has both supported and pushed me to complete this thesis.

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TABLE OF CONTENTS:

LIST OF TABLES:

LIST OF FIGURES:

CHAPTER I:

HUMAN DISTURBANCE AND THE ACTIVITY PATTERNS OF BAIRD'S TAPIR (*TAPIRUS BAIRDII*) AND JAGUARS (*PANTHERA ONCA*) IN PROTECTED AREAS OF NORTHWEST BELIZE.

Introduction

When humans are viewed as a threat, wild animals may change their behavior to avoid us (Frid & Dill 2002, George & Crooks 2006, Larson *et al*. 2016, Vistnes & Nellemann 2008). Avoidance can take the form of spatial movement away from anthropogenic disturbance, or temporal partitioning such that humans and wildlife use the same habitat but at different times (Nix *et al.* 2018). Where a pervasive human footprint negates the opportunity for complete spatial separation (Frid & Dill 2002, Gaynor *et al.* 2018) and humans are mostly active during the day, normally diurnal species may shift to nocturnal movement and foraging to minimize interaction with us (Bridges & Noss 2011, Gaynor *et al.* 2018, Rowcliffe *et al.* 2014). Although hunting and harassment are well known drivers of change in wildlife activity, nonlethal human activities can also affect behavior (Gaynor *et al.* 2018, Frid & Dill 2002, Larson *et al.* 2016). As both lethal and nonlethal human activities impact wildlife activity pattern, an understanding of how human presence influences wildlife activity is necessary for the planning and management of protected areas (Massara *et al.* 2018).

Large herbivores are often hunted for sustenance and are harassed by humans to deter them from foraging on crops. Simultaneously, these prey species may experience hunting

pressure from natural predators, like big cats. At the same time, the large predators themselves

may be persecuted and as such show avoidance behavior of humans. Thus, despite anthropogenic

threats, prey species may opt to associate more with humans when the risk of non-human

predation is even greater, especially if predators avoid humans (the "human shield", Atickem *et

al.* (2014) or "predator shelter" (Shannon *et al.* (2014) hypothesis). For example, moose (*Alces

alces*) choose birthing sites near long stretches of road avoided by bears in Yellowstone National

Park (Berger 2007). Alternatively, natural predation pressure could increase in human disturbed

areas. For example, predation rate increases when pumas (*Puma concolor*) stop feeding on their

kills sooner in response to human voices (Smith *et al.* 2017), and consequently must kill prey

more often in home ranges with greater human density (Smith *et al.* 2015). Such fitness

tradeoffs faced by prey and predators require long-term field study to elucidate, and are likely

idiosyncratic to particular ecological communities, making them impractical targets for

conservation management. However, if the activity patterns of prey and predators are indicative

of their interactions under different disturbance regimes, camera trapping may be an efficient

means of monitoring direct and indirect behavioral disturbance effects on species of conservation

concern (Oberosler *et al.* 2017, Patten 2018).

Tapirs (Perissodactyla, Tapiridae) are a tropical, herbivorous taxon of great conservation

concern. Tapirs disperse the seeds of rainforest plants and create depressions in the soil for

wallowing, thus acting as ecological engineers (Garcìa *et al.* 2012, Garcìa-Marmolejo *et al.*

2015, O'Farrill *et al.* 2013, Paolucci *et al.* 2019). However, their large size and wide ranging

movements make them susceptible to mortality by human-related causes, and the populations of

all four tapir species are declining (www.iucnredlist.org).

The endangered Baird's tapir (*Tapirus bairdii*) is the largest terrestrial mammal in a Neotropical biodiversity hotspot (Garcìa *et al.* 2016). The species' historic range covered all of Central America and into South America (Garcìa *et al.* 2016), most of which currently experiences varying degrees of human activity, such as: settlement and road construction, agricultural development, ecotourism, and archaeological and biological research activities in protected areas. Human exploitation has reduced the species' range by 50% in the past 30 years (Schank *et al.* 2015) due to deforestation for timber extraction and the creation of agricultural fields (Garcìa *et al.* 2012, Garcìa *et al.* 2016). Mortality due to vehicle collision is also a conservation concern for tapir populations in Belize (Poot & Clevenger 2018) and Brazil (Medici & Desbiez 2012) and is more likely to occur at night when poor visibility constrains drivers ability to slow soon enough to avoid impact with tapirs that are crossing roads.

Tapirs may innately fear humans because they were hunted by ancient civilizations (Black *et al.* 2012, Emery 2004, Tykot 2002), and are still hunted presently by Amazonian tribes (Robinson & Bennett 2013, Welch 2014) and elsewhere throughout Central and South America (de Azevedo Chagas *et al.* 2015, Garcìa *et al.* 2016, Koster 2006), despite legal protections (Garcìa *et al.* 2016, Stanton 2012). Retaliatory killing of crop-raiding tapirs has been recorded in Belize, Mexico, and Nicaragua (Waters 2015). In Belize, where the Baird's tapir is protected by national laws for its status as a national symbol (Waters 2015), it remains legal to shoot any tapir that threatens crops (Liverpool 2000, Waters 2007). While tapir hunting has been reported in villages surrounding the area of the current project (Waters 2007), there is miniscule, if any, hunting pressure on tapirs at all study sites (M. J. Kelly, *pers. comm.*, September 8, 2017). Due to the large size of adult tapirs, they are rarely killed by jaguars, and are not considered a significant part of the jaguar diet in Belize (Harmsen *et al.* 2011). Nevertheless, the threat posed

by jaguars to young or weak tapirs, and the risk of significant injury if attacked, would suggest that tapir should avoid them. Pumas in Central America have been found to primarily prey on small to medium sized animals including coati, raccoon, great curassow, peccary and white-tailed deer (Azevedo *et al.* 2016, Hernández-SaintMartín *et al.* 2015), and so were not examined as potential predators in this study.

Generally, humans, tapirs, and jaguars are thought to prefer different activity periods. Human activity in habitat reserves is typically diurnal (e.g., 78-100% of camera captures, Massara *et al.* 2018). Tapirs are thought to restrict daytime activity to avoid the risk of hyperthermia (Cruz *et al.* 2014, Eisenberg 1989 *in* Foerster & Vaughan 2002), and forage most actively during crepuscular periods (Carbajal-Borges *et al.* 2014, Cruz *et al.* 2014, Foerster & Vaughan 2002, Pérez-Irineo & Santos-Moreno 2016). Jaguars are often cathemeral but may exhibit a more crepuscular or nocturnal pattern as reflective of their main prey species in a particular habitat, when avoiding high daytime temperatures (Astete *et al.* 2008), or in response to human disturbance (Foster *et al.* 2010).

The objectives of this study were to: 1) Describe the activity patterns of these three species (i.e., tapirs, jaguars, humans) over four years in four areas of NW Belize protected from hunting but varying in the level of anthropogenic impact on the habitat, 2) Determine if there is a relationship between tapir activity pattern and human-mediated habitat alteration (specifically timber extraction), and 3) Investigate whether tapir activity patterns appear to be directly or indirectly influenced by the timing and rate of human activity. Because human disturbance is highly variable, it is difficult for wildlife to habituate to us (Buchholz & Hanlon 2012), potentially inducing greater chronic stress than natural predation risk (Zbyryt *et al.* 2018), we

predicted that both tapirs and jaguars would become more nocturnal in sites with logging and with high human activity.

<u>Methods</u>

Study area

This study was conducted across four sites in the Orange Walk District of Northwest (NW) Belize (Fig. 1.1 & Table 1.1). Two of the sites, Hill Bank (HB 17°35'27.96" N, 88°41'59.64" W) and La Milpa (LM 17°50'26.28" N, 89°1'5.88" W) are within the 260,000-acre (1,052 km^2) Rio Bravo Conservation and Management Area (RBCMA). Timber extraction occurs at HB but not at LM. Gallon Jug (GJ 17°33'33.48" N, 89°2'21.48" W) is a 33,000 acre (133.5 km^2) private agricultural estate with more than 900 head of cattle, a coffee plantation, timber extraction and an eco-lodge. Yalbac Ranch and Cattle Company (YB 17°25'00" N, 88°57'00" W) manages 131,117 acres (458 km2) of the Yalbac and Laguna Seca lands for timber extraction. Broadleaf forest is the most prevalent habitat type at each site with pine savannah in the SE section of Hill Bank. The average minimum and maximum temperatures are 20.5ºC and 31.3ºC, respectively, and the rainy season runs from June to December, with an average annual rainfall of 1524 mm (National Meteorological Service of Belize 2018). The northwestern portion of Belize averages 12 hours and 16 minutes of daylight per day throughout the year (US Naval Observatory 2016). Elevation across the four sites ranges from 4 to 250m above sea level.
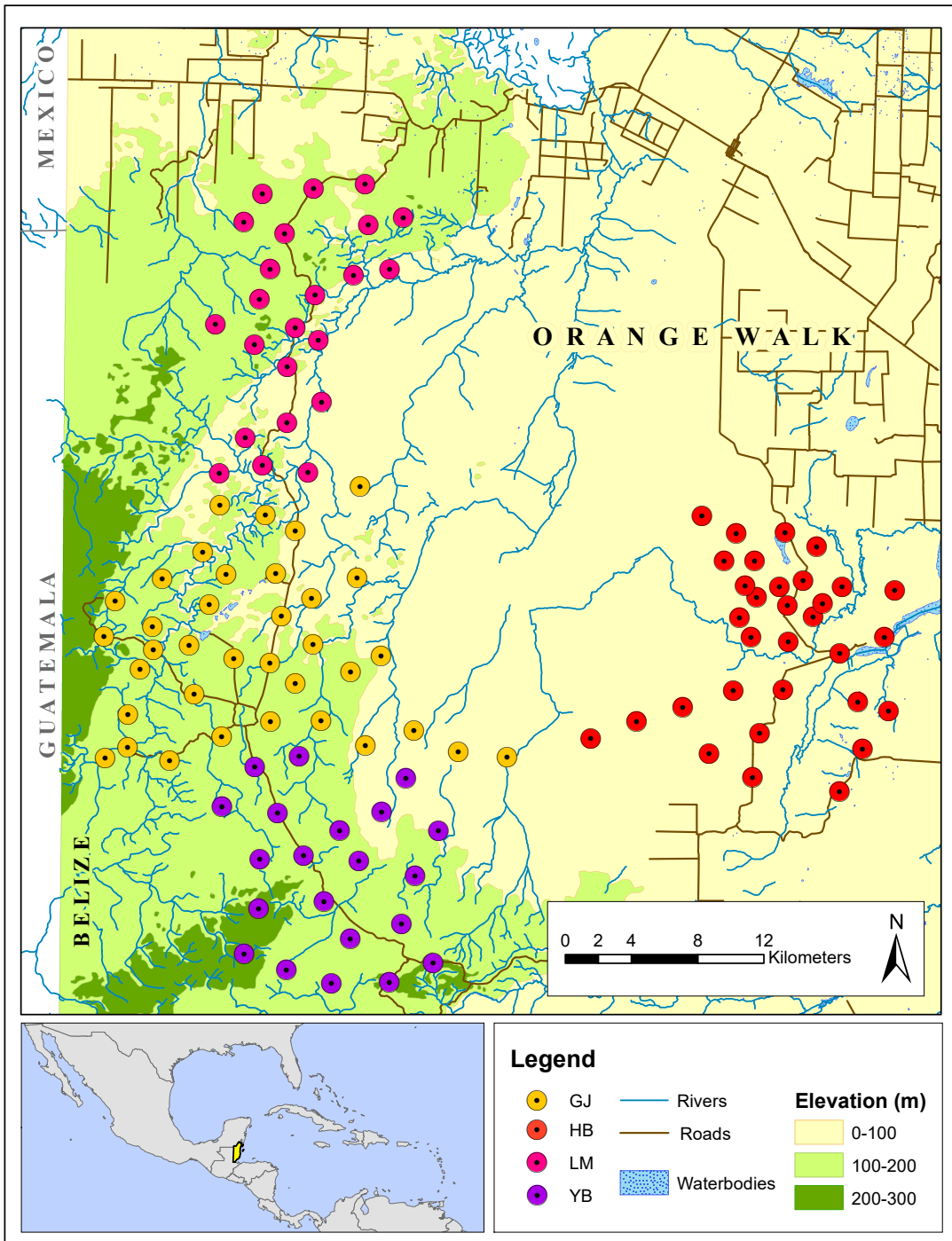
FIGURE 1.1. The colored circles on the map correspond to 112 camera trap station locations for the four study sites in NW Belize in 2016. Together, the orange, purple, and red circles represent the extractive logging area, with the pink circles representing the unlogged area. The location of Belize within Central America is shown in the inset, with Belize highlighted in yellow.

TABLE 1.1 Yearly survey parameters for the Gallon Jug (GJ), Yalbac (YB), Hill Bank (HB) and La Milpa (LM) study sites in NW Belize. Note: the YB survey began in 2014. The area if each site per year is given as the minimum convex polygon (MCP) The ratio of people/km² is based on the number of permanent residents at each site.

Site	Year	MCP area surveyed (km²)	Ratio of people/km²	Sampling Period	No. of camera traps	Total trap nights	Average trap nights/camera	Average human trap rate/camera
	2013	188.77	1: 1.72	20 May – 12 Aug	29	2076	71.6	0.44
GJ	2014	288.96	1: 2.63	16 May – 08 Aug	35	2645	75.6	0.50
	2015	288.96	1: 2.63	28 June – 27 Sept	33	2138	64.8	0.67
	2016	291.54	1: 2.65	05 May – 03 Oct	36	2467	68.5	1.03
	-	-	-	-	-	-	-	-
YB	2014	51.10	1: 1.25	06 July – 19 Oct	09	715	79.4	0.49
	2015	64.02	1: 1.56	08 May – 03 July	10	523	52.3	0.75
	2016	148.32	1: 3.62	20 May – 3 Sept	21	655	65.5	1.16
	2013	152.47	1: 15.25	05 Nov – 13 Jan	26	1605	91	0.22
HB	2014	152.47	1: 15.25	20 July – 25 Oct	26	2195	84	0.12
	2015	206.96	1: 20.70	08 April – 28 June	31	2105	68	0.64
	2016	207.49	1: 20.80	21 May – 02 Aug	32	1750	56	0.33
	2013	140.04	1: 14.0	26 May – 13 Aug	22	1666	75.7	0.77
LM	2014	140.04	1: 14.0	19 April – 09 July	23	1656	74.5	0.76
	2015	140.04	1: 14.0	04 July – 27 Sept	23	1849	80.4	0.31
	2016	140.04	1: 14.0	23 July – 09 Oct	23	1653	71.9	0.49

Sampling design

In 2014 and 2015, I worked as a member of the VA Tech Jaguar Project assisting with camera trap surveying at GJ, LM, YB, and HB. For the current study, I also used survey data that was collected before and after the field seasons I participated in. From 2013-2016, between 22-36 camera trap stations were established at GJ, LM and HB (see Table 1.2 for camera types) for a minimum survey period of two-three months per site (Table 1.1). Camera surveying of Yalbac began with 9 camera trap stations in 2014 and increased to a total of 21 stations by 2016. Cameras were arranged in a grid with each camera approximately 2-3 km apart based upon the home range of the native cat species, primarily jaguar and puma. This camera grid spacing has been successfully used in studies of other tapir species (Cruz *et al.* 2014, Linkie *et al.* 2013).

TABLE 1.2 Models of trail cameras used in the 2013-2016 camera surveys.

Brand	Model
Moultrie	Digital Multicam II Game Camera
	Game Spy D40
	M-550 Trail Camera
Reconyx	PC85 RapidFire Pro
	HC500 HyperFire
	PC800 HyperFire Pro
	PC90 Covert Pro
	PC900 Professional
	PC800 Professional
	RC55 RapidFire
HCO Scoutguard	SGC860C
Panthera	V4

Camera traps consisted of two cameras facing each other across a trail, road, or an old logging road. Cameras were placed on tree trunks or stakes about 35 cm off the ground and were not baited. Cameras remained active 24 hours per day and were set to take 3 photos when triggered by motion with a 15 second delay between trigger events. Date and time were recorded on each image allowing for each species to be classified based on the period of most frequent activity during the diel cycle (Table 1.3). Camera stations were checked every 10-14 days to replace batteries and exchange memory cards. A given species was considered present in a photo if any identifiable part of the animal was visible (e.g. human foot, tapir ear, or jaguar tail). To reduce pseudoreplication and the inclusion of non-independent data points in statistical analyses, images of the same species were considered independent capture events if they occurred at least 30 minutes apart, as suggested by Linkie & Ridout (2011), or if they could be identified as distinctly different individuals.

TABLE 1.3 Species' activity pattern in each survey area were assigned a classification based on what percentage of that species' total captures fell within a given time period. These classifications were adopted from Massara *et al.* (2018).

Classification	Percentage of total captures	Time period between:
Nocturnal	> 60%	1 h after sunset to 1 h before sunrise
Crepuscular	50%	1 h before and after sunrise and sunset
Diurnal	> 60%	1 h after sunrise to 1 h before sunset
Cathemeral	Approximate uniform activity	Throughout the 24 h diel cycle

Statistical analyses

All statistical analyses were run in RStudio version 1.0.136 (RStudio Team 2016). To correct for changing day length across each study season, image capture times were converted to solar time using the 'solaR' R package (Perpiñán 2012). Tapir, jaguar, and human activity

patterns were classified using nonparametric kernel density estimation, which treats images as random samples from an underlying continuous distribution (Ridout & Linkie 2009). The Rao spacing test (Agostinelli 2017) was used to test the null hypothesis that captures were randomly distributed across the 24-hour cycle before categorizing each species as diurnal, nocturnal, or cathemeral. The R package 'overlap' (Meredith & Ridout 2018, Ridout & Linkie 2009) was used to estimate the coefficient of overlap measure (Δ). A coefficient of overlap value of 1 signifies complete overlap in activity patterns of two species and a value of 0 signifies no shared period of activity. Ten thousand bootstrap samples were used to obtain 95% confidence intervals.

Ridout and Linkie (2009) suggested using the $\hat{\Delta}_1$ estimator for coefficient of overlap when the smallest sample size is less than 75 and $\hat{\Delta}_4$ when the smallest sample size exceeds 75. As sample sizes in this study varied depending on how the data was pooled, both estimators were used for all activity pattern comparisons. The difference between the $\hat{\Delta}_1$ and $\hat{\Delta}_4$ values (average difference = 0.58%, SD = 0.42%) was not great enough to impact the biological inferences made from the results, and therefore, for consistency I present only $\hat{\Delta}_4$ values.

To achieve ample sample sizes for activity pattern comparisons at each study site and across the entire region, the data were pooled two different ways: 1) tapir, jaguar and human records were each pooled by site across all four years (e.g., GJ 2013-2016), and 2) species records of all four sites were pooled within each year to characterize activity patterns at the regional scale (e.g., GJ+YB+HB+LM in 2014).

Data were also pooled to compare species activity patterns relative to the practice of timber extraction. GJ, YB, and HB data were pooled together as the logging area and compared to LM, the only site without logging. Lastly, to account for differences in human activity at

individual camera stations (for instance, those on main roads vs. hiking trails), the frequency of

human records per trap night (human activity) at each station was calculated and the stations

were divided into two groups. High human activity camera stations averaged 1 or more human

records per trap night with anything less than that being considered a low trap rate (Fig. 1.2; see

Table 1.4 for types of human activity per study site per survey year).
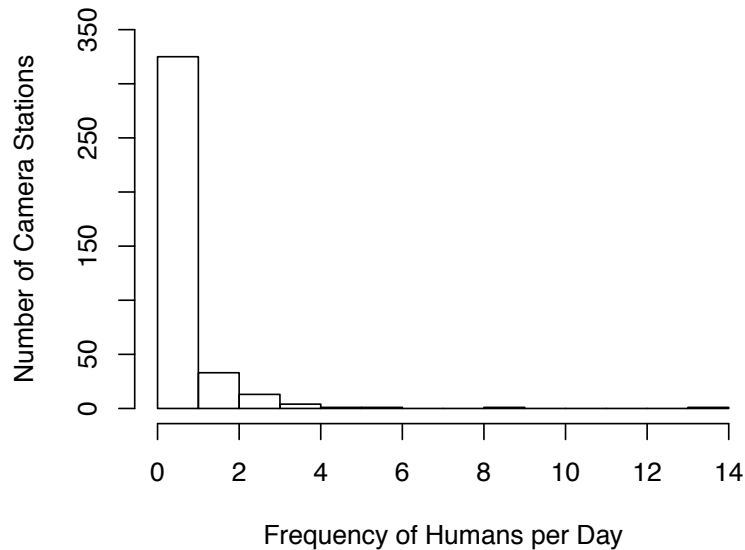
Figure 1.2 Histogram of the frequency of human activity per camera station. There were 379 total camera stations active during the camera survey from 2013-2016. Of those, 55 exhibited a frequency of 1 or more humans per day and were classified as high human activity stations. The remaining 324 stations averaging less the 1 human capture per day were classified as low activity.

To test for statistical differences in activity pattern distributions of each species between

high and low human activity and between logged and unlogged areas (e.g., tapir in logged vs.

unlogged areas) I used the Mardia-Watson-Wheeler W test from the R package 'circular'

(Agostinelli 2017). This nonparametric test allows for comparison of two circular distributions

and assumes that the test statistic "W" follows a χ^2 distribution. A randomization test was used

to evaluate the difference in activity pattern overlap of tapirs with jaguars and with humans in

logged/unlogged areas and at high/low human trap rate camera stations. For all statistical

analyses alpha = 0.05.

TABLE 1.4 Number (and %) of various types of human activity out of the total human records at each study site per survey year. A dash means none of the given type of activity was recorded.

Site	Year	Mode of Travel	Human Activity Type				Totals
			Research	Work	Tourist	Unknown	
	2013	Foot	243 (26.79)	3 (0.33)	70 (7.72)	22 (2.43)	338 (37.27)
		Bike	1 (0.11)	-	1 (0.11)	-	2 (0.22)
		Vehicle	148 (16.32)	155 (17.09)	84 (9.26)	167 (18.41)	554 (61.08)
		Horse	-	1 (0.11)	12 (1.32)	-	13 (1.43)
		Unknown	-	-	-	-	0
	2014	Foot	264 (19.82)	62 (4.65)	137 (10.29)	8 (0.60)	471 (35.36)
		Bike	-	-	15 (1.13)	-	15 (1.13)
		Vehicle	174 (13.06)	202 (15.17)	26 (1.95)	404 (30.33)	806 (60.51)
		Horse	-	2 (0.15)	-	-	2 (0.15)
		Unknown	-	-	-	38	38 (2.85)
GJ	2015	Foot	241 (16.86)	-	6 (0.42)	97 (6.79)	344 (24.07)
		Bike	-	-	6 (0.42)	-	6 (0.42)
		Vehicle	111 (7.77)	230 (16.10)	2 (0.14)	708 (49.5)	1051 (73.55)
		Horse	-	-	2 (0.14)	-	2 (0.14)
		Unknown	-	-	-	26 (1.82)	26 (1.82)
	2016	Foot	150 (5.90)	43 (1.69)	41 (1.61)	35 (1.38)	269 (10.58)
		Bike	-	-	9 (0.35)	-	9 (0.35)
		Vehicle	44 (1.73)	405 (15.93)	13 (0.51)	1737 (68.33)	2199 (86.51)
		Horse	-	-	-	-	0 (0.00)
		Unknown	-	-	-	65 (2.56)	65 (2.56)
YB	2014	Foot	64 (18.29)	-	-	2 (0.57)	66 (18.86)
		Bike	-	-	-	-	0
		Vehicle	24 (6.86)	194 (55.43)	-	65 (18.57)	283 (80.86)
		Horse	-	-	-	-	0
		Unknown	-	-	-	1 (0.29)	1 (0.29)

Group	Year	Mode					
YB	2015	Foot	66 (16.75)	9 (2.28)	-	6 (1.52)	81 (20.56)
		Bike	-	-	-	-	0
		Vehicle	27 (6.85)	43 (10.910)	1 (0.25)	218 (55.33)	289 (73.35)
		Horse	-	-	-	-	0
		Unknown	-	-	-	24 (6.09)	24 (6.09)
	2016	Foot	161 (21.16)	24 (3.15)	1 (0.13)	7 (0.92)	193 (25.36)
		Bike	-	-	-	-	0
		Vehicle	28 (3.68)	29 (3.81)	-	489 64.26)	546 (71.75)
		Horse	-	-	-	-	0
		Unknown	-	-	-	22 (2.89)	22 (2.89)
HB	2013	Foot	223 (65.82)	-	-	17 (4.80)	240 (67.80)
		Bike	-	-	1 (0.28)	-	1 1 (0.28)
		Vehicle	19 (5.37)	31 (8.76)	3 (0.85)	52 (14.69)	105
		Horse	-	-	3 (0.85)	-	3 (0.85)
		Unknown	-	-	-	5 (1.41)	5 (1.41)
	2014	Foot	158 (59.18)	-	7 (2.62)	18 (6.74)	183 (68.54)
		Bike	-	-	1 (0.37)	-	1 (0.37)
		Vehicle	7 (2.62)	10 (3.75)	-	51 (19.10)	68 (25.47)
		Horse	-	-	2 (0.75)	-	2 (0.75)
		Unknown	-	-	-	13 (4.87)	13 (4.87)
	2015	Foot	202 (15.11)	17 (1.27)	3 (0.22)	46 (3.44)	268 (20.04)
		Bike	-	-	1 (0.07)	-	1 (0.07)
		Vehicle	50 (3.74)	206 (15.41)	-	806 (60.28)	1062 (79.43)
		Horse	-	-	-	-	0
		Unknown	-	-	-	6 (0.45)	6 (0.45)
	2016	Foot	190 (32.42)	4 (0.68)	8 (1.37)	7 (1.19)	209 (35.67)
		Bike	-	-	-	-	0
		Vehicle	84 (14.33)	15 (2.56)	-	237 (40.44)	335 (57.17)
		Horse	-	-	-	-	0
		Unknown	-	-	-	41 (7.00)	41 (7.00)

13

Year	Mode					
2013	Foot	396 (30.91)	13 (1.01)	71 (5.54)	8 (0.62)	488 (38.10)
	Bike	-	-	1 (0.08)	-	1 (0.08)
	Vehicle	28 (2.19)	264 (20.61)	44 (3.43)	456 (35.60)	792 (61.83)
	Horse	-	-	-	-	0
	Unknown	-	-	-	-	0
2014	Foot	213 (16.97)	5 (0.40)	13 (1.04)	48 (3.82)	279 (22.23)
	Bike	-	-	-	-	0
	Vehicle	55 (4.38)	207 (16.49)	3 (0.24)	631 (50.28)	896 (71.39)
	Horse	-	-	-	-	0
	Unknown	-	-	-	80 (6.37)	80 (6.37)
2015	Foot	237 (41.07)	6 (1.04)	15 (2.60)	25 (4.33)	283 (53.38)
	Bike	-	-	-	-	0
	Vehicle	24 (4.16)	69 (11.96)	-	180 (31.20)	273 (47.31)
	Horse	-	-	-	-	0
	Unknown	-	-	-	21 (3.64)	21 (3.64)
2016	Foot	115 (14.22)	2 (0.35)	-	15 (1.85)	132 (16.32)
	Bike	-	-	1 (0.12)	-	1 (0.12)
	Vehicle	83 (10.26)	70 (8.65)	3 (0.37)	499 (61.68)	655 (80.96)
	Horse	-	-	-	-	0
	Unknown	-	-	-	21 (2.60)	21 (2.60)

LM

Results

From 2013-2016, across all study sites there were 546 independent captures of tapirs, 1099 of jaguars, and 14,181 of humans (Table 1.5) with 25,664 total trap nights (Table 1.1). LM had the fewest detections of tapirs each year, with an average sample size of 12.25 tapirs/year (SD = 5.8), followed by YB with 27.25 tapirs/year (SD = 22.0), GJ with 36.75 tapirs/year (SD = 16.4) and HB with most tapir detections on average at 60.25 tapirs/year (SD = 23.8) (Table 1.5). Of the 546 tapir captures, only 336 tapir photos were clear enough to determine tapir sex. Of these 76.5% were males and 23.5% were females. Only 3 of the 79 females captured on camera showed accompanying juveniles, and none of those young had the pelage markings that would indicate that they were less than six months old (Nowack 1999).

At all four sites, and across all four years, kernel density estimates of tapir activity pattern showed an overall tendency towards nocturnal behavior (Figs. 1.3 & 1.4) with the majority of tapir captures occurring between 1900 h and 0500 h (GJ – 87%, LM – 88%, HB – 93%, YB – 96%) and activity peaks between 0230 h to 0330 h and 2000 h to 2100 h. At all study sites, humans exhibited a diurnal pattern with multiple activity peaks from 0800 h to 1800 h. Overall, jaguars showed a nonrandom distribution of activity across the 24-hour day-night cycle (Table 1.6), but this pattern did not meet the criteria established by Massara *et al.* (2018) to label felid species as being cathemeral, crepuscular, diurnal or nocturnal (Table 1.3). When broken down by site, jaguars were cathemeral with a pattern of steady activity throughout the 24 hours of a day at all sites with the exception of YB where jaguars exhibited more nocturnal activity (59%) than diurnal (24%) or crepuscular (17%).

TABLE 1.5 Number of records for each species at each study site by survey year. Independent records were totaled for each site and are listed in the column under each species along with the total number of photos taken shown in parentheses. Researchers were included in the human records.

| Study Site	Survey Year	# of Photos		
		Baird's Tapir *Tapirus bairdii*	Jaguar *Panthera onca*	Human *Homo sapiens*
GJ	2013	12 (48)	75 (253)	907 (3131)
	2014	52 (269)	151 (566)	1332 (12,420)
	2015	32 (387)	84 (454)	1429 (7154)
	2016	51 (389)	93 (464)	2542 (11,450)
	Totals	147 (1093)	403 (1737)	6210 (34,155)
YB	2013	-	-	-
	2014	17 (92)	47 (108)	350 (2330)
	2015	28 (140)	23 (110)	394 (2058)
	2016	64 (571)	68 (389)	761 (5506)
	Totals	109 (803)	138 (607)	1505 (9894)
HB	2013	37 (254)	98 (274)	354 (5555)
	2014	62 (439)	119 (415)	267 (3917)
	2015	68 (756)	54 (183)	1337 (7760)
	2016	74 (417)	64 (296)	586 (2389)
	Totals	241 (1866)	335 (1168)	2544 (19,621)
LM	2013	19 (81)	40 (121)	1281 (10,488)
	2014	16 (174)	44 (141)	1255 (8304)
	2015	10 (79)	75 (328)	577 (4183)
	2016	4 (22)	64 (437)	809 (6140)
	Totals	49 (356)	223 (1027)	3922 (29,115)
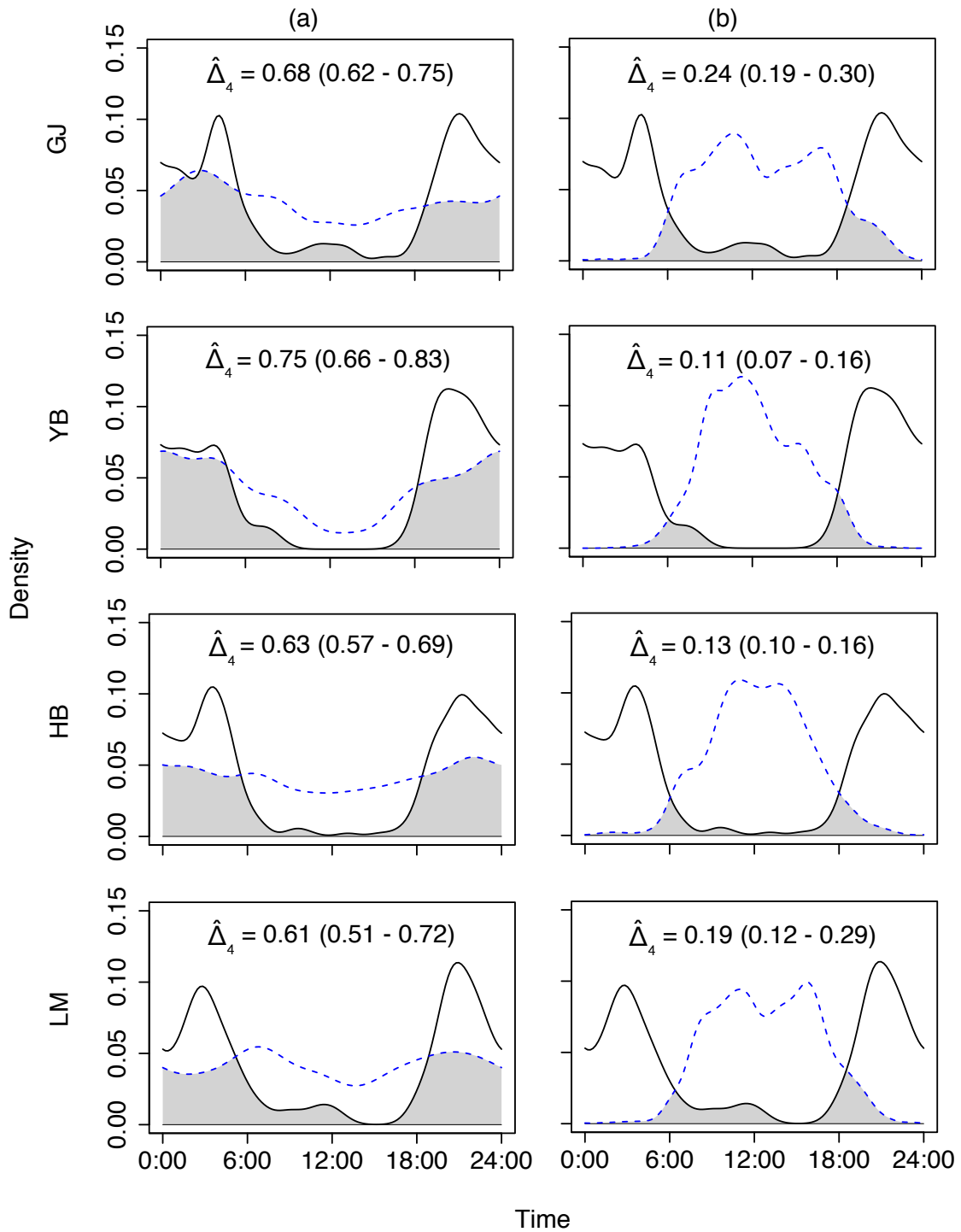
FIGURE 1.3 Estimates of the overlap in daily activity patterns of Baird's tapirs with (a) jaguars and (b) humans in four study sites in NW Belize: GJ, YB, HB, and LM. The solid lines are kernel density estimates for tapirs and the dashed lines are those of jaguars and humans. The shaded region in each plot represents the coefficient of overlap. The estimate of overlap $\hat{\Delta}_4$ is given in the top of each plot with 95% bootstrap confidence intervals in parentheses.
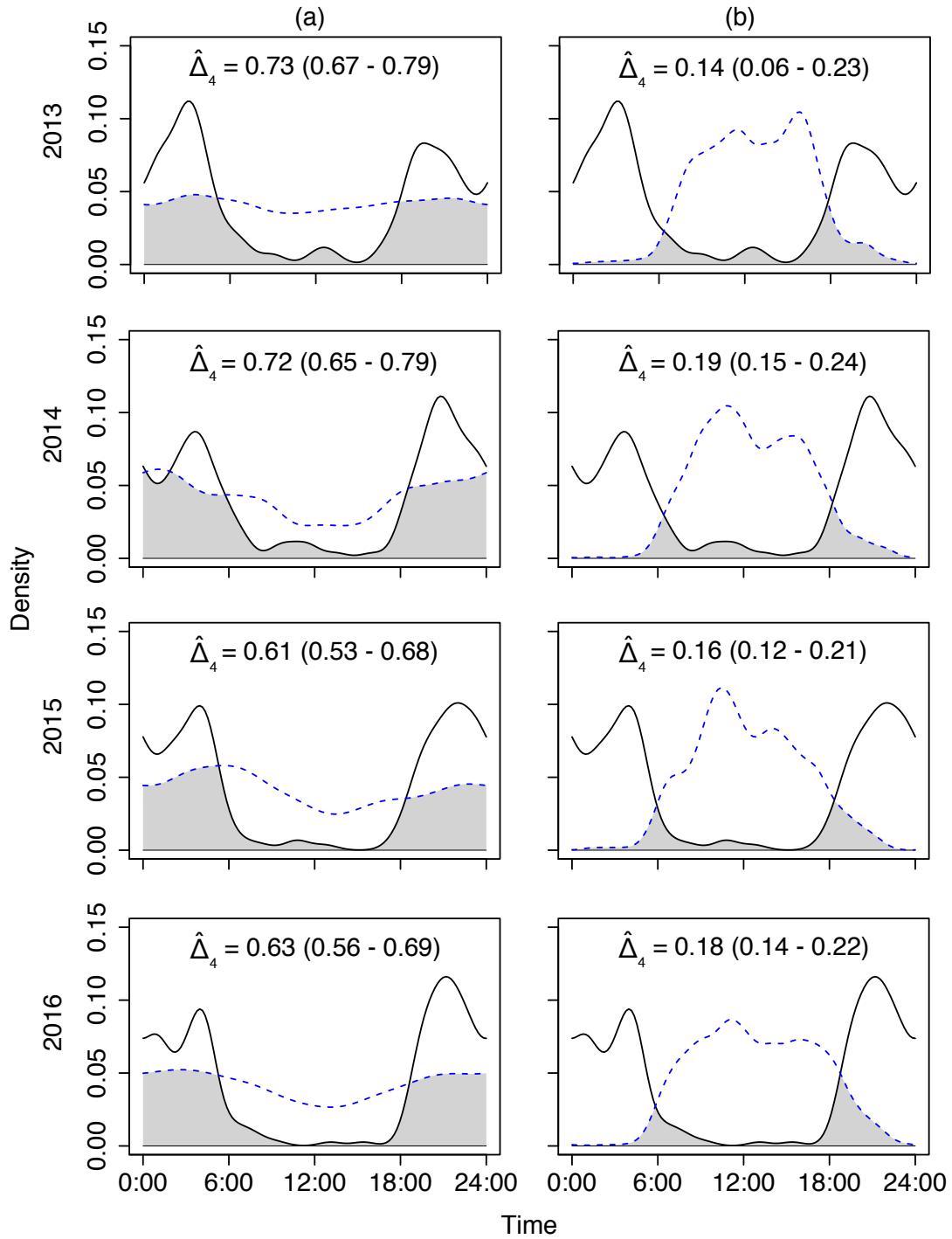
FIGURE 1.4 Regional estimates of the daily activity patterns of Baird's tapirs with (a) jaguars and (b) humans across four study sites in NW Belize by survey year. The solid lines are kernel density estimates for tapirs and the dashed lines are those of jaguars (left) and humans (right). The shaded region in each plot represents the coefficient of overlap. The estimate of overlap $\hat{\Delta}_4$ is given in the top left of each plot with 95% bootstrap confidence intervals in parentheses.

TABLE 1.6 Number (and %) of independent captures of tapirs, jaguars and humans in logged and unlogged study sites, camera stations with low and high frequency of human activity, as well as the entire region from 2013 to 2016. The Rao's spacing test was used to determine if each species exhibited a uniform activity pattern (cathemeral) throughout the day. Values with an * varied significantly from the uniform distribution (alpha = 0.05). Exact p-values were not displayed with Rao's spacing test. The bolded Rao test statistic values (U) indicate that jaguars at the unlogged study site and the low-human-frequency camera stations exhibited a cathemeral activity pattern. At all study sites and at the logged sites, jaguars showed a nonrandom pattern of activity, but did not meet the required criteria to categorize the activity pattern as cathemeral, nocturnal, diurnal or crepuscular (Table 1.3).

Species	Area	Nocturnal	Crepuscular	Diurnal	Rao's Test
Tapir	Unlogged	39 (79.59) *	6 (12.24)	4 (8.16)	$U = 180$
	Logged	395 (79.32) *	80 (16.06)	23 (4.62)	$U = 191$
	Low	371 (78.44) *	76 (16.07)	25 (5.29)	$U = 188$
	High	62 (83.78) *	10 (13.52)	2 (2.70)	$U = 207$
	All	434 (79.49) *	86 (15.75)	27 (4.95)	$U = 192$
Jaguar	Unlogged	86 (38.57)	47 (21.08)	90 (40.36)	$U = \mathbf{134}$
	Logged	429 (48.97)	152 (17.35)	295 (33.68)	$U = 140$
	Low	395 (43.84)	173 (19.20)	333 (36.96)	$U = \mathbf{135}$
	High	120 (60.61) *	26 (13.13)	52 (26.26)	$U = 148$
	All	515 (46.86)	199 (18.11)	385 (35.03)	$U = 140$
Human	Unlogged	150 (3.82)	383 (9.77)	3389 (86.41) *	$U = 278$
	Logged	520 (5.07)	1197 (11.67)	8542 (83.26) *	$U = 322$
	Low	135 (2.53)	332 6.23)	4860 (91.23*	$U = 299$
	High	535 (6.04)	1248 (14.10)	7071 (79.860*	$U = 317$
	All	670 (4.72)	1580 (11.14)	11,931 (84.13) *	$U = 332$

At all sites, the tapir-jaguar activity overlap was high ($\hat{\Delta}_4 > 0.60$; Fig. 1.3). Tapir-human overlap was higher in GJ and LM than in HB and YB. YB had the highest overlap of tapirs with jaguars ($\hat{\Delta}_4 = 0.75$), and the lowest overlap of tapirs with humans ($\hat{\Delta}_4 = 0.11$). Activity pattern overlap was similar across all years except 2014 in which there was a 9% average increase in tapir-jaguar overlap ($\hat{\Delta}_4 = 0.72$) and in 2015 a 3% average decrease in tapir-human overlap ($\hat{\Delta}_4 = 0.16$) (Fig. 1.4).

There was no significant difference in the tapir activity pattern distribution between logged and unlogged sites ($W = 0.06$, df = 2, $p = 0.97$; Fig. 1.5a). However, jaguars in logged sites showed significantly more nocturnal activity between 2400 and 0500 hours than at the unlogged site ($W = 7.20$, df = 2, $p < 0.05$; Fig. 1.5b). Human activity pattern distributions also significantly differed between these areas, with humans at the unlogged site exhibiting a second peak in activity between 1500 and 1800 hours that was not observed at the logged sites ($W = 27.70$, df = 2, $p < 0.001$; Fig. 1.5c). Between logged and unlogged sites, there was not a significant difference in tapir-jaguar overlap (randomization test, $p = 0.33$) and tapir-human overlap (randomization test, $p = 0.59$) (Fig. 1.6).
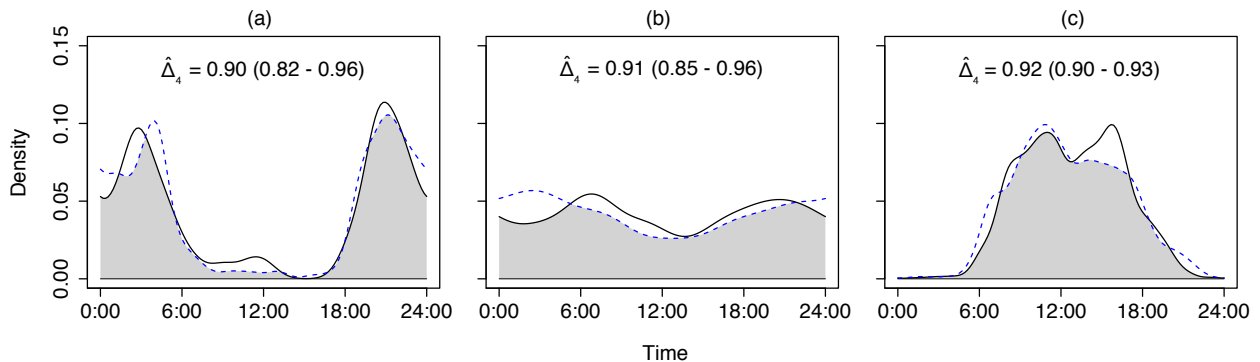

FIGURE 1.5 Estimates of the overlap in daily activity pattern of single species between areas with (dashed line) and without (solid line) extractive logging: a) tapirs, b) jaguars, and c) humans. The shaded region in each plot represents the coefficient of overlap. The estimate of overlap $\hat{\Delta}_4$ is given in the top of each plot with 95% bootstrap confidence intervals in parentheses.
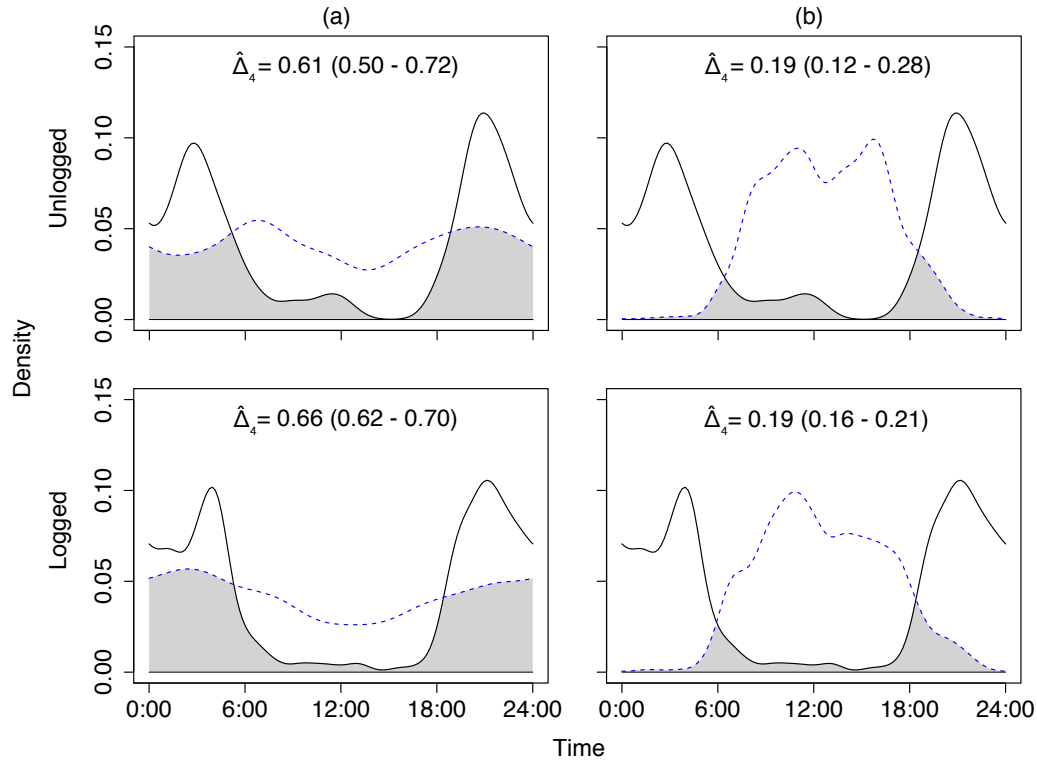
FIGURE 1.6 Estimates of overlap in daily activity pattern of tapirs with a) jaguars and b) humans at sites with (GJ, HB, YB) and without extractive logging (LM). The solid lines are kernel density estimates for tapirs and the dashed lines are that of jaguars and humans. The shaded region in each plot represents the coefficient of overlap. The estimate of overlap $\hat{\Delta}_4$ is given in the top of each plot with 95% bootstrap confidence intervals in parentheses.

There was also no significant difference in the pattern of tapir activity between camera stations of high and low human trap rate (Mardia-Watson-Wheeler test; $W = 3.60$, df $= 2$, $p = 0.17$; Fig. 1.7a). Jaguars again showed a significant difference in activity pattern, with jaguars at high human activity camera stations exhibiting more nocturnal activity between 2400 and 0500 hours and less daytime activity between 0600 and 1900 hours relative to low human activity camera stations ($W = 15.38$, df $= 2$, $p < 0.001$; Fig. 1.7b). A significant difference was also observed in humans at high and low human activity stations ($W = 856.3$, df $= 2$, $p < 0.001$). Humans activity at high activity cameras stations was spread more broadly throughout the 24 hours of the day, whereas at low human activity stations human activity exhibited a distinct peak

just before noontime (Fig. 1.7c). There was no significant difference in tapir-jaguar

(randomization test, $p = 0.17$) or tapir-human (randomization test, $p = 0.41$) activity pattern

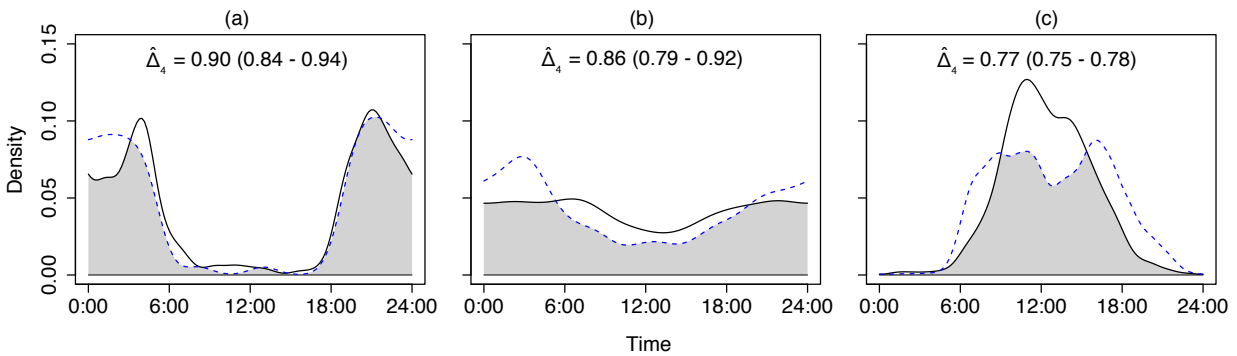overlap between high and low human trap rates (Fig 1.8).

FIGURE 1.7 Estimates of the overlap in daily activity pattern of single species between camera sites with low (solid line) or high (dashed line) frequency of human activity: a) tapirs, b) jaguars, and c) humans. The shaded region in each plot represents the coefficient of overlap. The estimate of overlap $\hat{\Delta}_4$ is given in the top of each plot with 95% bootstrap confidence intervals in parentheses.
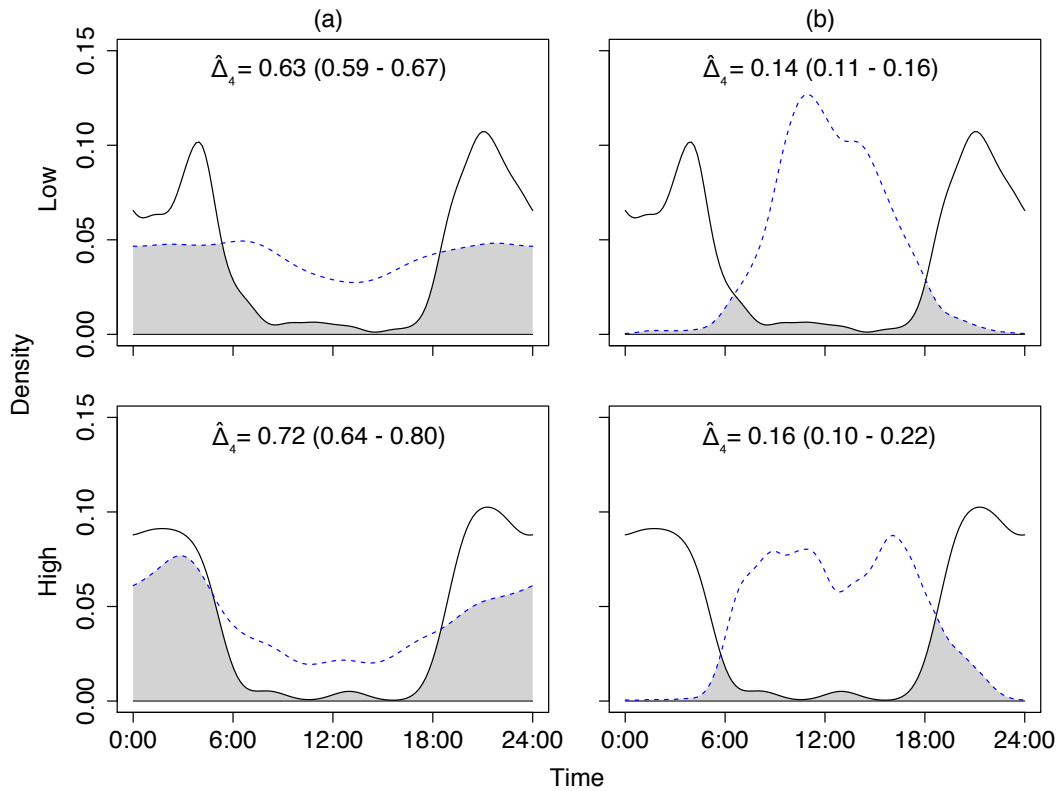
FIGURE 1.8 Estimates of overlap in daily activity pattern of tapirs with a) jaguars and b) humans at camera stations with a low or high frequency of human activity. The solid lines are kernel density estimates for tapirs and the dashed lines are that of jaguars and humans. The shaded region in each plot represents the coefficient of overlap. The estimate of overlap $\hat{\Delta}_4$ is given in the top of each plot with 95% bootstrap confidence intervals in parentheses.

DISCUSSION

Timber extraction & human trap rate

We found no significant difference in tapir activity pattern between the logged and

unlogged areas or between camera trap stations exhibiting high or low human trap rates.

However, both jaguars and humans have significantly different activity patterns between these

areas, suggesting that jaguars may be altering their behavior in response to human activity.

Similarly, Massara *et al.* (2018) found that the ocelot (*Leopardus pardalis*), another Neotropical

cat species, adopted a more nocturnal pattern of activity in areas where the landscape had

anthropogenic alterations due to human settlements and agriculture. The high overlap of tapir-jaguar activity patterns in all activity pattern comparisons ($\hat{\Delta}_4 > 0.60$) implies that tapirs are indifferent to the presence of this predator and suggests that it is unlikely that tapirs are relying on human activity to shield them from jaguars. The increased nocturnal pattern of jaguars in YB results in an increase in tapir-jaguar activity overlap ($\hat{\Delta}_4 = 0.75$) and the lowest tapir-human overlap ($\hat{\Delta}_4 = 0.11$) of the four study sites, providing support that both jaguars and tapirs are potentially altering their behavior in response to the human presence at that site.

Despite the expectation that tapirs would exhibit a nocturnal activity pattern in the logged sites and at camera stations with a high human trap rate, tapirs were nocturnal at all sites. This suggests that even low levels of human disturbance/presence may impact tapir activity. However, the $\hat{\Delta}_4$ tapir-human overlap (11-24%, Figs. 1.3 and 1.4) shows that tapir avoidance of humans is not absolute. Tapirs occasionally exhibit diurnal activity (best visualized in Fig. 1.4, years 2013 and 2014). Of the 546 tapir captures, 25 (5%) occurred between 0700 h and 1700 h. Over half of those 25 tapirs were active in GJ and YB, which have the highest human densities per km^2 of the four sites (Table 1.2) and the highest average human trap rate per camera over the cumulative years of this study, suggesting potential habituation or tolerance.

In the case of true habituation, tapirs would have been expected to maintain a crepuscular activity pattern. This is especially so as this study occurred during the rainy season in Belize, which in Costa Rica has been found to correlate with tapir activity becoming more crepuscular and diurnal (Foerster & Vaughan 2002). Tapirs in all study areas have maintained a bimodal activity pattern reflective of crepuscular activity, but both activity peaks have shifted about 1.5 h into the nocturnal period of time. Although Foerster & Vaughan (2002) suggested that tapirs become nocturnal to avoid hyperthermia, Cruz *et al.* (2014) did not find an effect of temperature

on lowland tapir activity patterns in Argentina. The cameras in our study were not calibrated for

temperature monitoring. Despite the lack of difference in tapir activity pattern at high and low

human activity camera station and between the logged and unlogged areas, the nocturnal activity

observed in this study differs from what has previously been found of Baird's tapirs in areas with

little to no human disturbance (Carbajal-Borges *et al.* 2014, Pérez-Irineo & Santos-Moreno

2016). This suggests that tapirs are behaviorally responding to human activity.

Because all captured tapir images were of adult males and females, and large-sized

juveniles, it is possible that our findings are not representative of females with small young that

are more susceptible to predation. The high $\hat{\Delta}_4$ tapir-jaguar activity pattern overlap observed in

the current study is similar to a study by Harmsen *et al.* (2011) in the protected Cockscomb

Basin Reserve of south-central Belize. A positive relationship was found between tapir and

jaguar activity patterns, although in that study area jaguars exhibited a nocturnal activity pattern

(Harmsen *et al.* 2009). Interestingly, the cathemeral jaguar activity pattern exhibited at most

study sites in the current study in NW Belize aligns with that of prior studies in areas lacking

anthropogenic influence (Ecuador - Blake *et al.* 2014, Mexico - Hernández-SaintMartín *et al.*

2013, Bolivia - Romero-Munoz *et al.* 2010). In areas with human presence and anthropogenic

change, jaguars have shown either nocturnal or diurnal activity patterns (Brazil - Astete *et al.*

2008, Belize - Dobbins *et al.* 2017). It is possible that the human-mediated change in the current

study sites is not enough to elicit a change in jaguar activity patterns, or it could be that jaguars

have habituated to the levels of human activity and human-mediated habitat changes in this part

of Belize and are thus indifferent. Finally, as logging season usually finishes in late May or early

June (around the time the camera trapping study starts), it is possible that, since logging trucks

are not present during camera surveys, the season when tapirs and jaguars are most likely to

avoid humans has been missed. Expanding the camera survey to encompass the logging season

would allow for a future study comparing species activity during active and in-active logging.

Future considerations

Much of Belize (~74%) is suitable tapir habitat (Schank *et al.* 2015) and Baird's tapirs

appear tolerant of human activity in the absence of direct persecution by humans. Despite

exhibiting an overall nocturnal activity pattern, evidence of diurnal tapir activity at all sites

suggests that there is potential for tapir habituation to frequent human activity related to

ecotourism, agriculture, and, perhaps most notably in the Neotropics, sustainable logging

practices. A possible benefit of wildlife habituation or tolerance is that, though it may result in

changes to wildlife activity, individual animals may look to use suitable habitat in the vicinity of

humans. This would be advantageous in countries where wildlife reserves bring in substantial

revenue from ecotourism and viewing wildlife in native habitat (Lynam *et al.* 2012, Malo *et al.*

2011). For example, in our GJ study site, white-tailed deer and ocellated turkeys are

commonplace, showing little fear of humans, and the area is well-known by tourists for its

wildlife viewing. However, for appropriate conservation of large tropical mammals, we must

also consider the adverse impacts that accompany habituation and an increased tolerance to

humans.

In the presence of humans, wildlife may alter their behavior or activity pattern (Frid &

Dill 2002, Gaynor *et al.* 2018, George & Crooks 2006, Larson *et al.* 2016, Vistnes & Nellemann

2008). For instance, after closing the largest protected area in Thailand for 6+ months,

researchers observed a 45% increase in leopard (*Panthera pardus*) detection rates and a shift

from nocturnal to diurnal activity in the absence of visitors (Ngoprasert *et al.* 2017). Similarly,

in Argentina, guanaco (*Lama guanicoe*) sightings at a World Heritage Site are less likely on days with high visitor numbers, suggesting that guanacos actively seek undisturbed areas (Malo *et al.* 2011). Any such deviation from typical behavior comes with inevitable tradeoffs resulting in changes to resting or foraging habits, territory marking, and/or seeking out mates (Gaynor *et al.* 2018, Larson *et al.* 2016).

Yet, the effects of human disturbance on animals are not always visible or predictable. Lunde *et al.* (2016) found that impala (*Aepyceros melampus*) foraging near main roads of Serengeti National Park exhibited higher stress hormone levels than those near less-traveled roads. In the presence of humans, wildlife may exhibit heightened vigilance, which reduces the amount of time spent on other fitness enhancing behaviors (Gaynor *et al.* 2018, Larson *et al.* 2016). However, some ungulate species have been found to exhibit decreased vigilance after prolonged exposure to human stimuli (Brown *et al.* 2012), which could potentially leave them more susceptible to predation. Conversely, predators that follow their habituated prey can be seen as a threat to farming communities and local settlements, potentially putting predators into direct conflict with people (Morrison *et al.* 2016).

Additionally, habituation is not always exhibited by all species in the same region. In Thailand, sambar (*Rusa concolor*) and red muntjac (*Muntiacus muntjak*) experience poaching pressure and as such avoid roads and villages, while Asian tapirs (*Tapirus indicus*) experience little to no hunting pressure, and thus are not sensitive to roads and human settlements (Lynam *et al.* 2012). Furthermore, while dispersal and migration in highly fragmented ecosystems are challenging (Morrison *et al.* 2016), habituation can make these even more arduous and potentially deadly behaviors. If wildlife habituate to humans with nonlethal intentions (e.g., tourists, researchers, and other inhabitants of forest reserves) and then travel outside a protected

area, then wildlife will not know to avoid humans that see them as a nuisance, threat, and source of revenue and/or food. Traversing fragmented landscapes also exposes wildlife to the potential for vehicular collisions, which in Belize and Brazil are a substantial threat to the viability of tapir populations (Poot & Clevenger 2018, Medici & Desbiez 2012).

Indeed, Baird's tapirs occupy habitat adjoining heavily impacted areas, for example near Belize's international airport (Poot & Clevenger 2018). The dark coloration and lack of pelage markings on adult tapirs makes them difficult to see in low lighting. Additionally, Colino-Rabanal *et al.* (2018) found that nights with brighter moonlight correlate with an increase in ungulate-vehicle collisions in three out of the four ungulate species they studied, presumably due to the increase in ungulate movement on nights with better visibility. Brown *et al.* (2012) found that increased vehicle traffic resulted in lower vigilance of elk (*Cervus canadensis)* and pronghorn (*Antilocapra americana*). This, in combination with our findings that Baird's tapir exhibit activity peaks during nocturnal hours, suggests that they might inadvertently suffer greater road mortality than if they were crepuscular.

CHAPTER II:

THE ASSOCIATION OF PARASITE LOAD AND BODY CONDITION OF BAIRD'S TAPIRS WITH THE FREQUENCY OF HUMAN ACTIVITY IN A NEOTROPICAL FOREST.

Introduction

The health (ability to maintain physiological homeostasis, Ryser-Degiorgis 2013) of an individual animal is a product of its genetics, behavior (Wittrock *et al.* 2019), and the availability and quality of resources in relation to stressors (Clutton-Brock & Sheldon 2010, Ryser-Degiorgis 2013), such as predation (Weinstein & Lafferty 2015) and disease (Cross *et al.* 2009, Hing *et al.* 2016). These same factors also affect population dynamics (Bonenfant *et al.* 2009, Cross *et al.* 2009), through their impact on recruitment (Irvine 2006), dispersal, and population densities (Clutton-Brock & Sheldon 2010). Human alteration of natural habitats, for example through habitat restructuring (Cove *et al.* 2013, García-Marmolejo *et al.* 2015), habitat fragmentation (Garcìa *et al.* 2016, Rhodes *et al.* 2017), pollution and the introduction of invasive species (Larson *et al.* 2016, Martin *et al.* 2011) and novel predators (Atickem *et al.* 2014, Shannon *et al.* 2014), is well known for causing rapid changes in animal health with negative population-level conservation outcomes (Hing *et al.* 2016, Frid & Dill 2002).

Even in the absence of anthropogenic habitat destruction or species community alteration, however, human activity can be harmful to wildlife, especially if the disturbance is chronic (Hing *et al.* 2016, Jolles *et al.* 2015, Zbyryt *et al.* 2018). For instance, impala (*Aepyceros melampus*) in

Serengeti National Park exhibit higher stress hormone levels when foraging near high-traffic roads than near less-traveled roads (Lunde *et al.* 2016). Similarly, Formenti *et al.* (2018) found Apennine chamois (*Rupicapra pyrenaica ornate*) to maintain elevated stress hormone levels during months when hikers and domestic sheep (*Ovis aries*) and goats (*Capra hircus*) were present. Elevated androgens and glucocorticoids can weaken immune function and make animals more susceptible to parasitic infection and/or disease (Beldomenico & Begon 2015, Jolles *et al.* 2015, Glaser & Keicolt-Glaser 2005). These studies substantiate the rising concern of conservation and public health entities regarding the cross-transmission of parasites and diseases between domestic animals and wildlife (Hing *et al.* 2016, Martin *et al.* 2011, Weinstein & Lafferty 2015).

Cross-transmission of parasites and disease from domestic animals to wildlife has been detrimental to wild ungulate populations including bighorn sheep (*Ovis canadensis*) in the United States (Jolles *et al.* 2015), Alpine chamois (*Rupicapra rupicapra*) in Switzerland (Jolles *et al.* 2015; Martin *et al.* 2011), and elk (*Cervus canadensis*) and bison (*Bison bison*) in the United States and Canada (Meagher & Meyer 1994). Such infections may be tolerated without obvious harm under ideal conditions (Hart & Hart 2018, Irvine 2006, Weinersmith & Early 2016), but result in reduced body condition under stressful conditions (Coulson *et al.* 2018). Among free-living ungulates a negative relationship between ungulate body condition and parasite load has been shown in feral horses (*Equus ferus*) in Nova Scotia (Debeffe *et al.* 2016), Soay sheep (*Ovis aries*) in Scotland (Coltman *et al.* 2001), moose (*Alces alces*) in Norway (Davidson *et al.* 2015), and red deer (*Cervus elaphus*) in Scotland, the latter species' body condition suffering greatly from even low level infection with gastrointestinal nematodes (Irvine

et al. 2006). In contrast, there have been no studies of the effect of parasites on the body condition and population viability of wild Neotropical ungulates.

Neotropical ecosystems are experiencing high rates of land use change in the form of deforestation for timber extraction, urban expansion (Rhodes *et al.* 2017), and agriculture (García-Marmolejo *et al.* 2015, Taber *et al.* 2016). For large, wide-ranging ungulates the reduction in resources and increased proximity to humans can amplify hunting pressure (Taber *et al.* 2016) and expose native ungulates to novel diseases via livestock (Medici *et al.* 2006, Walker & Morgan 2014). However, there is limited understanding of how these factors are affecting Neotropical ungulate populations, and anthropogenic impacts on Neotropical ecosystems are expanding faster than field studies can generate informative results (Taber *et al.* 2016).

Monitoring the body condition of free-living wildlife is difficult. Capture and handling of wild ungulates is costly, often requires personnel trained in the use of veterinary pharmaceuticals, can result in animal and handler injury (Quse & Fernandes-Santos 2014) and may not be permitted by government agencies, local communities or landowners. Close inspection of only hunter-killed animals may be informative, but may also skew our perception of population dynamics, since hunters preferentially target larger individuals whose impressive horns or antlers may be the product of better health (Allendorf & Hard 2009). The use of remotely captured images removes the potential for this bias and provides a noninvasive way to score body condition of individual animals (Pérez-Flores *et al.* 2016, Schiffman *et al.* 2017, Wolf *et al.* 2018). Camera trap surveys of wildlife occurrence and abundance are now widespread in the tropics (Schank *et al.* 2017) and may provide a means of monitoring ungulate population viability, particularly in zones of cohabitation with domestic livestock and/or chronic disturbance by people, when species of conservation concern are at risk.

Baird's tapirs are endangered Neotropical ungulates that are difficult to observe given their dense habitat and elusive behavior (Garcìa *et al.* 2016). Baird's tapirs eat the roots, leaves, stems, bark, flowers, fruits and seeds (Garcìa *et al.* 2012; O'Farrill *et al.* 2006; O'Farrill *et al.* 2013) of some 90+ species of ferns, vines, palms and hardwood trees (Cove *et al.* 2013; Naranjo 2009). Thus, they play a crucial role in tropical forest ecosystems as seed dispersers (Garcìa *et al.* 2012, O'Farrill *et al.* 2013). Suitable habitat in the tapir's geographic range is heavily fragmented (Garcìa *et al.* 2016), and there is concern that some populations may become genetically isolated (Mangini *et al.* 2012, Naranjo & Bodmer 2002), making those populations more susceptible to parasitic infection and disease (Medici *et al.* 2006). Additionally, there is evidence of parasite cross-transmission between domestic cattle and tapirs in Mexico (Cruz *et al.* 2006, Romero-Castañón *et al.* 2008). As conservationists attempt to secure a landscape of protected reserves for Baird's tapir, with wildlife corridors to connect them (Mendoza *et al.* 2013), practical methods for monitoring the condition and health of wild-living tapirs are urgently needed. Thus, using noninvasive camera trap surveys is an ideal approach to monitor the behavior and body condition of Baird's tapirs.

The objectives of this study were to determine if the frequency of human activity in NW Belize correlates with 1) fecal parasite load of tapirs and 2) tapir body condition, 3) to compare two chemicals for long-term preservation of fecal samples in field studies, 4) to investigate more thoroughly the types of parasites harbored by Belizean tapirs, and 5) if any parasites may be shared with domestic horses and cattle.

Methods

Sample collection

Tapir fecal samples were collected while walking trails encompassed by a camera survey grid in May-July of 2017 at the Gallon Jug (GJ), La Milpa (LM) and Yalbac (YB) study sites, such that each transect was visited every 2-3 days. A fourth site (Hill Bank) was not accessible due to hurricane damage. A detailed description of these sites and the camera grid is provided in Chapter 1. Forty-two total tapir fecal samples were collected (Figure 2.1), with an estimated 12km walked per sample collected during the course of six weeks. Horse and cattle fecal samples were collected from pastures in GJ after the direct observation of a cow or horse defecating. For collection of all species' fecal samples, the sample was collected from the center of the dung pile and processed by the following protocol.

Tapir feces are distinct and have large boli with partially digested plant matter. Enough boli (2-3 depending on size) to fill a 284 mL plastic screw-top container were collected from the center of the dropping pile. Samples were processed following protocol used by Mikota & Gage (2006) to process elephant fecal samples as follows: a) Water was added to fill the container, b) the container was shaken vigorously for one minute to homogenize the fecal mixture, c) a metal spoon was used to break up large pieces if necessary, after which the container was shaken for an additional 30 seconds, and repeated until homogenized, d) the fecal mixture was filtered through a wire mesh strainer (0.8 mm) into a plastic cup, while using a spray bottle to rinse large debris and allow small debris and fine sediment to pass through the strainer, and e) the filtrate was allowed to settle in the cup for 5 hours before carefully pouring off the water layer.

The fecal sediment was sub-sampled for three purposes: 1) DNA extraction, 2) preservation of parasites in ethanol, and 3) preservation of parasites in formalin. For DNA

extraction, from the sediment, two 150 mg samples were placed in replicate bead beating tubes

from ZYMO Research Quick DNA Fecal/Microbiome DNA Miniprep kits (Zymo Research,

Irvine, CA) with 750 µL of Shield Reagent (Zymo Research, Irvine, CA) and vortexed for two

minutes. The Shield Reagent guarantees preservation of DNA for 1 month at ambient

temperatures. Due to delays in exporting the samples from Belize to the USA, they were held at

ambient temperatures (in Belize) for 6 months before they could be refrigerated at -20ºC. In the

USA, DNA extractions from fecal samples were completed following the protocol of ZYMO

Research Quick DNA Fecal/Microbiome DNA Miniprep kits (Zymo Research, Irvine, CA). The

remaining sediment was weighed and divided between two 15 mL plastic centrifuge tubes, one

containing 10% formalin and the other 95% ethanol. Two different storage solutions were used

to investigate their relative preservation abilities for worms and eggs/embryonic larvae (Hu *et al.*

2016, Nielson *et al.* 2010). Samples in both storage solutions were used to quantify parasite

load.

Parasite Quantification

To isolate parasite eggs, embryonic larvae and worms from the fecal sediment, each

sample was gently shaken (resuspended) and then 1 mL of the resuspended fecal sample was

added to 14 mL of sugar solution (700 g table sugar and 1 L reverse osmosis (RO) water) in a 15

mL glass centrifuge tube and topped off with sucrose solution such that a glass coverslip adhered

to the liquid when placed on the tube's opening. Each sample underwent centrifugation for 10

minutes at 2000 rpm in a swinging bucket centrifuge (International Equipment Company,

model# HNS11). The parasites that floated to the top were transferred with the coverslip to a

glass microscope slide. After allowing the contents to become still for 10 minutes, each slide

was systematically examined at 100X magnification on a compound microscope (Olympus Optical Corporation, Limited, model# BX40F4).

Quantification of parasite eggs was done as follows. In horse and cattle samples, eggs were first categorized to the lowest taxonomic level possible by using the veterinary manual by Foreyt (2013). Then, eggs were counted for each taxonomic category. Unidentified eggs (from tapir samples) were photographed and counted after being described by shape, size, color, thickness of wall, and appearance of cell mass. Worms were measured and described based on appearance of external features, as internal features were not clearly discernable.

The sediment of each centrifuged tube was then examined to quantify nematode worms in the following way. First, the sugar solution supernatant was poured off of each sample. Then, the fecal sediment in each centrifuged tube was resuspended in RO water using a knitting needle to scrape the sediment plug from the bottom of the tube. The resuspended sediment was poured out into a glass petri dish that had been marked with 1 cm wide lanes and allowed to settle before being viewed at 35X magnification under a dissection microscope (Labomed Incorporated, model# 4144000). Worms were separated by morphotype into 1.5 mL Eppendorf tubes containing 100% ethanol and stored at -20°C. Worms were categorized into 6 worm morphologies (Table 2.1). Fecal parasite load was measured as the sum of all types of eggs and worm morphologies (except worm morphology D) in a 1 mL sample of formalin-stored tapir feces. If there were any worm morphotypes found in a 1 mL ethanol-stored fecal sample that were not found in the corresponding formalin-stored sample, then those worm counts were added to the formalin sample count to obtain a total parasite burden value. Sequencing results identified worm morphology D as nonparasitic of animals, so morphology D counts were excluded from total parasite load.
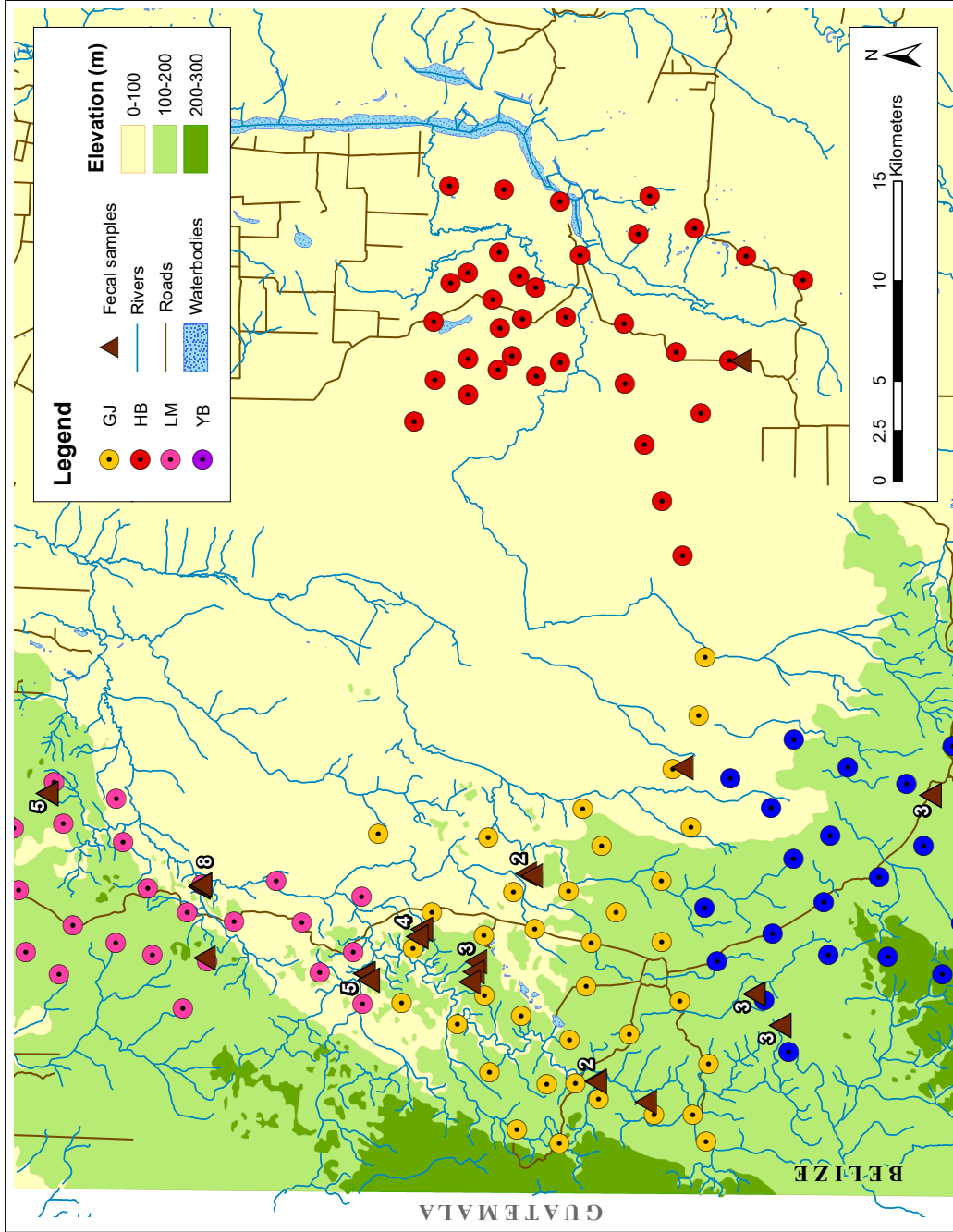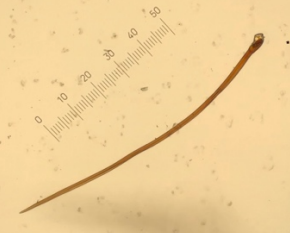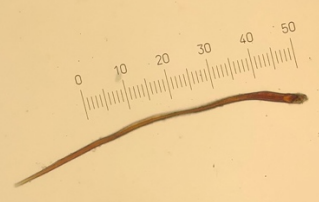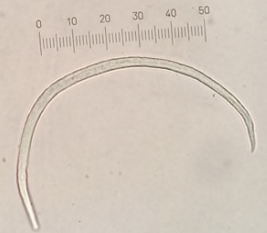
FIGURE 2.1 Map of tapir fecal sample collection locations relative to the 2017 camera survey grid. In some locations, samples could not be differentiated due to the clustering of several samples within 200 meters of each other. In these situations, a numerical value denotes the number of samples found at a given collection site. If no number is present, only one sample was collected.

TABLE 2.1 Descriptions of the six-nematode worm morphotypes observed in both formalin and ethanol preserved tapir fecal samples. Each line of the scale in these photos represents 10 µm.

Worm Morph Type	Description	Photo at 100X magnification
A	Clear protrusion from mouth; long, slender body with amber coloration & smooth sides	
B	Long, slender body with amber coloration & smooth sides; mouth protrusion absent	
C	Body shaped as an elongated tear drop, with mouth parts at the wider end; amber coloration with a darkened body cavity posterior to mouth; smooth sides	
D	Clear; smooth body with a short tail	
E	Clear; smooth body with a long, whip-like tail	
F	Clear; smooth body with long whip-like tail and ridges along sides of body	

Individual worm lysis & PCR

Individual worms pulled from the ethanol-preserved samples were placed in 0.2 mL PCR strip tubes. Before lysing the worms, the ethanol was allowed to evaporate until the worms were fully dry. Following the methods of Chalasani (2016) a lysis mastermix (LMM) solution was created containing 0.3 µL of proteinase K (Viagen Biotech, Inc., cat# 501-PK) and 19.7 µL of a direct-to-PCR mousetail lysis solution (Viagen Biotech, Inc. cat# 101-T) per worm. Twenty µL of the LMM was added to each 0.2 mL sample tube containing a worm. The samples were incubated at 55°C for 16 hours, followed by a heat inactivation at 85°C for 1 hour. The lysed samples were then diluted with 180 µL of sterile water per sample before using 2 µL for PCR.

A PCR mastermix (PMM) was created consisting of 5.0 µL sterile water, 4.0 µL 5X GoTaq Flexi buffer (Promega, #M891A), 3.2 µL dNTPs, 1.6 µL $MgCl_2$ (Promega, #A351H) 1.0 µL of 20mg/mL BSA, 0.5 µL of both the forward and reverse primer (10 µM) and 0.2 µL of GoTaq Flexi DNA polymerase (Promega, #M829B) per sample. Sixteen µL of the PMM was used with 4 µL of DNA per sample. Two primer pairs targeting the 18S rRNA region of nematodes were used for PCR (Table 2.2). The settings for the NEM primer pair were 95°C for 3 mins to start, then 95°C for 1 min, 52°C for 30 sec, 72°C for 1 min repeated 40 times followed by 72°C for 5 min and then 12°C infinitely. The second pair was the 18S primer set, which again began with 95°C for 3 mins. Then, 95°C for 1 min, 55°C for 30 sec, and 72°C for 1 min repeated 40 times followed by a final elongation at 72°C for 5 min and 12°C infinitely. Negative and positive (from fecal DNA samples) controls were used in each PCR.

PCR products were loaded alongside a 100bp ladder into 2% agarose gel containing GelRed (Pheonix Research, #RGB-4103). TAE buffer was used for electrophoresis. Samples were electrophoresed at 110 volts for 35 minutes and then the gel was viewed under UV light.

exoSAP-IT (Affymetrix, #75001) was used to cleanup positive PCR product before sending for

Sanger sequencing at the Yale DNA Analysis Facility on Science Hill.

TABLE 2.2 Primer sequences used to amplify the 18S rRNA region of individual nematodes pulled from tapir fecal samples.

Primer name	3'-5' sequence	Fragment length (bps)	Reference
NEM1217F NEM1619 R	CGN BCC GRA CAC YGT RAG GGA AAY AAT TDC AAT TCC CKR TCC	402	Huggins *et al.* 2017
18S 965 F 18S 1573 R	TTG ATC CCG CCA TAG ACT AGC GG TAA TGC AGG GAC GGG AAA CAT	646	Powers *et al.* 2009

Body condition scoring

The photo data used in Chapter 1 were also used to score the body condition of individual

tapirs. Only tapirs whose body was fully photographed (in a single image or in series) were

scored. All females were excluded from statistical analyses as there was no way to determine if

a female was pregnant, and the weight gained during pregnancy may have interfered with body

condition assessment. To prevent pseudoreplication, only scores of males that were individually

identifiable by scar patterns were used.

Using the scale refined by Pérez-Flores *et al.* (2016), body condition was scored by

visually assessing the presence of fat and muscle associated with the bone structure of six

anatomical regions (head, neck, shoulder, spine, ribs, pelvis). Each anatomical region was

scored on a scale of 1-5 with each number corresponding to the following categories of

condition: emaciated (1), thin (2), fair (3), good (4), and obese (5). The scores of all 6

anatomical regions were totaled for each tapir and overall body condition was assessed by the following total point ranges set by Pérez-Flores *et al.* (2016): obese (28-30), good (22-27), fair (16-21), thin (10-15), and emaciated (6-9). See Figure 2.2 for photo examples of the overall body condition categories observed in this study.

FIGURE 2.2 Examples of tapirs scored from the current study as (a) thin, (b) fair, (c) good, and (d) obese body condition. None of the tapirs in this study were found to be emaciated. Moving through the photos from (a) to (d), the most noticeable changes in condition can be seen with the increase in muscle and fat present around the neck, the upper shoulder, and along the ribs.

Frequency of Human Activity

Due to the variability in human usage of roads and trails across field seasons, the human trap rate (the frequency of human records per trap night) was calculated every year from 2013-2017 for each individual camera trap station. The 2017 data were only used in the comparison of human trap rate to parasite load. Comparisons of body condition to parasite load use body condition scores from tapirs captured in 2013-2016. Each scored tapir was assigned the human trap rate corresponding to the station and year in which the tapir was photographed. For clarity, for the remainder of this chapter I will refer to human trap rate as simply human activity.

Statistical Analyses

The frequency of human activity at camera stations where tapir body condition was scored and at the stations nearest to fecal collection locations did not meet the assumption of normality required for parametric statistical tests. This same problem was encountered with the body condition score data as well. The normal distribution could not be achieved even after the data were transformed using square root and logarithmic transformations; therefore, the nonparametric Kendall's tau correlation was used to test for a relationship between fecal parasite load relative to body condition and to human activity.

To do so, each scored male tapir was assigned the parasite load of the nearest fecal sample, and human trap rate (from 2017) was assigned to each fecal sample based on the camera station nearest to where it was collected. Kendall's tau correlation was also used to test for a relationship between body condition and human activity, using the human trap rates that corresponded to each camera station in the year that the tapir being scored was photographed. Differences in sample preservation between ethanol and formalin were assessed with four

different comparisons: 1) parasite richness in ethanol versus in formalin, 2) overall egg count/mL per chemical (all parasite eggs regardless of different egg types), 3) overall worm count/mL per chemical, and 4) pairwise comparison of individual worm morphologies. ANOVA was used to test parasite richness. As overall egg and worm counts and counts of the individual worm morphologies were not normally distributed before or after being transformed, the nonparametric Wilcoxon signed-rank test was used to assess egg and worm counts in ethanol and formalin.

Results

Parasite load, body condition & human activity

Parasite load and body condition did not have a significantly directional relationship (Fig. 2.3) (Kendall's tau correlation: $r_\tau = -0.10$, $p > 0.05$, n = 29), nor did parasite load and human activity (Fig. 2.4) ($r_\tau = 0.02$, $p > 0.05$, n = 42). Body condition score of identifiable male tapirs negatively correlated with human activity at the stations at which they were photographed (Kendall's tau correlation: $r_\tau = -0.37$, $p < 0.01$, n = 29; Fig. 2.5a). Though there was a smaller effect when unidentifiable males are included in the analysis, the relationship remained significantly negative ($r_\tau = -0.19$, $p < 0.05$, n = 72; Fig. 2.5b). Body condition of all scored female tapirs did not show a significantly directional relationship with human activity ($r_\tau = -0.05$, $p > 0.05$, n = 37; Fig. 2.6).
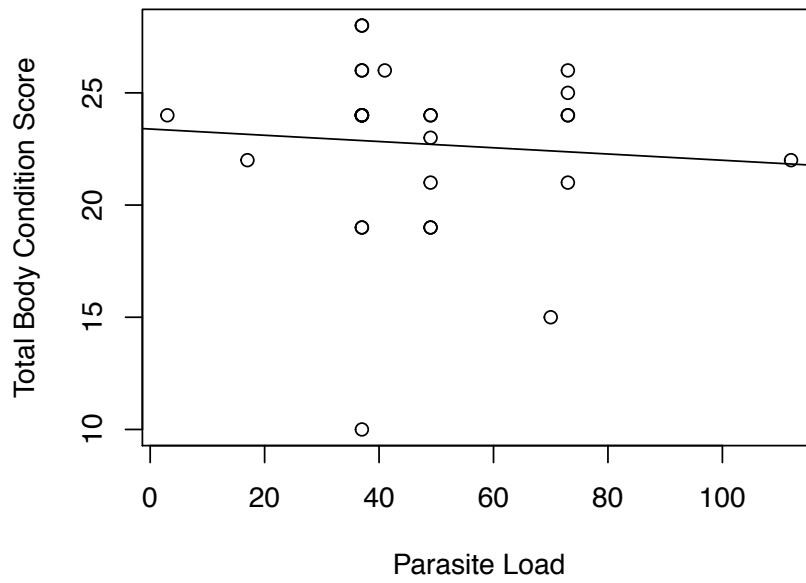
FIGURE 2.3 The body condition of tapirs was not associated with the number of parasites in the feces found nearest to the location where each tapir was photographed ($p > 0.05$).
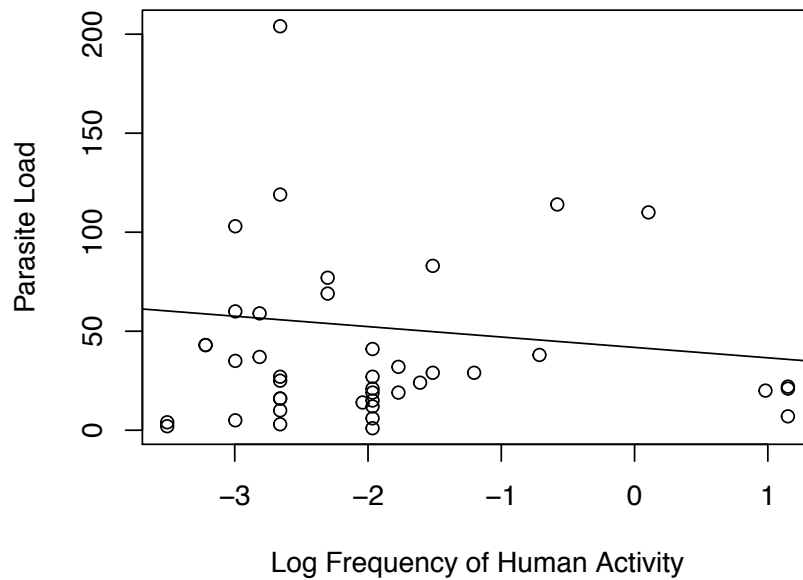

FIGURE 2.4 Human activity at the station nearest each fecal sample was not significantly associated with the number of fecal parasites ($p > 0.05$).
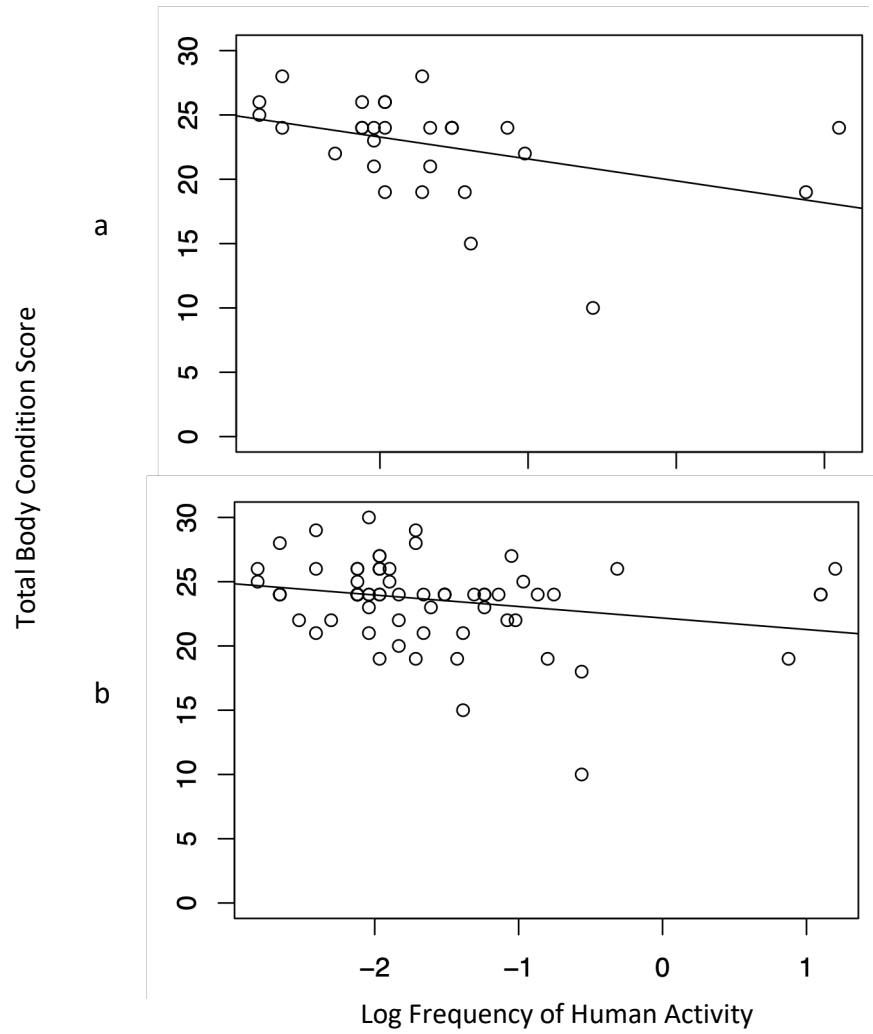
FIGURE 2.5 Body condition is negatively associated with human activity ($p < 0.05$); a) 29 individually identified male tapirs and b) 72 male tapirs including identifiable individuals and unidentified individuals. Human activity was measured at the camera station where each tapir was photographed.
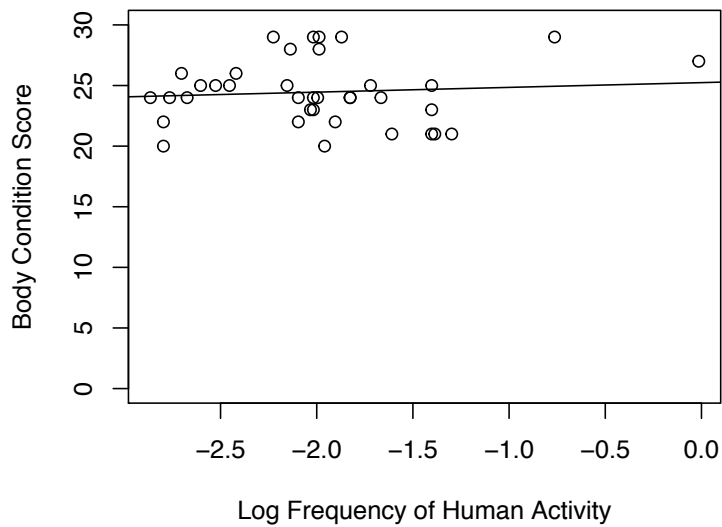
Figure 2.6 Body condition of all scored female tapirs does not show a significantly directional relationship with human activity ($p > 0.05$).

Ethanol versus formalin preservation

Formalin preserved-samples contained significantly higher parasite richness than ethanol samples (ANOVA; $F_{1,82} = 21.81$, $p < 0.001$, n = 42; Fig. 2.7), with formalin samples averaging a parasite richness of 4.29 (SD = 1.71) and ethanol samples averaging 2.79 (SD = 1.18). There was also a significant difference in the total number of eggs found in formalin ($\bar{x} = 6.31$, SD = 9.86) versus ethanol samples ($\bar{x} = 1.14$, SD = 1.66) ($z = 0.69$, $p < 0.001$), but the total number of worms did not vary significantly ($z = 0.21$, $p > 0.05$) (Appendix: Figs. 1 and 2). Morphology C was the only worm morphology that differed significantly between preservation methods ($z = 0.72$, $p < 0.001$) with ethanol-stored samples averaging 1.25 morphology C worms (SD = 2.26) and formalin-stored samples averaging 9.03 (SD = 14.67).
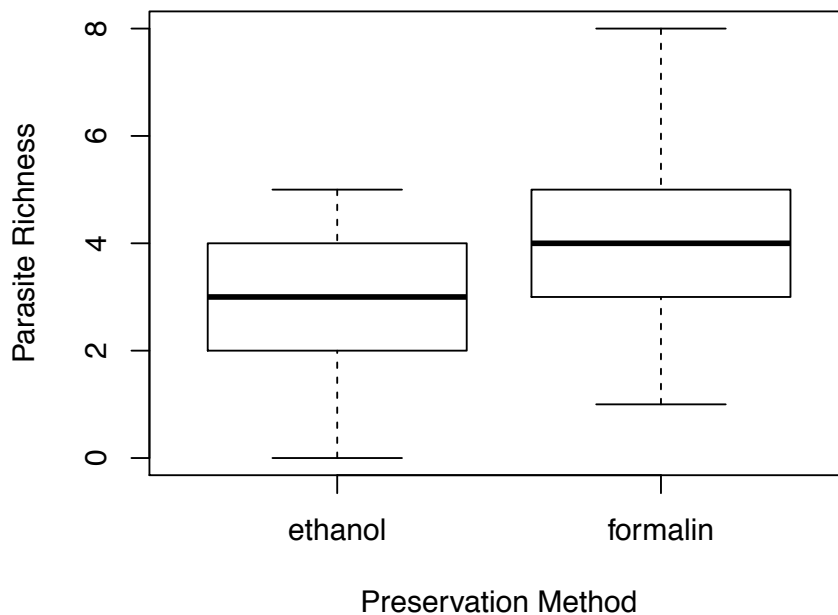
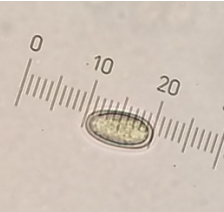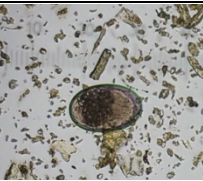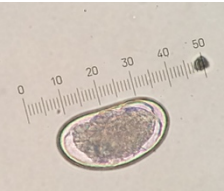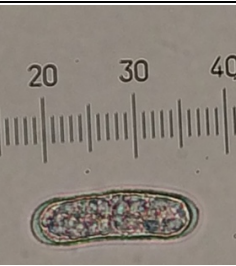FIGURE 2.7 Ethanol-stored samples exhibited less parasite richness than formalin-stored samples.

Molecular assessment of tapir nematodes
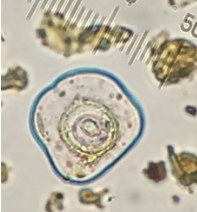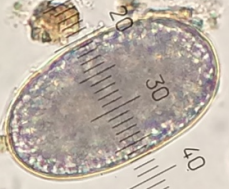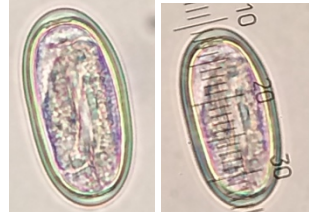
DNA was successfully lysed, amplified and sequenced from 4 individual nematodes all of morphology D (Table 2.1). The results of a BLAST search suggest that none of these nematodes are parasitic, but rather are within 3 genera of free-living, soil-dwelling worms (Table 2.3). The 13 different egg types found in the tapir fecal samples were not of similar size to any parasite eggs from the horse and cow fecal samples (Table 2.4).

TABLE 2.3 BLAST search results showed that the four sequenced nematodes that were classified as morphology D represent 3 genera of free-living, soil inhabiting nematodes.

Worm ID (from tapir fecal)	Most similar to GenBank Accession #	BLAST result	Similarity (%)
T34_D06	EU880004.1	*Labronemella ruttneri*	608/614 (99)
T34_D07	AY284825.1	*Oxydirus oxycephalus*	1046/1048 (99)
T39_D23	EU880034.1	*Tylencholaimus spp.*	1083/1083 (100)
T39_D26	EU880004.1	*Labronemella ruttneri*	612/617 (99)

TABLE 2.4 Thirteen different egg types were found in the tapir fecal samples. Each line on the scale of the 100X magnification photos is equal to 10 μm, and 2.5 μm on the 100X magnification photos.

Photo and magnification	Egg size (μm)	No. (and %) of samples present in
a. 400X	25 x 12.5	2 (5)
b. 100X	130 X 80	4 (10)
c. 100X	185 X 125	5 (12)
d. 400X	40 x 20	7 (17)
e. 400X	42.5 x 80	3 (7)
f. 400X	45 x 12.5	8 (19)

g. 400X	47.5 x 20	2 (5)
h. 400X	50 x 22.5	13 (31)
i. 400X	40 x 12.5	5 (12)
j. 400X	62.5 x 25	3 (7)
k. 100X	35 x 35	1 (2)
l. 400X	72.5 x 42.5	4 (10)
m. 400X	57.5 x 30	8 (19)

Morphological comparison of tapir and domestic animal parasites

There were 3 worms found out of 16 horse fecal samples that were unidentifiable due to condition, and no worms found in the 29 cattle fecal samples. In horse samples, parasite eggs were predominantly those of large and small strongyles (75%, 1201 out of 1611 total eggs), which are a group of parasitic nematodes that commonly infect ungulates. Tapir worm morphologies E and F have physical similarities similar to those of equine strongyle worms (smooth or ridged cuticle along the body, whip-like tail). Eighteen of the 42 tapir fecal samples (43%) contained morphology E or F worms, and 8 (44%) of those 18 samples were collected from GJ, which is the only site with horses.

Three egg types were identifiable to species: *Fasciola hepatica, Strongyloides westeri*, and *Trichostrongylus axei*. The most common parasite eggs in cattle fecal samples were *Eimeria spp.* (68%, 999 out of 1472 total eggs). Two parasite eggs were identifiable to species in the cattle samples, *Trichuris ovis* and *Moniezia benedeni*. The parasite eggs of which the species was unknown were identified to the lowest taxonomic group possible and are listed in Tables 2.5 (equine parasites) and 2.6 (bovine parasites).

TABLE 2.5 Examples of parasite eggs found in equine fecal samples. All photos were taken at 400X magnification. Each line on the photo scales represents 2.5 μm.
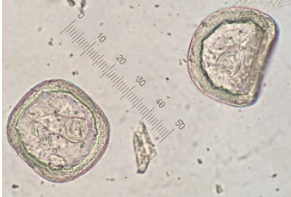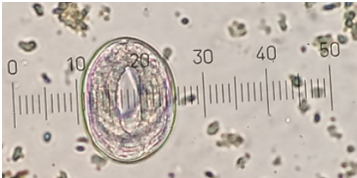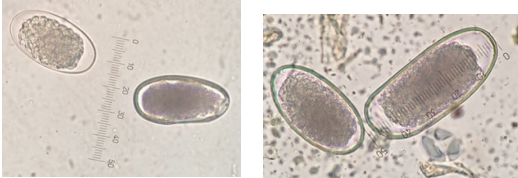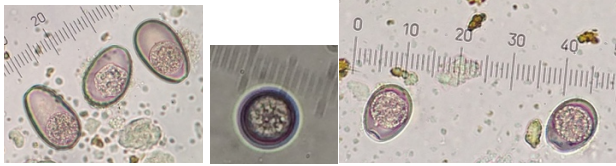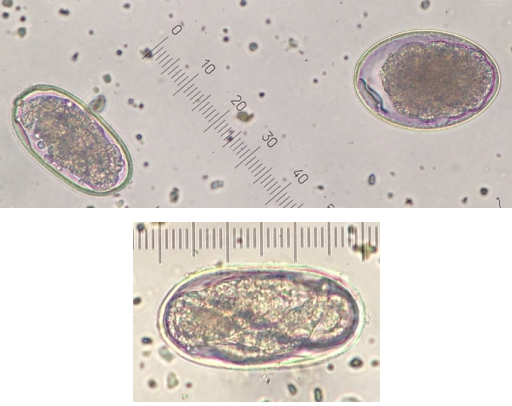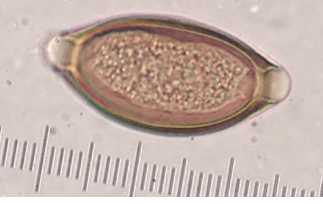
Taxonomic group (common name)	Photo example(s)	No. (and %) of samples present in
Anoplocephala sp. (Tapeworm)		6 (38)
Fasciola hepatica (Liver fluke)		4 (25)
Strongyloides westeri (Equine threadworm)		1 (6)
Superfamily Strongyloidea (Large & small strongyles)		11 (69)
Trichostrongylus axei		3 (19)

TABLE 2.6 Examples of parasite eggs found in bovine fecal samples. All photos were taken at 400X magnification. Each line on the photo scales represents 2.5 μm.

Taxonomic group (common name)	Photo example(s)	No. (and %) of samples present in
Bunostomum sp. (Hookworm)		6 (21)
Eimeria spp. (Coccidia)		21 (72)
Moniezia benedeni (Tapeworm)		2 (7)
Superfamily Trichostrongyloidea (Includes: barber's pole worms, brown stomach worms, hair worms, thin-necked intestinal worm, cattle bankrupt worm and lungworm)		11 (38)
Trichuris ovis (Whipworm)		1 (3)

Discussion

Parasite load, body condition & human activity

Throughout an animal's life, there are situations where periods of stress lasting months can be expected, and an associated decrease in body condition (Barboza *et al.* 2004) and increase in parasite load has been observed (Debeffe at al. 2016). For example, during the rutting season, body condition of male reindeer (*Rangifer tarandus*) declines (Baboza *et al.* 2004) and territorial male Alpine chamois (*Rupicapra rupicapra*) sustain heightened stress hormone levels and experience an increase in counts of parasite larvae present in feces (Corlatti *et al.* 2012). Similarly, spikes in stress and reproductive hormones in pregnant springbok (*Antidorcas marsupialis*) and zebra (*Equus quagga*) correlate with immunosuppression and increased susceptibility to gastrointestinal helminth infections (Cizauskas *et al.* 2015). Human activity may also be stressful to animals.

In my study, tapirs in areas with higher levels of human activity appear to be in poorer body condition. There are three possible explanations to explain this negative correlation between human activity and body condition. First, human activity may be stressful to tapirs and cause behavioral or physiological changes that cause chronic decline in condition. Second, human activity levels may indicate the degree of habitat alteration, and poor condition is the direct result of reductions in the quantity or quality of forage for tapirs where humans occur frequently. Third, human activity may not have a causal affect on tapir condition at all. Instead it may be that individual tapirs who are already in poor condition, due to advanced age or subordinate social status for example, are excluded by other tapirs from better habitats were humans occur less commonly. Thus tapirs in poor condition may simply have no other choice but to associate with humans.

The lack of an effect of human activity on tapir parasite load and of parasite load on body condition could be attributable to limitations in the study design. Several fecal samples were collected from within 100-200 meters of one another (Fig. 2.1). This resulted in clusters of fecal samples being nearest to the same camera station, therefore those samples were assigned the same value for frequency of human activity. Body condition was assessed from photos taken from 2013-2016 but parasite load was estimated from the nearest 2017 fecal sample. Thus, the parasite count may not actually represent the health of the same individual animal whose condition had been assessed in previous years. Lastly, the few accounts of Baird's tapir home range vary from $0.67km^2$ to $23.9km^2$ (Foerster & Vaughan 2002, Naranjo 2002; Reyna-Hurtado *et al.* 2016), which suggests that the a fecal sample collected near a high human activity station could be from the same tapir as a sample collected near a low human activity station.

A main assumption of my analyses in this chapter has been that increasing fecal parasite load corresponds with decreasing individual health as has been demonstrated in previous studies of wild ungulates (Beldomenico & Begon 2016, Coulson *et al.* 2018, Jolles *et al.* 2015). However, the ecological relationship between parasite and host is a complex one and the presence of parasites does not always represent danger to the carrier. Tapirs could act as an intermediate host by moving the parasites nearer to their target host or harboring parasitic worms until they have undergone a life stage change (Chubb *et al.* 2010, Leung & Koprivnikar 2016). Additionally, parasitic infection can be beneficial to tapirs if the parasites enhance the tapir fitness (Fellous & Salvaudon 2009, Weinersmith & Earley 2016). Alternatively, the shedding of worms in the feces could indicate that the tapir immune system is resisting parasitic infection (Fellous & Salvaudon 2009, Maizels & McSorley 2016).

Though counting parasite load is a commonly used method for assessing gastrointestinal helminth infections (Cain *et al.* 2018, Coulson *et al.* 2018, Debeffe *et al.* 2016), it does not allow for a reliable estimate of how many worms are infecting an animal as a single nematode worm can shed thousands of eggs in a day (Gadberry *et al.* 2011). Fecal parasite load also does not allow for assessment of helminths infecting other internal organ systems that could be detrimental to an individual's health and potentially impact body condition. Another concern is that the total estimate of parasite burden probably includes counts of non-parasitic nematodes (see next section Parasites of tapirs), and therefore would not necessarily reflect the degree of parasitic damage to the tapir host. Nevertheless, the literature suggests that parasitic effect on host condition is not always apparent. Cain *et al.* (2018) found no correlation between body condition and fecal parasite load in two horse populations (one domestic, one feral) in Louisiana.

Although my data set is inadequate for explaining the cause of poor condition in tapirs that live around high levels of human activity, it does raise management concerns about human disturbance in protected areas and points to a priority topic of future research.

Parasites of tapirs

There are many practical challenges to studying the parasitology of wild animals, especially little-studied tropical species. Although previous researchers have described the endoparasite community of Baird's tapir, they have relied on dissections of the gastrointestinal tract to obtain adult helminths (Güiris-Andrade *et al.* 2018) or have collected fecal samples totaling less than half of what was used in the current study (Cruz *et al.* 2006, Romero-Castañón 2008). I was able exclude the presence of parasites from domestic livestock based on known morphologies, and also identified six putative parasites based on morphology. Tapir worm

morphologies E and F appear similar to equine pin worms (*Oxyuris equi*) and also to larval stage large (*Strongylus spp.*, *Triodontophorus spp.*) and small (*Trichonema spp.*) strongyles. While no eggs within tapir fecal samples closely resemble the size or appearance of equine pin worm eggs, egg types 'e' and 'l' (Table 2.4) appear similar to and are within the appropriate egg size ranges (Foreyt 2013) typical of large and small strongyles.

The DNA sequences of four individuals of one morpho-species (type D) demonstrated the inadequacy of this morphological method for discerning separate worm species and for identifying which are parasitic. A BLAST search yielded sequence similarities suggesting that these four worms belong to three different genera of free-living, soil-dwelling nematodes, rather than a single parasite of the tapir gastrointestinal tract. This observation suggests that the five other morphologies identified in tapir fecal samples probably represent more than five nematode genera, although it is still possible that the remaining worms identified as separate morphologies are just different life stages of a single nematode species. To resolve this issue the remaining morphotypes will need to be sequenced and identified by their nucleotide sequences. Additionally, greater efforts to link whole intestinal "worms" to their egg and larvae types that appear in fresh feces would allow researchers to know whether fecal parasite counts are indicative of the types and biomass of parasites that are causing damage to the host.

Preservation comparison

Preservation of fecal samples is usually necessary in the study of parasitology because the locations where fecal samples can be collected from wild animals are rarely nearby the laboratory supplies and equipment needed to quantify parasite numbers. Preservation methods will vary depending on whether it is necessary to maintain parasite morphology, inactivate

potential pathogens, or allow for subsequent extraction of DNA that can be amplified by PCR. Ten percent formalin has been demonstrated as the most efficient form of storage for parasite eggs (Hu *et al.* 2016, Huber 1998, Foreyt 1986), as was supported by the present study. However, formalin is not suitable for downstream use of DNA in PCR and nucleotide sequencing, thus ethanol would be considered a better preservative for this purpose (Hale *et al.* 2015). In my samples, 10% formalin maintained the external structure of worms quite well, but no internal structures remained fully intact and those that were partially visible were not enough to taxonomically identify the nematodes. The structure of the buccal cavity, length of the esophagus, shape and number of intestinal cells, presence/absence of a tail sheath, and the location and appearance of genital openings are among the features important for taxonomic identification of parasitic nematodes (Anderson *et al.* 2009, Foreyt 2013). For this reason, 70% ethanol is used by some researchers to preserve feces (Huber 1998). While the 95% ethanol used in the current study is better suited to DNA preservation (Hale *et al.* 2015, Foreyt 1986), its desiccating quality resulted in most worms having a shriveled appearance, distorting any internal structures that may have been present, again making them unsuitable for effective identification by the morphological features on taxonomic identification keys.

There were also differences in where worms were found in formalin vs. ethanol-stored samples. In formalin samples, morphs D, E, and F (all clear worms) floated and were counted during egg counts. However, in ethanol samples morphs D, E, and F were all found in the sediment. This made locating and accurately counting them more difficult. Almost no eggs were found in ethanol sample floats. However, considering that ethanol preserved nematodes sank to the sediment, it seems likely that parasite eggs could have sank as well. Unfortunately, at

35X, the maximum magnification of the dissection scope was not nearly high enough to make parasite eggs visible in the sediment.

Differential performance of ethanol and formalin as fecal sample preservatives has been previously reported (Hu *et al.* 2016, Nielson *et al.* 2010). Because of the differences in parasite loads recorded in this study, I strongly suggest that future field studies of wildlife fecal parasite load employ two methods of sample preservation as was done here. If projects are limited to using only one chemical, the best choice will be dependent on the proposed project goals. Formalin supplied far better parasite egg counts, but formalin is not ideal if DNA sequencing is proposed as it is a known PCR inhibitor.

Though more evidence is needed to better understand the relationships between human activity, parasite load, and body condition, it is apparent from my study that, by whatever means, there is a negative relationship between human activity and tapir health. It is worth noting that the sites used in this study are remote compared to the vast stretches of Belize that are more populated, more easily accessible to the general public, and/or more broadly developed for agriculture. While I found no conclusive evidence of parasite cross-transmission between horses and tapirs, it may be more likely in areas with less defined and maintained boundaries between horses and tapirs. To develop a better understanding of the connection between human activity and human-mediated habitat changes to tapir health, conservation efforts would benefit from expanding the focus of the current study to encompass the more populated areas that tapirs may utilize.

CHAPTER III: THESIS CONCLUSION

For species like Baird's tapirs that are confined to a relatively small geographic range and where nature preserves are separated by large tracts of developed land, the effects of human disturbance are especially relevant to conservation. The ongoing development of countries within biodiversity hotspots will inevitably continue to increase the exposure of wildlife to people. Because of the variable impacts of human disturbance on different species and across locations, the effects of the human activity and human-mediated habitat changes observed in this study on wildlife behavior and health need to be considered for Neotropical species of concern to ensure their conservation.

To slow species decline and ideally increase populations, resource management and species recovery plans must be informed by well-designed scientific study. This is a challenge in itself, as we are losing species faster than we can study them, which emphasizes the need to have purposeful study design that addresses questions relating to both the direct and indirect effects of humans and human-associated stimuli on wildlife. However, collecting the appropriate data to answer such questions is not easy and often exposes the species of concern to additional disturbance via research methods. The methods used in my study are noninvasive in that they do not require direct capture and handling of tapirs. However, researcher presence on roads and trails contributes to my measurement of human activity and could impact the frequency at which tapirs use the trails. In Belize, the Baird's tapir's status as a national symbol and the conservation campaigning of the Belize Zoo has promoted its ecological importance to Belizeans

and tourists alike. Future studies of elusive animals like tapirs may benefit from educating

locals, particularly those that work in agriculture or tourism, in the relevance of the research and

then training them to participate in sample collection as they go about their daily routine. This

would reduce the disturbance of additional researchers while also empowering locals by giving

them a vested interest in conservation of the species.

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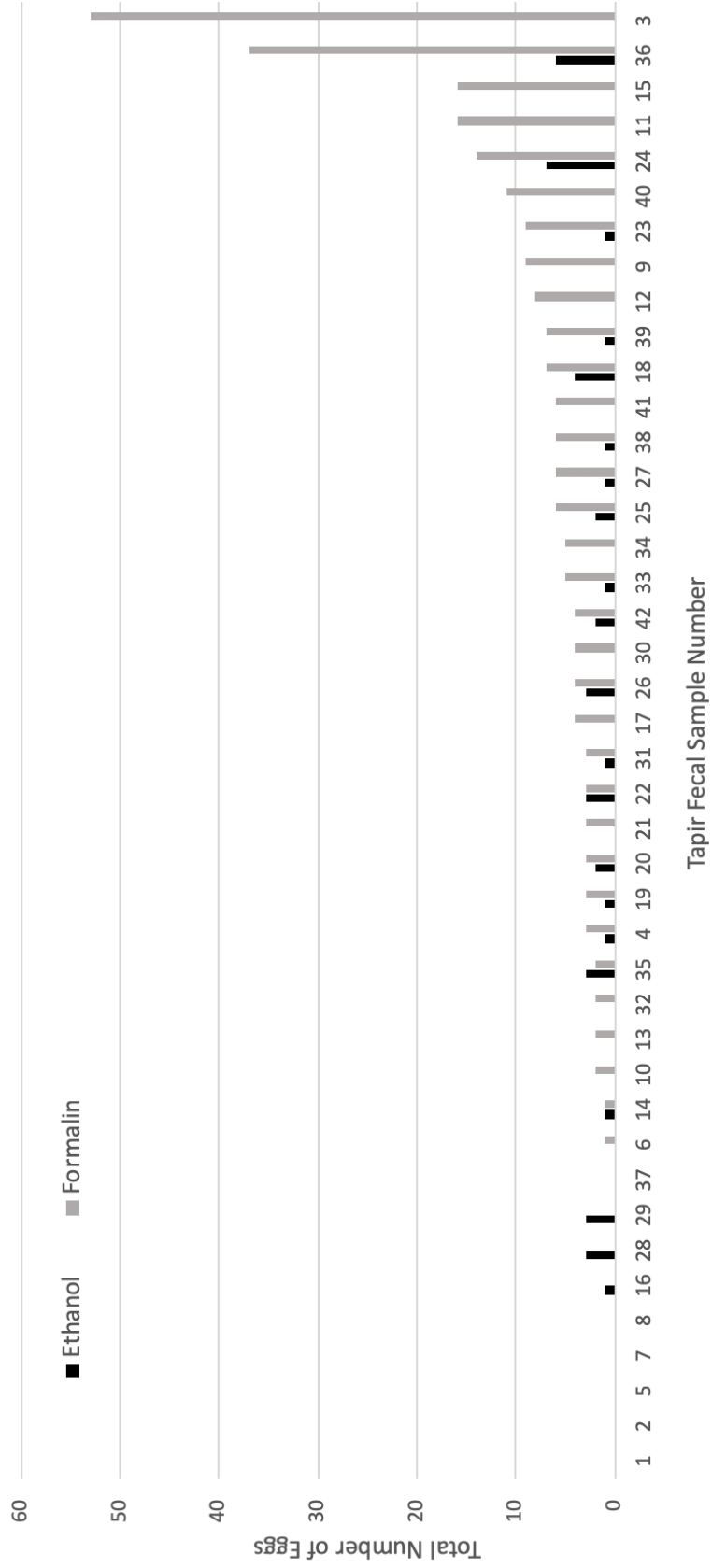
APPENDIX

FIGURE 1 Comparison of total eggs found in formalin versus those in ethanol. Samples are ordered from least to greatest number of eggs in the formalin-stored samples. Of the 36 fecal samples containing eggs, 15 samples were positive for eggs in formalin but not in ethanol.
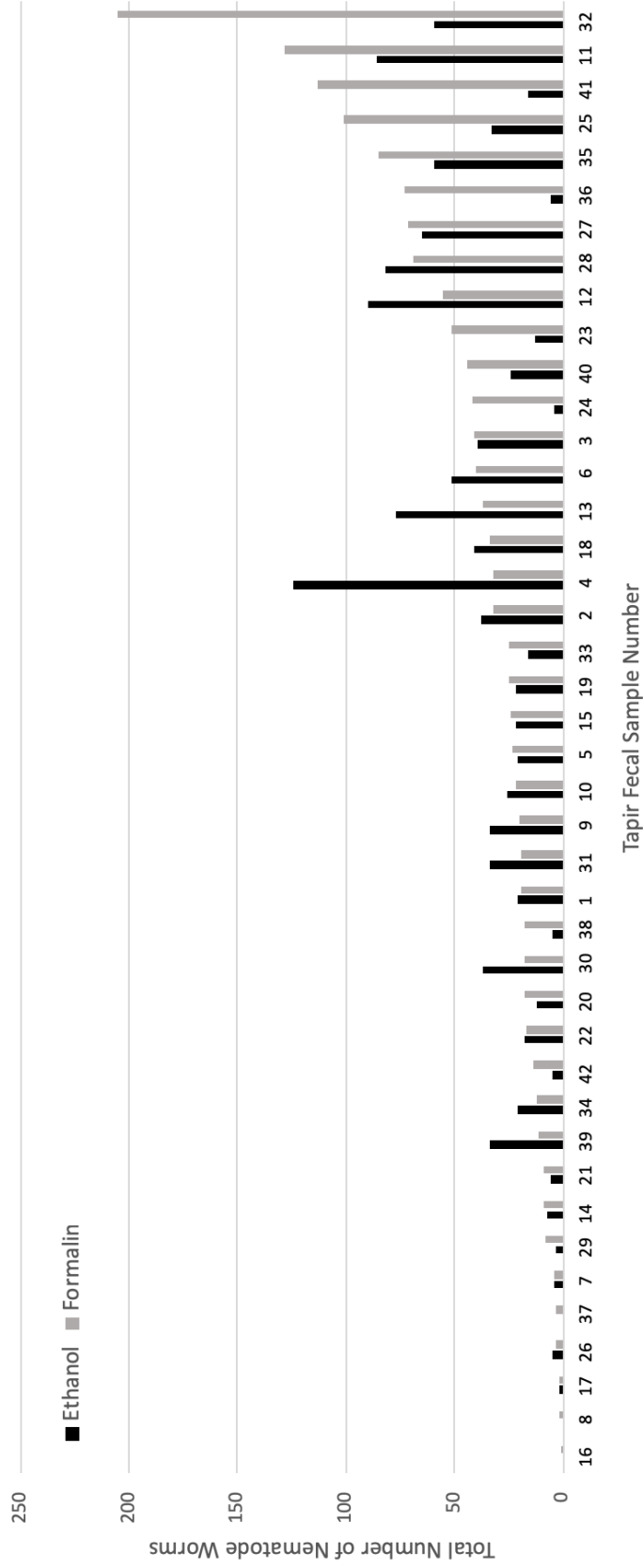
FIGURE 2 Comparison of total number of nematode worms found in formalin versus ethanol. Samples are ordered from least to greatest number of worms in the formalin-stored samples. Contrary to the pattern observed with egg counts, nematode worm counts did not significantly vary between the two preservative chemicals.

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