

**Do Social Interactions Decrease Basal Stress Levels In Breeding
Female Yellow-Bellied Marmots?**

Student: Xochitl Ortiz Ross

Mentor: Daniel T. Blumstein

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Abstract

In order for sociality to exist, the benefits must outweigh the costs. Benefits include better territorial defense, co-operative foraging, and predator avoidance. Costs include increased disease transmission, intraspecific competition for resources or mates, and reproductive suppression. One way to examine the costs and benefits of group living is to assess the impact of social stressors on basal glucocorticoid (GC) levels. In certain species, social interactions such as grooming have been shown to decrease basal GC levels. Since elevated levels of GC have been shown to increase mortality and decrease reproductive rates, decreased stress levels could be a benefit of social living. We investigated whether social interactions decreased stress levels in breeding female yellow-bellied marmots. We found that relationship strength, not the number of relationships, decreased stress levels in females both before and soon after their litter emergence, supporting our hypothesis that decreased basal stress levels can be a benefit of social group living.

Introduction

The evolution of social behavior has been of great interest to biologists for decades (Alexander 1974) and many studies have documented a variety of costs and benefits of social living (Alexander 1974). Some of the benefits of sociality include improved survival rates of adults (in baboons, *Papio hamadryas ursinus*; Silk et al. 2010) and offspring (in yellow-bellied marmots, *Marmota flaviventris*; (Walter 1990), territorial defense (in African lions, *Panthera leo*; Mosser and Packer 2009), ability to engage in co-operative foraging (in coastal otters, *Lontra canadensis*; Blundell et al. 2004), and predator avoidance (in white-nosed coati, *Nasua narica*; Hass and Valenzuela 2002). However, sociality also has costs which include increased risk of disease transmission (in barn swallows, *Hirundo rustica*; Shields and Crook 1987) and intraspecific competition for resources (in African lions, *Panthera leo*; Packer et al. 1990). Furthermore, many of the benefits listed are the result of co-operative behaviors, which come at an additional cost. In fact, co-operating individuals pay a cost for another's benefit (Nowak 2006). For sociality to persist in a population, the benefits must outweigh the costs.

Changes in basal glucocorticoid (GC) levels are often used to assess social and environmental stressors (Wey et al. 2015), and can be used to evaluate the costs and benefits of social living. Stress is the body's reaction to a suboptimal state (von Holst 1998). In the presence of a stressor, the hypothalamic-pituitary-adrenal (HPA) axis produces GC hormones, which diverts energy to cope with the stressor and restore homeostasis (Reeder and Kramer 2005). Elevated GCs are usually associated with acute short-term stressors (von Holst 1998; Smith et al. 2012), but long-term stressors can greatly alter an animal's life history strategies.

Affiliative social behavior has been shown to decrease stress levels across taxa (e.g. grooming behavior in *Macaca sylvanus*; Shutt et al. 2007). Because high stress levels are often associated with higher mortality and lower reproductive rates, a decrease in stress levels through social interactions could be of great benefit to social animals and outweigh some of the costs of social living. We investigated how social interactions affect stress levels in the yellow-bellied marmots of the Rocky Mountain Biological Laboratory, Colorado, USA.

Yellow-bellied marmots have been studied at this site for over 54 years. They are facultatively social animals (Armitage 2014) that live in matriline (female-dominant social groups) of varying sizes. Although sociality has been deemed to arise for group thermoregulation in many species of ground squirrel (Armitage 2014), this is not the case for yellow-bellied marmots. Although these animals live in social groups, they do not appear to behave co-operatively, which begs the question: why live in social groups? We hypothesized that one way these animals are benefiting from social group living is through decreased stress levels.

In this study we focused on breeding females. Previous studies have demonstrated the impact of maternal stress on offspring, especially on rodents (Liu et al. 1997; Bauer et al. 2015). Maternal stress can affect offspring survival and/or their ability to cope with stressors (CITE) and this matters because... A degree of maternal programming has also been shown to last across generations (Bjorklund 2006; Richards 2006) and could, therefore lead to greater evolutionary implications. Can social stressors affect maternal life history trajectories? In this study we hope to highlight the effect of social interactions

on maternal stress levels and further the understanding of how this affects offspring survival and evolutionary trajectories.

Methods

We analyzed data collected from 2005 to 2015 on the yellow-bellied marmots of the Rocky Mountain Biological Laboratory, Gothic, Colorado, USA (38°57'N, 106°59'W). Marmots were observed daily during times of peak activity (0700-1000, and 1600-1900 h) from mid-April to mid-September. We focused on breeding females with confirmed litters, and used data from four geographically different colony areas, each with multiple social groups.

Quantifying stress

We estimated basal stress levels by quantifying fecal glucocorticoid metabolites (FGMs). Marmots were trapped biweekly (details in Blumstein et al. 2016), and in each trapping session we collected all available fecal samples. Samples were immediately stored on ice and frozen at -20C within 2 hours, and FGMs were extracted within 6 months of collection (Blumstein et al. 2005; Smith et al. 2012). Prior work has shown that the majority of individuals show no distress from trapping, but those who did had a significant increase in FGM levels upon recapture the following day (Smith et al. 2012). Thus, to reduce the influence of trapping on FGM levels, we excluded samples collected within 24 hours of a previous capture. Fecal samples were only collected from mid-April until the end of July/beginning of August. Details of sample preparation and glucocorticoid assay are in Appendix A of Blumstein et al. (2006).

To determine whether social interactions increased basal stress level we calculated the FGM levels in breeding females (a) annually (defined here as mid-May to the end of July/beginning of August), (b) before and after a given female's litter emerged, and (c) the date closest to the weaning/litter emergence date.

Group and litter size

Yellow-bellied marmot colonies are geographic areas that contain one or more social groups. These are usually smaller groups of marmots that live in close proximity to each other (usually a matriline) and tend not to interact with neighboring groups

(Armitage 2014). We assigned individuals to groups annually in four geographically discrete colony sites based on space use overlap (Smith JE, Strelloff CC, Blumstein DT, unpublished data). Using Socprog (Whitehead 2009), we calculated the simple ratio index (SRI, Cairns and Schwager 1987) from live-trapping and observation data for each pair of marmots. We then used the random walk algorithm on Map Equation (Rosvall and Bergstrom 2008; Rosvall et al. 2009) to identify the number and identity of the individuals that belonged to a particular social group. The number of individuals in each of these social groups determined group size. Only individuals that had been trapped or seen more than five times that year were included in the group calculations to avoid incorporating transient individuals that were only passing by and were not part of the group. We determined emergence litter size by genotyping individuals and assigning pups to mothers using 12 microsatellite loci and 95% confidence assignments at the trio level using CERVUS 3.0 (Kalinowski et al. 2007). Further details can be found in in Blumstein et al. (2009).

Behavioral observations

Social interactions were quantified using all-occurrence sampling by observing marmots at colony sites during hours of peak activity (0700-1000, and 1600-1900 h) daily during the active season (mid-April to mid-September). Animals were observed through binoculars and x15-45 spotting scopes from a 20-150m distance to avoid altering marmot behavior (Blumstein et al. 2016). Recorded observations included marmot presence, type of social interactions (details in Blumstein et al. 2009), and the initiator, recipient, and winner of each interaction. Between 2005 and 2015 observers spent 60 hours observing and quantifying behavior in the four colonies and observed 22,612 social interactions between adults and yearlings. For this study we focused on female-female affiliative interactions because these are more likely to prove beneficial to individuals. Interactions with pups were not considered since observations of these interactions were few and of a different nature.

Predator index

Predator presence varies by group and may explain substantial variation in stress as has been documented in European rabbits (*Oryctolagus cuniculus*; Monclús et al.

2006), and yellow-bellied marmots (Monclús et al. 2011). Following Monclús et al. (2011), we calculated a predator index by quantifying the proportion of observation sessions where we detected predators. Predators included: red fox (*Vulpes vulpes*), coyote (*Canis latrans*), American badger (*Taxidea taxus*), American black bear (*Ursus americanus*), domestic dog (*Canis lupus familiaris*), and several species of raptors and weasels. We then used a median cut to separate colonies into low and high predator presence.

Social attributes

We calculated association matrices from the set of affiliative social interactions between females. From these association matrices we calculated a set of social attributes that have been shown in previous work to capture various dimensions of marmot social variation (REFS). The social attributes included were degree (in and out), strength (in and out), closeness centrality, eigenvector centrality, betweenness centrality, clustering coefficient, and embeddedness. Degree is the number of unweighted, directed interactions initiated or received by an individual (Wasserman and Faust 1994). Strength is the sum of the weights of affiliative interactions with the focal individual and is therefore calculated using weighted, directed networks (Barrat et al. 2004). Closeness centrality is the reciprocal of the sum of the shortest path lengths between the focal and other individuals (Wasserman and Faust 1994; Wey et al. 2008) and was calculated using unweighted, undirected networks (Fuong et al. 2015). Eigenvector centrality is the eigenvector associated with the maximal eigenvalue of an adjacency matrix (Bonacich 2007) and is also calculated using unweighted, undirected networks (Fuong et al. 2015). Betweenness centrality is based on the number of shortest paths between pairs of individuals in the focal individual's network (Wey et al. 2008). The clustering coefficient reflects the density of the network around the focal individual (Wey et al. 2008). Embeddedness was calculated by identifying the cohesive substructures of the network (Moody and White 2003) and was unweighted and undirected (Fuong et al. 2015). Social attributes were calculated in igraph package 0.7.0 (Csardi and Nepusz 2006) and R 3.3.1 (R Development Core Team 2016).

Because many of these social attributes are correlated (Wey and Blumstein 2012), we used a principal components analysis to reduce these down to three uncorrelated

measures (PC1, PC2, and PC3; extractions based on eigenvalue > 1 with varimax rotation; SPSS 21.0; IBM Corp., Armonk, NY; IBM Corp 2012).

Statistical analysis

To determine whether social interactions increased basal stress levels in breeding female yellow-bellied marmots, we fitted a set of linear mixed effects models. We first $\log_{10}$ transformed our FGM data, which included (1) annual mean, (2) mean before litter emergence, and (3) mean closest to wean date. Our initial models included PC1, PC2, PC3, group size, litter size, and predator index as fixed effects, and unique identity (UID) and year as random effects to account for pseudoreplication and annual variation. Following Zurr et al. (2009), we then simplified these models by removing non-significant variables (i.e., those with $p > 0.05$), refitted the reduced model, and interpreted those results. We evaluated the assumptions of these models by checking the distribution of the data (to check for normality) and plotting qq plots and histograms of the residuals. Following these results we $\log_{10}$ transformed PC2, and group size. We also scaled our continuous fixed effects around a mean of 0 for better comparison of effects (except for our PC factors, which were already thus scaled). All our analyses were carried out in R 3.3.1 (R Development Core Team 2016) packages lme4 1.1-12 (Bates et al. 2014), and lmerTest 2.0-32.

Results

Our final data set included a set of 88 breeding events with social network measurements that came from 53 unique females comprising 37 social group years. Associated with these 88 breeding events were 214 individual FGM samples. Groups averaged 29.5 individuals (± 17.4 ; range 3-34). FGM levels averaged 0.743 ngL (± 0.473 ngL; range 0.18-2.94 ngL). Because we only collected fecal samples mid-May until the beginning of August, most of the FGM samples analyzed were collected prior to the litter emergence date (110 samples before and 66 after).

We interpreted our three principal components (PC1, PC2, and PC3) as (1) popularity, which included degree (in and out), closeness (in and out), and eigenvector centrality; (2) relationship strength, which included strength (in and out); and (3) group

cohesiveness, which included clustering, embeddedness, and betweenness (Table 1). These explained 74.6% of the variation.

After removing non-significant fixed effects from our initial models (Table 2), and accounting for litter size ($p = 0.002$; estimate = 0.172; SE = 0.055), we found that females in stronger affiliative relationships had significantly lower FGM levels before their litters emerged ($p = 0.008$; estimate = -0.313; SE = 0.114). Also, females in stronger relationships had significantly lower FGM levels on the sample taken closest to the weaning date ($p = 0.018$; estimate = -0.251; SE = 0.102). After controlling for litter size ($p < 0.001$; estimate = 0.125; SE = 0.033), we found that females in more cohesive groups had significantly lower annual mean FGM levels ($p = 0.015$; estimate = -0.079; SE = 0.032).

Discussion

Overall, females in stronger social relationships had reduced FGM levels before and after they weaned their litters, and females in more cohesive groups had reduced FGM levels throughout the year.

We also found that females with larger litters have higher FGM levels before wean and throughout the year. Thus, while producing more pups is associated with increased FGM levels – our measure of stress – mothers with stronger, more meaningful, relationships can buffer these effects. Importantly, social isolation, not simply being in a small group, increases stress or, conversely, having meaningful relationships, not being in a large group, reduces stress. In contrast to some previous findings (REFS), our results suggest that marmots do derive benefits from sociality, but these are limited to a tightly knitted network of group mates. Maldonado-Chaparro et al. (2015) demonstrated that yellow-bellied marmots actively seek to increase their social interactions in smaller groups, but appear to avoid them in larger groups, suggesting that individuals may only care to maintain a limited number of social relationships. Our study provides evidence that female breeders do not interact equally with everyone in their social group. Therefore, each individual may have a smaller subset of individuals with whom they interact more often and create stronger bonds; these could be the relationships from which they benefit.

By limiting their interactions with individuals outside of their smaller network (but still included in their group) marmots may be avoiding the costs that would come with larger groups (Maldonado-Chaparro et al. 2015). Therefore, although individuals may live in larger groups, these group sizes may not strongly affect them because they rely more on their smaller social network within that group. However, more analysis must be made to distinguish between the strength and number of interactions to validate this idea.

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Tables and figures

Table 1 - Rotated (varimax rotation) principle component scores from the principle component analysis. Values in bold indicate the attributes that define the principal components PC1, PC2, and PC3.

Social attributes	PC 1 (popularity)	PC 2 (strength)	PC 3 (cohesiveness)
Indegree	0.849	0.181	-0.141
Outdegree	0.837	0.276	0.009
Incloseness	0.865	0.122	-0.128
Outcloseness	0.839	0.197	0.149
Eigenvector centrality	0.848	-0.038	0.116
Instrength	0.213	0.902	0.066
Outstrength	0.202	0.913	0.095
Betweenness centrality	0.188	-0.188	-0.628
Embeddedness	0.214	0.284	0.724
Clustering coefficient	0.020	-0.347	0.798

Table 2. Model selection for fixed effects. Models in bold are the reduced/final models used in the analysis. All models included the random effects of ID and year.

Model	df	AIC
Mean before weaning		
PC1 + PC2 + PC3 + group size + litter size + predator index	57	103.7
PC2 + litter size	61	97.7
Mean closest to weaning date		
PC1 + PC2 + PC3 + group size + litter size + predator index	35	64.8
PC2	40	60.5
Annual mean		
PC1 + PC2 + PC3 + group size + litter size + predator index	78	68.9
PC3 + litter size	82	63.8

Table 3 – Fixed effects used for each final model.

Model	Estimate (SE)	<i>P</i>
Fixed effects		
Mean before		
PC 2 (strength)	-0.149 (0.056)	0.009
Litter size	0.100 (0.030)	0.001
Mean closest to wean		
PC 2 (strength)	-0.149 (0.056)	0.009
Annual mean		
PC 3 (cohesiveness)	-0.783 (0.153)	<0.001
Litter size	0.069 (0.018)	<0.001

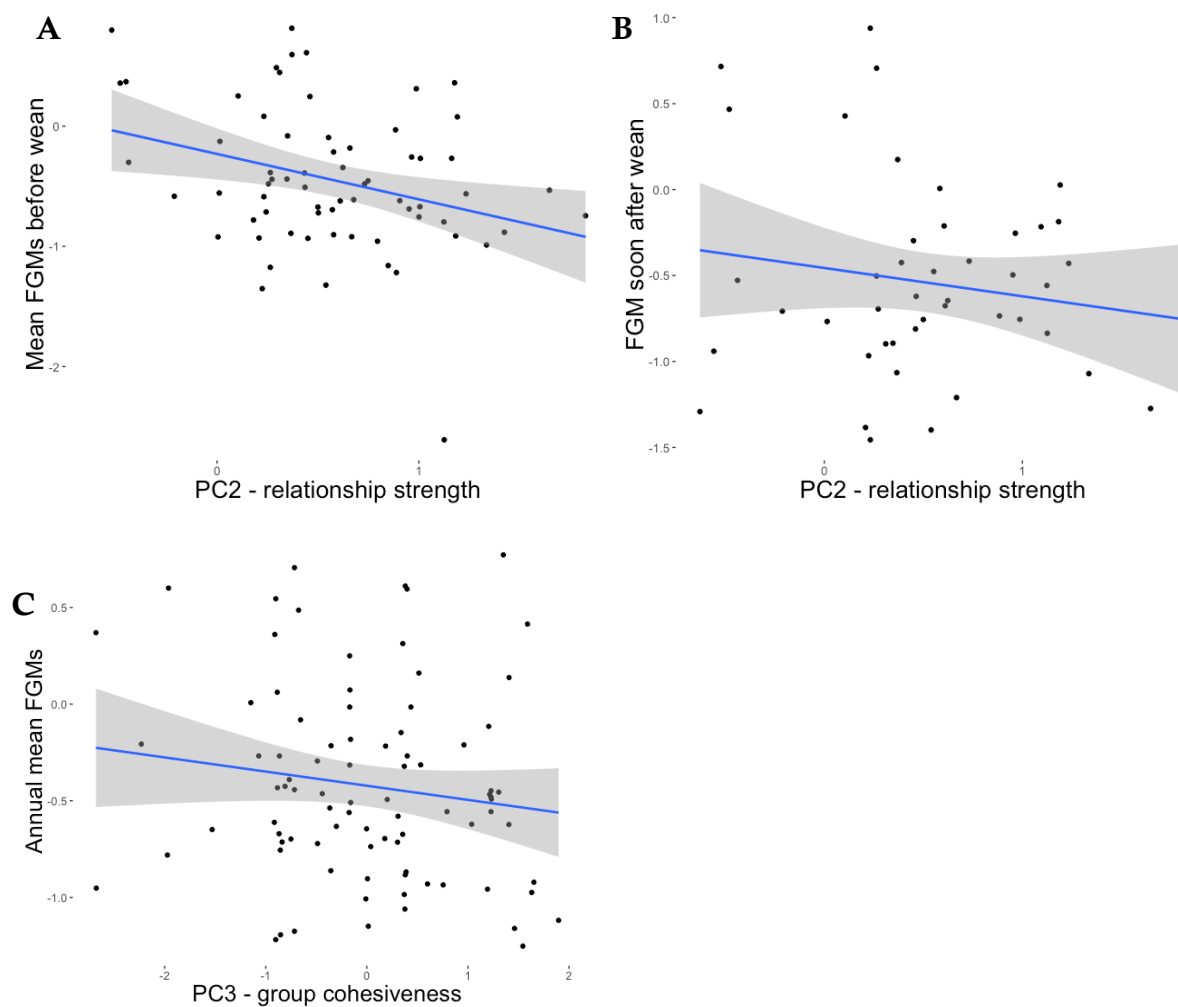


Fig 1. Graphs illustrating linear regressions for some of the significant effects of our models.



Fig 2. Map illustrating trap locations for the bench-river colony, one of the four colonies analyzed.