

An integrative study of marmot responses to ecotourism

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Abstract

Human disturbance in the form of ecotourism alters behavior many species and may impact fitness but few studies take an integrative approach to quantify impact in various ways to test for variation in physiological, behavioral, and consequential life history and demographic traits. We extended a previous study on antipredator behavior for yellow-bellied marmots (*Marmota flaviventris*) by controlling for natural predation and by splitting the data by marmot age group. In addition, we tested the assumption between altered behavior and a decreased fitness by analyzing different physiological traits. Using the same 2009 disturbance data of vehicles, hikers, bicycles, and dogs as the previous study, we analyzed the relationship between the different types of disturbance with the variables of stress, mass gain, summer survival, and annual survival from that same year. We fit models to each age class for each type of disturbance for each variable. We found that, across all variables, juvenile marmots were most sensitive to human disturbance. Behavioral traits were more sensitive to variation in human disturbance than physiological traits. Mass gain rate, a fitness correlate, was relatively impacted by disturbance, but does not impact overall marmot fitness. Despite behavioral and some physiological impacts of human visitation, variation in visitation does not explain marmots' likelihood of surviving the summer or to the next year. This suggests that marmots are compensating for behavioral modifications, the mechanisms of which are not yet known.

Introduction

Ecotourism and nature-based recreation, although commonly supported and encouraged, is not free from creating anthropogenic disturbances and negative consequences on wildlife (Geffroy et al. 2017). The potential of human impact on wildlife must be considered. Human behavior can evoke a predator response from animals (Amo et al. 2006). Motorized vehicles, bicycles, and hikers have the potential to influence animal behavior, physiology, and population dynamics which may lower an animal's fitness (Stabach et al. 2015). Previous work has shown the impact of ecotourism on marmot antipredator behaviors, specifically flight initiation distance and vigilance while foraging (Li et al. 2011). Li et al. found that as disturbance increased, marmots permitted closer approaches from humans and in areas with high levels of bikers and vehicles, marmots foraged less and were more vigilant.

We aimed to build a more comprehensive understanding of human disturbance by quantifying the impacts of anthropogenic disturbance on a series of physiological responses as well as a series of demographically important responses. Thus, we examined the relationship between human visitation to marmot colonies and fecal glucocorticoid metabolites, neutrophil: lymphocyte ratios, mass gain rates, summer survival, and annual survival.

For this study, we used archival data gathered in and around the Rocky Mountain Biological Laboratory in Gothic, Colorado. This species was chosen due to extensive archived data that allows for the statistical testing of different variables. Yellow-bellied marmots are a convenient model species because they live in colonies that have varying levels of anthropogenic

disturbances and inhabit an area (Upper East River Valley, Gothic CO) of well documented human impact.

Fecal glucocorticoid metabolites

The measurement of fecal glucocorticoid metabolites (FGM) is an effective and noninvasive technique to examine the production of stress hormones in individual animals. When an animal perceives an experience as stressful, glucocorticoids are released by the hypothalamic pituitary adrenocortical axis (Smith et al. 2012). FGMs increase when stressors are applied. These glucocorticoids are beneficial and allow an animal to utilize energy to escape or fight off a predator. The response to a stressor allows an animal to reset their physiology to a homeostatic state. (Geffroy et al. 2017). Previous work shows monitoring of FGM levels in wild mammals such as Kenyan white-bearded wildebeest (*Connochaetes taurinus*; Stabach et al. 2015) and western lowland gorillas (*Gorilla gorilla gorilla*; Shutt et al. 2014) to evaluate stress levels due to human impacts. The importance of studying FGM levels in mammals is that increased stress in animals can cause negative behavioral and immunological consequences (Keay et al. 2006). Because energy is limited, energy allocated to the flight or fight response from increased stress inhibits energy devoted for growth or reproduction (Geffroy et al. 2017).

Neutrophil: lymphocyte ratios

An assay of FGMs are effective for determining acute short-term stress. To determine the measure of chronic stress on an animal, neutrophil to lymphocyte (N:L) ratios in blood samples should be examined along with FGMs. High levels of FGM cause neutrophil rates to be higher in relation to lymphocytes (Swan and Hickman 2014). Elevated N:L ratios in laboratory rats (Swan and Hickman 2014) and laboratory mice (Hickman 2017) are associated with chronic stress. We therefore examined blood slides from individual marmot samples to count N:L ratios as a metric of chronic stress.

Rate of mass gain between June and August

Human impacts cause disturbances in marmot foraging behavior (Li et. al. 2011) which could be linked to a decrease in rate of mass gain. Previous work has shown that Amazonian hoatzin (*Opisthocomus hoazin*) chicks have a lower body mass in areas of ecotourism (Mullner 2003) and common wall lizards in areas of high ecotourism during the summer have a lower body mass than individuals in areas of low tourism (Amo et al 2006). Marmots must forage efficiently to gain mass during their relatively short active season so as to increase their chance of surviving a 7-month hibernation (Armitage 2014). Adult marmots with larger body masses going into hibernation have lower mortality rates (Ozgul et al. 2010). We therefore focused on mass gain rate because those animals that gain mass quickly should be in better condition and thus more likely to survive winter. Thus, a positive correlation between lower rates of mass gain and increased human activity could indicate the harmful impact that humans contribute towards marmot fitness.

Survival

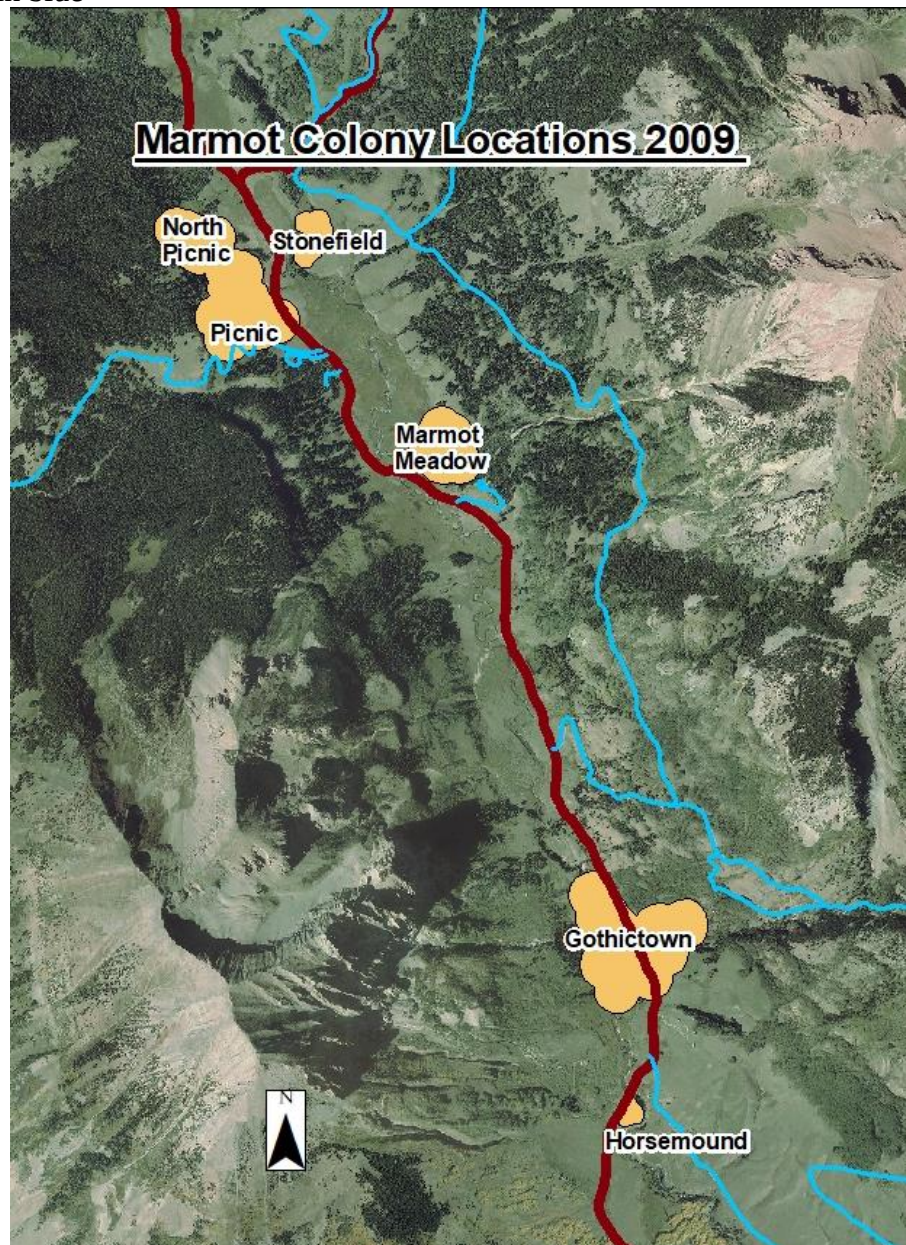
Ecotourism can directly interfere with antipredator behaviors and thus increase prey vulnerability to predators (Li et al. 2011, Geffroy et al. 2015). Thus, the sum total of human disturbance could conceivably influence summer survival. Annual survival may also be reduced if ecotourism's immediate effects have consequences. If there are no relationships between variation in

disturbance and survival, this suggests that marmots are able to compensate for disturbance-driven physiological or behavioral changes.

Quantifying impact

In this study, human impact is quantified through counting the number of vehicles, bikers, pedestrians, and dogs within a certain distance of marmot colonies. The counts of the types of human disturbances represents the impact of ecotourism. All were done at the height of the tourist season (July) and this represent maximum impacts.

Figure 1: Map of six marmot colonies studied in orange, Gothic country road in Red, trails and small roads in blue



Methods

A. Data Collection

Human disturbance data collection (Li et al. 2011 methods)

Human disturbance data was collected from 7:00 to 18:00 every other day for 12 days from late June to mid-July in the East River Valley. The marmot colony sites used were Gothictown, Marmot Meadow, Horse mound, Picnic, North Picnic, and Stonefield (Figure 1). Any vehicle, pedestrian, dog, or bicycle that passed within 50, 100, 200, and 300m of a marmot colony was recorded. For each colony, each disturbance type was recorded as a number of observations per hour. A rate per hour was calculated and then averaged. Using these data we compared five different way to quantify disturbance.

First, following Li et al., we calculated a weighted estimate of disturbance for 300m. To do so, we split up distances from a certain colony into different meter zones. Zone 1 was the distance of 0-50m from a colony, Zone 2 was 50-100m from a colony, Zone 3 was 100-200m from a colony, and Zone 4 was 200-300m. Each zone count was made into a rate by dividing by the minutes spent observing. Each zone was then divided by its zone number to give it a weight. Closer disturbances have a larger weight than further ones. We added the weighted values and took the mean of that value.

$$\left(\frac{\left(\frac{N_{0-50m}}{\text{mins}} \right)}{1} + \frac{\left(\frac{N_{50-100m}}{\text{mins}} \right)}{2} + \frac{\left(\frac{N_{100-200m}}{\text{mins}} \right)}{3} + \frac{\left(\frac{N_{200-300m}}{\text{mins}} \right)}{4} \right) / 4$$

Where N= the raw counts of disturbance counts and
mins= the amount of time spent counting disturbances at that site in minutes

Second, we calculated an unweighted average for areas within 100 m and 300 m of a colony. We calculated this index by taking the mean of all the counts of each disturbance level for each zone for each colony.

$$\left(\frac{N_{0-50m} + N_{50-100m}}{\text{mins}} \right) \\ \& \\ \left(\frac{N_{0-50m} + N_{50-100m} + N_{100-200m} + N_{200-300m}}{\text{mins}} \right)$$

Third, we rank transformed 100m and 300m into a rank order of disturbance. By doing so we were able to view the disturbances relative to each other, as opposed to them being continuous values. Each of these methods makes different assumptions about how marmots' perceive impacts. By comparing them we were able to gain more insights into how, precisely human activities impact a wild animal.

Predator index

A predator index for each colony was calculated to control for natural predation effects on tested variables. Predators were recorded as being either present or absent (0 or 1) during an observation period. The total number of observation periods during which a predator was detected was divided by the sum of the observation periods for each colony. Using a median split, each colony was assigned a high or low estimate of predator visitation in 2009.

Marmot physiology and survival

To collect data on marmots, individuals were captured in Tomahawk live traps using horse feed as bait. Marmots were placed in handling bags to be safely processed. Individuals were given numbered ear tags known as their unique ID (UID) and marks were applied with black non-toxic dye. Fecal samples were collected from individuals if available and labeled with UID, date, time, location, and furmark. Up to 3mL of blood was collected from individuals via their hind leg and labeled the same. Blood is not collected from juveniles. The mass of each individual was taken after trapping. Near daily observations and our record of capture is used to monitor both summer and annual survival.

Sample processing

Fecal Glucocorticoid metabolites: Fecal samples were processed based on the methods in Smith et. al 2012 to determine levels of the stress hormone.

N:L ratios: Blood collected from individuals was placed onto labeled push slides in a line with capillary tubes and a push slide was used to draw a thin layer of blood over then slide. These push slides were examined under a microscope to count and determine the ratio of neutrophil to lymphocyte.

Antipredator Behavior Data (data and methods from Li et al 2010).

We retested Li et al.'s study of the impact of human disturbance on marmot antipredator behaviors with the only differences being adding a predator index to the analyses model and splitting the age groups.

FID: to determine an animal's flight initiation distance, an observer approached an individual marmot at a constant pace of 0.5m/s and dropped a flag to mark when the marmot fled. A laser rangefinder was used to measure start distance (initial distance from the marmot to observer), flight initiation distance (the distance from the dropped flag to the initial location of the marmot), and distance to burrow (the distance between the initial position of the marmot to the nearest burrow). A clinometer measured the initial incline of where the marmot was originally located at the approach before fleeing.

Time allocated to vigilance and foraging: 2009 observers conducted 2 minute focal observations on foraging marmots from 20 May- 30 Aug between 7:00 and 16:00. Using

established protocols (Li et al. 2011), we estimated the amount of time in sight that marmots allocated to vigilance and foraging.

B. Statistical Analysis

We fit a series of models to study the impact of human visitation on FGMs, N:L ratios, mass gain rate, annual survival, summer survival, FID, foraging, and vigilance as dependent variables. Marmots were split by age group; adult, yearling, or juvenile because of the different constraints and pressures experienced by each age group. The models were fitted for each disturbance index, with each type of disturbance, for each variable.

We had repeated measures of physiological and behavioral traits on individuals, so we fit linear mixed effect models to explain variation in N:L ratios, FGM, FID, time allocated to foraging and time allocated to vigilance after controlling for the fixed effects of sex, predator index and disturbance type and the random effect of marmot identity. In addition, for FID, we also included starting distance, incline of the marmot's initial surface, and the distance from marmot to burrow because these have been shown to explain additional variation in FID (Li et al. 2011).

We had a single measurement for mass gain, so we fitted a general linear model with the following independent variables: sex, predator index, and disturbance type.

Survival is binary, therefore we fitted logistic regressions to explain variation in summer and annual survival. The fixed effects for summer survival included sex, predator index, and type of disturbance. The fixed effects for annual survival also included August body mass because of its importance in explaining overwinter survival (Ozgul et al. 2010).

Results

Across all models for each 5 disturbance indexes, we found that juveniles were the most significantly impacted by the different disturbances (Figure 2). Any way examined, we also found that variation in neither summer nor annual survival was explained by any of our disturbance metrics. Finally, when we looked at the results of the different metrics of disturbance, we found that unranked, unweighted disturbances within 300m led to the most statistically significant relationships. For the remainder of the paper we focus on these responses. Additionally, because the associations between light, heavy, and total vehicles and our dependent variables were similar, we focus on total vehicles, bicycles, pedestrians, and dogs.

Figure 2: Total analyses with 5 disturbance indexes (final version will have clearer image). Red cells signify an insignificant ($p > 0.1$) relationship between disturbance and dependent variable after controlling for other fixed and random effects. Green cells signify a significant ($p < 0.05$) relationship. Yellow signifies ($0.05 < p < 0.1$). The plus or minus signifies the direction of the effect on the dependent variable.

2009		CORT	NLR	FID	FORAGE	VIGILANCE	MASS GAIN	ANN SURVIVAL	SUMM SURVIVAL
Weighted	A	Vehicles				+			
		Bicycles		-	-	+			
		Pedestrians			-		+		
		Dogs			-				
	V	Vehicles			-				
		Bicycles			-				
		Pedestrians							
		Dogs	+		-				
	J	Vehicles	+		-	+	+		
		Bicycles	-		-	+	+		
		Pedestrians		-	-	+			
		Dogs	-		-		-		
100 M	A	Vehicles			-	+			
		Bicycles			-				
		Pedestrians			-		+		
		Dogs			-				
	V	Vehicles			-				
		Bicycles			-				
		Pedestrians	+						
		Dogs	+	-	-				
	J	Vehicles	+		-	+	+		
		Bicycles	-		-	+	-		
		Pedestrians		-	-	+			
		Dogs	-		+	-	-		
100 M	A	Vehicles		-	-	+			
		Bicycles		-	-				
		Pedestrians			-		+		
		Dogs			-				
	V	Vehicles			-				
		Bicycles			-				
		Pedestrians		+					
		Dogs	+	-	-				
	J	Vehicles	+		-	+	+		
		Bicycles	-		-	+	-		
		Pedestrians		-	-	+			
		Dogs	-		+	-	-		
100 M	A	Vehicles		-	-	+			
		Bicycles		-	-				
		Pedestrians			-		+		
		Dogs			-				
	V	Vehicles			-				
		Bicycles			-				
		Pedestrians		+					
		Dogs	+	-	-				
	J	Vehicles	+		-	+	+		
		Bicycles	-		-	+	-		
		Pedestrians		-	-	+			
		Dogs	-		+	-	-		
100 M	A	Vehicles		-	-	+			
		Bicycles		-	-				
		Pedestrians			-		+		
		Dogs			-				
	V	Vehicles			-				
		Bicycles			-				
		Pedestrians		+					
		Dogs	+	-	-				
	J	Vehicles	+		-	+	+		
		Bicycles	-		-	+	-		
		Pedestrians		-	-	+			
		Dogs	-		+	-	-		
100 M	A	Vehicles		-	-	+			
		Bicycles		-	-				
		Pedestrians			-		+		
		Dogs			-				
	V	Vehicles			-				
		Bicycles			-				
		Pedestrians		+					
		Dogs	+	-	-				
	J	Vehicles	+		-	+	+		
		Bicycles	-		-	+	-		
		Pedestrians		-	-	+			
		Dogs	-		+	-	-		

Figure 3: Analysis of unweighted disturbances within 300m of a marmot colony . Colors and pluses and minuses as in Figure 2.

2009		CORT	NLR	FID	FORAGE	VIGILANCE	MASS GAIN	ANN SURVIVAL	SUMM SURVIVAL
300 M	Vehicles				-	+			
	Bicycles				+	-			
	Pedestrians				-		+		
	A Dogs				+	-			
	Vehicles				-				
	Bicycles				+				
	Pedestrians		+						
	Y Dogs	+	-		+				
	Vehicles	+		-	-	+	+		
	Bicycles	-			-	-	-		
	Pedestrians			-	-	+			
	J Dogs	-		+	+	-	-		
	Vehicles								
	Bicycles								
	Pedestrians								
	Dogs								

Physiology

FGMs: Variation in human disturbance was mostly significantly associated with juvenile and to some extent yearling FGM levels (Figure 3). An increase in dogs is positively correlated with a rise in yearling FGMs. Juvenile FGM levels are most impacted by disturbances. Bicycles and dogs are associated with reduced FGM levels while vehicles are associated with higher levels.

N:L ratio: We had no blood samples for juveniles, but for yearlings, more pedestrians are associated with higher N:L ratios (Figure 3).

Behavior

FID: Juveniles tolerated a closer approach before fleeing as vehicles and pedestrians increased. They also increased their FID as dog visitation increased (Figure 3).

Time allocated to foraging: Foraging was modified by all impacts (Figure 3). Adults, yearlings, and juveniles decreased their foraging time in the presence of vehicles and pedestrians (although the variation was not significant for the impact of pedestrians on yearling foraging). Adults, yearlings, and juveniles increase their foraging time with increased bicycles and dogs.

Time allocated to vigilance: Adults and juveniles increased vigilance as vehicles increased, and decreased vigilance as bicycles and dogs increased (Figure 3). Juveniles increased vigilance as pedestrians increased.

Population Dynamics

Mass gain: Adults' rate of mass gain is increased as pedestrian visitation increased (Figure 3). Juvenile rate of mass gain is increased in areas with greater vehicles and decreased in areas with bicycles and dogs

Survival: Any way examined, our measures of human disturbance had no significant effect on summer or annual survival (Figure 3).

Discussion

This is one of few studies of human impacts on wildlife to adopt an integrative view of human impacts that includes physiological, behavioral, and demographically important variables. We clearly see that different age animals are differentially impacted by human visitation and that

juvenile marmots are most sensitive to a variety of human disturbances. The effects of dogs are worthy of discussion. Relatively few dogs are seen at our study sites. The sample size for the disturbance impact number of dogs was relatively small, so results for that specific impact may be suspect. However, dogs can have a profound effect on the presence of wildlife in an area (Banks and Bryant 2007) so it's also possible that the effect is real. Marmots may view dogs as predators and execute antipredator mechanisms. The lack of significant relationships between potential impacts and our dependent variables for adults and yearlings may suggest that the older animals may habituate, move off, or die by the time they are adults.

Interestingly, many impacts that are found to alter behavior traits across most levels do not have a major impact on the physiological or more consequential traits. This possibly implies that although behaviors are being altered, marmots are not paying a large cost in terms of stress, rate of mass gain, or survival. These results are consistent with other disturbance studies on marmots. For instance, Olympic marmots (*Marmota olympus*) in low impact and high impact sites both have similar survival and reproductive rates (Griffen et al. 2007). Alpine marmots choose to live in areas of low human disturbance (Rodrigue et al. 1994) which suggests that disturbance is significant enough to change a suitable habitat for a marmot. Our study further supports the concept of marmots adjusting their behavior to compensate for potential negative consequences. Ecotourism impacts are not always detrimental to an animal (Knapp et al. 2013). These results, if consistent across time, could have implications for land management. Vehicular traffic is a stressor of marmots and modifies their behavior relatively more so than do pedestrians. Thus, if one is concerned about reducing the impact on marmots and other animals, this study could be used to prove the sustainable and low-impact of some disturbances and the possible negative influences of others. The implementation or removal of regulations for specific types of impacts could be utilized following these results.

Most importantly, this study proves that ecotourism is not negatively impacting fitness for marmots despite significant change in behavior for animals in the East River Valley for 2009. This study should be expanded to the range between 2010 and the present to find trends and to further support findings and commonalities.

References

- Amo L, Lopez P, Martin J. 2006. Nature based tourism as a form of predation risk affects body condition and health state of *Podarcis muralis* lizards. *Biol Conserv.* 131:402-409
- Andrews C, Viviani J, Egan E, Bedford T, Brilot B, Nettle D, Bateson M. 2015. Early life adversity increases information gathering in European starlings. *Anim Behav.* 109:123-132
- Banks PB, Bryant JV. 2007. Four-legged friend or foe? Dog walking displaces native birds from natural areas. *Biol Letters.* 3:611-613
- Geffroy B, Sadoul B, Ellenburg U. 2017. Physiological and Behavioral Consequences of Human Visitation. In: Blumstein DT, Samia DSM, Geffroy B, Bessa E, editors. *Ecotourism's Promise and Peril: A Biological Evaluation*. Cham, Switzerland: Springer. p. 10-22
- Griffin SC, Valois T, Taper ML, Mills LS. 2007. Effects of Tourists on Behavior and Demography of Olympic Marmots. *Conserv Bio.* 21:1070-1081
- Hickman DL. 2017. Evaluation of the neutrophil:lymphocyte ratio as an indicator of chronic distress in the laboratory mouse. *Lab Anim.* 46:303-307
- Keay JM, Singh J, Gaunt MC, Kaur T. 2006. Fecal glucocorticoids and their metabolites as indicators of stress in various mammalian species. *J Zoo Wildlife Med.* 37:234-244
- Knapp CR, Hines KN, Zachariah TT, Perez-Heydrich C, Iverson JB, Buckner SD, Halach SC, Lattin CR, Romero LM. 2013. Physiological effects of tourism and associated food provisioning in an endangered iguana. *Conserv Physiol.* 1:1
- Li C, Monclus R, Maul TL, Jiang Z, Blumstein DT. 2010. Quantifying human disturbance on antipredator behavior and flush initiation distance in yellow-bellied marmots. *Appl Anim Behav Sci.* 129:146-152
- Mullner A, Linsenmair KE, Wikelski M. 2003. Exposure to ecotourims reduces survival and affects stress response in hoatzin chicks. *Bio Cons.* 118:549-558
- Ozgul A, Childs DZ, Oli MK, Armitage KB, Blumstein DT, Olson LE, Coulson T,. 2010. Coupled dynamics of body mass and population growth in response to environmental change. *Nature.* 466:482-485
- Rodrigue I, Berre ML, Ramousse R. 1994. Habitat preferences of alpine marmots, *Marmota marmota*. *Canadian Jour Zoo.* 72:2193-2198
- Shutt K, Heistermann M, Kasim A, Todd A, Kalousova B, Profosouva I, Petrzalkova K, Fuh T, Dicky JF, Bopalanzognako JB, Setchell JM. 2014. Effects of habituation, research and ecotourism on faecal glucocorticoid metabolites in wild western lowland gorillas. *Bio Cons.* 172:72-79

Smith JE, Monclus R, Wantuck D, Florant GL, Blumstein DT. 2012 Fecal glucocorticoid metabolites in wild yellow-bellied marmots. *Gen Comp Endocr.* 178:417-426

Stabach JA, Boone RB, Worden JS, Florant G. 2015. Habitat disturbance effects on the physiological stress response in resident Kenyan white-bearded wildebeest (*Connochaetes taurinus*). *Bio Cons.* 182:177-186

Swan MP, Hickman DL, 2014. Evaluation of the neutrophil-lymphocyte ratio as a measure of distress in rats. *Lab Anim.* 43:276-282