

Does Stress Explain Variation in Marmot Parenting Behavior or Pup
Survival?

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Abstract

Raising offspring is one of the most important roles of mothers in many species. We studied maternal care in yellow-bellied marmots (*Marmota flaviventris*) and how it is affected by stress. Stress helps these rodents survive hazardous situations. To study this, we observed the marmots at The Rocky Mountain Biological Laboratory in Gothic, Colorado, USA. We recorded their alarm calling rates, social interaction rates, and vigilant behavior. We measured stress levels by analyzing the fecal glucocorticoid metabolites in feces that were collected from the marmots when they had been trapped. We found significant relationships between stress levels of female reproductive marmots and over-winter pup survival as well as between litter size and over-summer pup survival. Higher glucocorticoid levels, which signify higher stress levels, were indicative of lower over-winter pup survival and lower pup survival into the next year. Larger litter sizes were associated with lower summer survival rates. Stress is an important factor in the ability for mothers to raise their offspring into adulthood, sustaining her lineage.

Introduction

Stress can influence behavior in mammals from rats to humans (Katz, Roth, & Carroll, 1981; Cohen 1980). Stress is the body's response to increased physical or psychological demands. When these demands are increased, the body releases adrenocorticotropin from the anterior pituitary, glucocorticoids from the adrenal cortex, epinephrine from the adrenal medulla, and norepinephrine from the sympathetic nerves (Axelrod & Reisine 1984). Stress can be induced by potentially hazardous situations or by a change in the environment. Stressors include predators, food shortage, anthropogenic disturbances, biological invasions, climate change, social environment, and physical stimuli. Stress levels can also be influenced by year, season, time of day, sex, age, and reproductive state of the animal. Fecal glucocorticoid metabolites can be used to measure stress levels among vertebrates. When an animal perceives that it is in a stressful situation, the animal's hypothalamic pituitary adrenocortical axis is activated. This releases glucocorticoid hormones, which activate the mobilization of energy needed to address the stressful situation (Harris & Carr, 2016; Smith, Monclús, Wantuck, Florant, & Blumstein, 2012). Glucocorticoid deposits in feces indicate that the animal has been stressed (Smith et al. 2012).

It has been shown that maternal care behaviors can affect the survival, fitness, and behavior of offspring (Champagne, Francis, Mar, & Meaney, 2003). Providing maternal care, however, requires the mother to expend energy. How the mothers choose to spend their energy may be influenced by their levels of stress. The object of this study was to determine whether mothers who are more stressed provide better, worse, or similar maternal care to their offspring than mothers who are less stressed.

We focused on the following behaviors in yellow-bellied marmots (*Marmota flaviventris*): the rate of alarm calling, vigilance while foraging, and the rate of social interactions. Yellow-bellied marmots provide a good model organism for this study due to the

evident behavioral changes that they exhibit when exposed to stressful situations and the measurable nature of behaviors that are associated with maternal care (Smith et al. 2012). Prior work has demonstrated that glucocorticoid concentrations modulate the production of alarm calls (Blumstein, Patton, & Saltzman, 2006) and that adult females with pups call more than any other group once young emerge above ground. Alarm calling and behavioral vigilance are used to keep the pups safe from predators and other threats (Blumstein, Steinmetz, Armitage, & Daniel, 1997; Blumstein, Blumstein 2007; Blumstein, Lea, Olson, & Martin, 2010). Therefore, mothers who alarm call more often and are more vigilant are providing better maternal care than those who alarm call less often and are less vigilant. Another potential measure of maternal care is the rate of affiliative social behavior between mother and offspring. Rat studies have shown that affiliative maternal social behavior, specifically grooming, affects the offspring's sensitivity to stress (Liu, Diorio, Tannenbaum, Caldji, Francis, Freedman, Sharma, Pearson, Plotsky, & Meaney, 1997; Champagne et al. 2003). In our study, a higher rate of affiliative maternal social behavior is an indicator of better maternal care.

We predicted that mothers who are more stressed would alarm call more often, be more vigilant while foraging, and exhibit a higher rate of affiliative maternal social behavior than mothers who are less stressed. We would interpret this pattern as indicative of greater maternal care, and we evaluate this by looking at pup survival rates as a function of these behaviors and as a function of glucocorticoid levels. Mothers who are more stressed are probably raising their pups in an environment that is more dangerous. We predicted that a more dangerous environment would increase the mothers' stress levels, prompting behaviors like alarm calling and increased vigilance (Blumstein et al. 1997; Mateo 2007). A dangerous environment should necessitate better maternal care to increase the chances of the pups' survival and fitness.

Methods

Field Observations

To determine the relationships between stress levels and maternal care, we studied the behaviors and social interactions of yellow-bellied marmots in and around the Rocky Mountain Biological Laboratory (RMBL, 38°57'N, 106°59'W) in Gunnison County, Colorado, U.S.A. (Armitage, 2010; Blumstein, 2013; 2016) (*Figure 1*). For these analyses we focus on data collected on females with pups, studied over 11 years (2005-2015).

In order to determine the rate of alarm calling, affiliative maternal social interactions, and vigilant behavior; we observed marmots in the morning (0700-1000 hours) and in the afternoon (1600-1900 hours), when marmots are most active (Armitage 1962). We observed marmots at distances of 20-150 m (Huang, Wey, & Blumstein, 2011). During observation, we noted any alarm calling and social interactions. For alarm calls, we recorded the number of alarm calls in a bout, the individual who was producing the alarm call, their location, and the stimulus of the call. For each social interaction, we recorded the type of interaction, the instigator and the victim, as well as the winner and loser of the interaction (the winner being defined as the marmot that remained in the location where the interaction occurred and the loser being defined as the marmot that left the location where the interaction occurred) (Blumstein et al. 2016).

To measure vigilance, we conducted 2-min focal observations on individual marmots. We used 15-45x spotting scopes and recorded the following behaviors: quadrupedal and bipedal foraging, quadrupedal and bipedal looking (vigilance), locomotion, and out of sight (Blumstein, Runyan, Seymour, Nicodemus, Ozgul, Ransler, Im, Stark, Zugmeyer, & Daniel, 2004). If the individual being watched left the immediate area before two minutes had passed, the focal observations were ended. Focal observations were dictated into microcassette recorders and later scored in JWatcher 1.0 (Blumstein & Daniel, 2007). Observers were required to obtain ≥ 0.95 consistency while practicing with JWatcher before collecting data. Number of other marmots within 10 and 50 m, incline category (0-10°, 10-30°, >30°), and substrate (stones and talus, dirt and short vegetation, or tall vegetation) were recorded with focal observations. All observers were trained to identify each behavior, incline category and substrate with 100% accuracy (Blumstein et al. 2010).

Marmots were regularly live-trapped to collect fecal samples from identified subjects. To trap the marmots, we used Tomahawk traps, which we placed around marmot burrow entrances for a few consecutive days every other week. For every event in which an individual was trapped, we recorded its mass, sex, age, reproductive status (by scoring nipple development as 'present', 'swollen', or 'lactating'; Armitage & Wynne-Edwards, 2002), and collected feces if present. The first time each individual was trapped, we collected a hair sample, marked the individual with ear-tags, and dyed it with a unique fur mark for visual identification from afar (Armitage, 1982; Blumstein et al. 2016).

Following collection, feces were stored on ice in plastic bags, then stored at -20°C within two hours of collection. Steroid hormones, including glucocorticoid metabolites, were extracted from the feces within six months of collection at the University of California Los Angeles (details in Monclús et al. 2011; Smith et al. 2012). Details of sample preparation and glucocorticoid assay are in Appendix A of Blumstein, Patton, and Saltzman (2006). We used only the first fecal sample collected from each individual from each weekly trapping session for fecal glucocorticoid metabolite analysis to avoid detecting elevated fecal glucocorticoid metabolite levels than can be a result of repeated capture (Blumstein et al. 2016). This analysis yielded fecal glucocorticoid level data, which can be extrapolated to stress levels in the marmots (Smith et al. 2012). We used the mean of fecal glucocorticoid measurements of the mother before her pups emerged, the glucocorticoid measurement that was taken soonest after the pups emerged and the annual mean of the glucocorticoid measurements for the mother in our analysis.

When calculating survival rates, a pup is considered to have survived the summer if it remains alive from its emergence until the following September and is seen and marked in that month. A pup is considered to have survived the summer and the following winter if it was seen alive and marked in the following summer. We also isolated winter survival by counting the proportion of pups who survived winter given that they had survived summer. The number of pups that survived in a litter was divided by the total litter size from when they emerged to obtain a pup survival rate.

Predator index was calculated for each colony area by dividing the number of recorded predator sightings during observation of that area by the number of observation periods of that

area. Predators included red fox (*Vulpes vulpes*), coyote (*Canis Latrans*), American badger (*Taxidea taxus*), domestic dog (*Canis lupus familiaris*), and numerous raptors and weasels. A colony area has a “high” predator index if the result of the previous calculation was above the median value for all areas and “low” if it was below. Predator index was used as to account for variations in environment between the colony areas that could lead to variation in stress and glucocorticoid measurements.

We extracted fecal glucocorticoid metabolite, alarm calling, social interaction, vigilance, predator index, and survival data from a database that spans 11 years from 2005 to 2015. For each individual, we identified whether or not she had been observed alarm calling or exhibiting affiliative maternal behavior towards her offspring after her litter had emerged. We also calculated the proportion of time marmots were in sight during focal observations that they spent in a vigilant position (quadrupedal or bipedal looking). Individuals with a higher proportion of the focal observation time spent in a vigilant position are said to be more vigilant.

We then compared these three categories of behavioral data (alarm call rate, affiliative maternal social interaction rate, and vigilance score), independently, to the stress levels of the mothers as indicated by the glucocorticoid levels found in their feces. We fit linear mixed effects models to explain variation in each category of maternal care as a function of the log of fecal glucocorticoid metabolite measures. We tested for an association between stress and pup survival in a similar way. We analyzed significant variables by isolating all dependent variables that were significant at a 95% confidence level in the full linear mixed effect model and testing those variables on their own against the independent variable (Zuur et al. 2009).

Results

Our analysis revealed several negative correlations at a 95% confidence level. First, between the number of offspring that the female reproductive marmot had in a year and over-summer pup survival rate for her pups (*Figure 2*). Also, between the log of all three measures of glucocorticoid levels of the mother (mean before emergence date, soonest measurement after emergence date, and annual mean) and pup survival over summer and winter (year survival) (*Figure 3*). As well as, between the log of all three measures of glucocorticoid levels of the mother and pup survival over winter given summer survival (winter survival) (*Figure 4*) (*Table 1*) (*Table 2*) (*Figure 5*). None of the three behavioral measures that were tested (rate of alarm call bouts, social interaction rate, and vigilance score) were significantly correlated to fecal glucocorticoid levels of the mother.

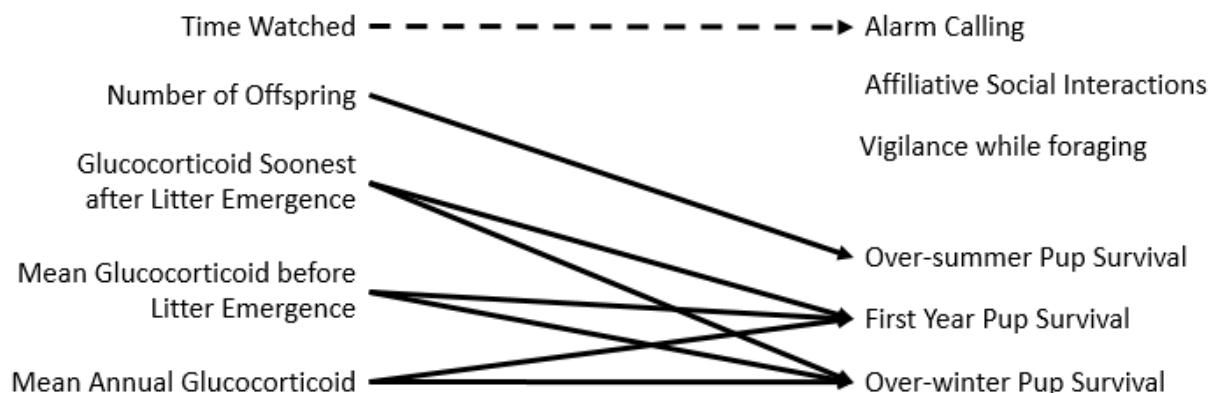


Figure 5. The solid black arrows indicate a negative correlation between the independent variable (on the left) and the dependent variable (on the right). Number of offspring is negatively correlated to the over-summer survival rate of that offspring. All three fecal glucocorticoid measurements are negatively correlated with pup year survival and pup winter survival. The dashed arrow represents a positive correlation between the time that an individual was watched and whether or not it was observed alarm calling. In this case, time watched is merely a nuisance variable.

Discussion

As shown in the results of this study, higher glucocorticoid levels measured in female reproductive marmots are associated with reduced summer and over-winter survival. Young marmots are the least likely group to survive hibernation, which occurs during the winter months. In this study, about 68% of the pups that survived the summer, survived the winter. Other studies have estimated that young marmots have as low as 50% survival rates through winter hibernation (Barash 1989; Lenti Boero 1994; Bryant 1996; Blumstein & Arnold 1998; Schwartz & Armitage 1998; Farand, Allaine, & Coulon 2002). It has been shown that the pre-hibernation mass of the marmot pups is positively correlated to over-winter survival (Armitage 2014). This is also true in the meadow jumping mouse (*Zapus hudsonius*) (Schorr et al.). This study reveals an additional factor to the hibernation survival rate of pups, maternal stress (as indicated by fecal glucocorticoid levels).

Mammal pups can be influenced by social interaction with their mother (Liu 1997). We did not, however, find a significant correlation between fecal glucocorticoid levels and any of the three maternal care behaviors that we measured, which includes social interaction. How, if there are no observable changes in maternal care behaviors, does maternal stress effect the pups? Environmental stressors could be both causing the mother to be stressed and endangering the pups, making maternal stress an indicator, not a causative factor, of pup hibernation survival. These environmental stressors could come in the forms of climate, human interaction, or many other variable aspects of environment. A harsh and cold winter climate, for example, could potentially make it more difficult for young marmots to regulate their body temperature during hibernation. Male meadow jumping mice have lower hibernation survival rates over winters with high snowfall (Schorr et al.). Predator density is not likely to affect hibernation survival,

because the pups are hidden in burrows from predators during this time. We did test for a correlation between pup survival and predator index and found that they were not correlated.

Another possibility is that there is a negative correlation between fecal glucocorticoid levels of the mother and pre-hibernation mass of her pups. It could be that a mother gets stressed when her pups are not getting enough food, possibly because the mother herself is also not getting enough food. This would account for high fecal glucocorticoid levels and smaller pups, who have been shown to have a smaller chance of surviving winter hibernation.

The other significant correlation that we found was that between number of offspring in the litter and pup over-summer survival rates. We found that pups in larger litters have a smaller chance of surviving the summer in the year that they were born. This could be due to the reduced attention that the mother is able to give to each individual pup. It could also have to do with competition for resources between the pups. In accordance with existing literature, we found that litter size does not affect hibernation survival (Armitage & Woods 2003).

These findings may be translatable to other species. Although marmots have a somewhat uncommon yearly cycle of hibernation, the life history decisions that they must make are common to many species. Most mothers in the wild must balance their energy between sustaining themselves and caring for their offspring. The decisions they make, which are often based on their environment, affect the lives of their offspring.

In future studies, it would be productive to study the presence of relationships between fecal glucocorticoid levels of the mother, pup survival, and various environmental factors, as well as between fecal glucocorticoid levels of the mother and pre-hibernation mass of her pups. It may also prove interesting to study the causes of pup deaths over the summer to determine whether or not these deaths could have been influenced by litter size.

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Tables and Figures

Stonefield Trap Sites 7/7/16



Figure 1. Stonefield Colony Area Trap Sites: One of our colony areas with three trap sites on July 7th, 2016

Model	Sample Size	df	AIC
<u>Alarm Call</u>			
glucocorticoid* + number of offspring + time watched + predator index	120	114	89.7
<u>Affiliative Social Interactions</u>			
glucocorticoid* + number of offspring + predator index**			
<u>Vigilance while Foraging</u>			
glucocorticoid* + number of offspring + predator index**			
<u>Over-summer Pup Survival</u>			
glucocorticoid* + number of offspring + predator index	115	110.63	80.7
<u>First Year Pup Survival</u>			
glucocorticoid* + number of offspring + predator index			
mean glucocorticoid before emergence	84	77.96	80.5
glucocorticoid soonest after emergence	115	115	92.2
annual glucocorticoid mean	115	7.749	93.8
<u>Over-winter Pup Survival</u>			
glucocorticoid* + number of offspring + predator index			
mean glucocorticoid before emergence	84	84	103.7
glucocorticoid soonest after emergence	115	115	128.7
annual glucocorticoid mean	115	115	127.9

Table 1. Models and Simplified Models of significant variables with sample size, degrees of freedom, and AIC. *represents all three measures of glucocorticoid (mean before, soonest after, and annual)
 **no significant variables

Independent	Dependent	Estimate (Std. Dev.)	P-Value
Number of Offspring	Over-Summer Survival	-0.03669 (0.01652)	0.0284
Mean Cort Before	Year Survival	-0.37449 (0.14700)	0.0128
Mean Cort Before	Winter Survival	-0.36297 (0.16940)	0.035
Cort After Emergence	Year Survival	-0.57594 (0.12255)	7.29e-6
Cort After Emergence	Winter Survival	-0.58882 (0.14362)	7.74e-5
Annual Cort Mean	Year Survival	-0.61615 (0.13965)	0.00243
Annual Cort Mean	Winter Survival	-0.6710 (0.1590)	4.93e-5

Table 2. The Estimates and P-Values of all correlations in a linear mixed effects model that were found to be significant at a 95% confidence interval. All correlations are negative. "Cort" means fecal glucocorticoid metabolites.

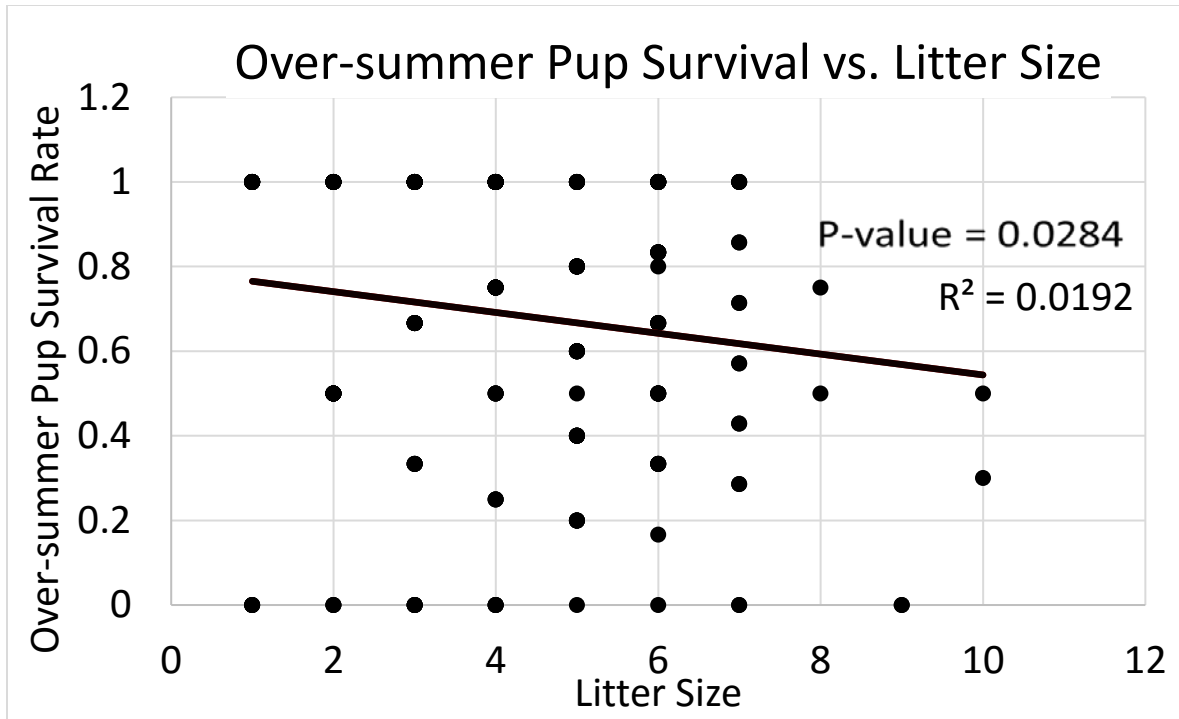


Figure 2. Over-summer Pup Survival as a function of Litter Size: There is a negative correlation between litter size and the proportion of pups from each litter that survived their first summer. The P-value and the R squared value are taken from the linear mixed effects model.

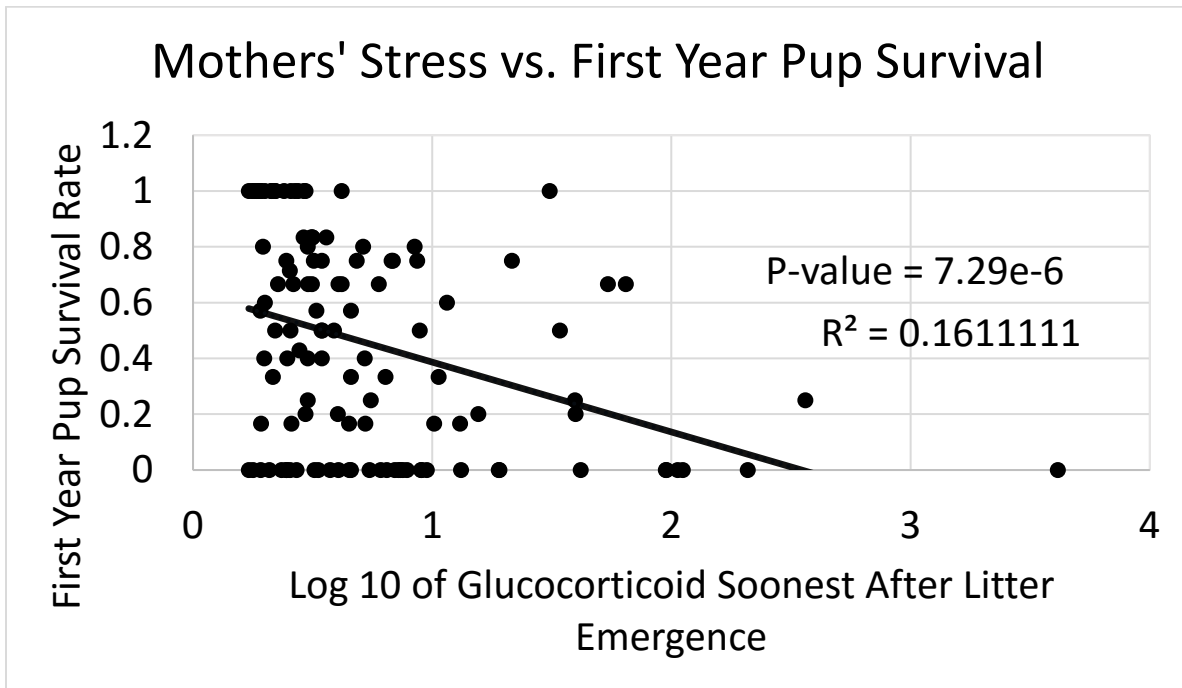


Figure 3. First Year Pup Survival as a function of the log of Mother's Glucocorticoid Measured Soonest after Litter Emergence: There is a negative correlation between the mother's glucocorticoid level and the proportion of pups from each litter that survived their first year. The P-value and the R squared value are taken from the linear mixed effects model.

Figure 4. Over-winter Pup Survival as a function of the log of Mother's Glucocorticoid Measured Soonest after Litter Emergence: There is a negative correlation between the mother's glucocorticoid level and the proportion of pups from each litter that survived their first winter if they survived their first summer.