

The importance of parasitism and body condition in explaining variation in anti-predator vigilance in the yellow-bellied marmot (*Marmota flaviventris*)

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Prior research has indicated that environmental factors such as substrate and incline influence the amount of time that yellow bellied marmots (*Marmota flaviventris*) allocate to vigilance while foraging. This study examined whether internal factors such as body condition and parasite infection also explained variation in marmot anti-predator vigilance. Adult and yearling marmots were live-trapped at Rocky Mountain Biological Laboratory (RMBL), Gunnison County, Colorado, USA during spring and summer months from 2004 to 2008 and sampled for blood, fecal and ectoparasites. The behavior of foraging marmots was recorded during 2 minute foraging animal samples. General linear models including internal and external state factors were fitted to three different measures of marmot vigilance behavior. Results indicate that body condition and infection with types of *Entamoeba*, *Eimeria*, and *Trypanosoma* may affect marmot vigilance behavior. These results suggest that marmot vigilance behavior is sensitive to changes in marmot internal condition and that parasites may modify marmot behavior.

Introduction:

During summer months, yellow-bellied marmots (*Marmota flaviventris*) greatest mortality risk is through predation (Van Vuren, 2001). Common marmot predators include coyotes (*Canis latrans*), badgers (*Taxidea taxus*), American martens (*Martes americana*) and raptors such as the golden eagle (*Aquila chrysaetos*) (Van Vuren, 2001). Predation risk is thought to affect a variety of marmot behaviors, including foraging (Armitage 1982; VanVuren, 2001). Marmots that feel particularly threatened may allocate more or less time to vigilance behavior while foraging. Threatened marmots may choose to minimize risk by allocating more time to vigilance or by foraging more intently and minimizing time exposed to predation risk. However, research indicates that risk assessment, and thus marmot vigilance behavior during foraging, is moderated by environmental factors such as local slope and substrate (Blumstein *et al.*, 2004). In addition to environmental factors, it is hypothesized that risk behavior is affected by internal factors such as body condition and parasite load. Research in the Belding's ground squirrel (*Spermophilus beldingi*) indicates that deterioration in body condition decreases ground squirrel vigilance while foraging (Bachman, 1993). Similarly, research in a variety of bird species preyed upon by the male sparrowhawk (*Accipiter nisus*), indicates that bird species with high incidence of *Plasmodium* infection exhibit greater risk behavior, initiating flight in response to potential threats significantly later than species with low levels of *Plasmodium* infection (Moller *et al.*, 2007).

Given that parasitized animals in weaker condition are at greater risk of predation (Temple, 1986) (Alzaga *et al.*, 2008), I hypothesize that variation in marmot vigilance behavior can be explained by marmot body condition and parasite infection. This study will examine whether infection with fleas (*Oropsylla stanfordi*), an ectoparasite, *Trypanosoma*, a blood parasite, and intestinal parasites such as *Entamoeba* and *Eimeria* explain the amount of time that marmots allocate to vigilance during bouts of foraging.

Materials and Methods

Marmots used in this study were trapped and observed in the East River Valley near Rocky Mountain Biological Laboratory (RMBL), Gunnison County, Colorado, USA between 2003 and 2008. Marmots were trapped in six colony locations near RMBL (Table 1). Blood samples, used to identify *Trypanosoma* blood parasites, and flea abundance (estimated by sample combing) were collected every two weeks during regularly scheduled trapping sessions. When available, fecal samples were also collected during trapping and used to identify presence of intestinal parasites such as *Entamoeba* and *Eimeria*. Marmot mass was measured and used to estimate marmot condition.

Blood samples were stained using a Fisher Scientific HEXA 3 stain kit and scored under for presence of *Trypanosoma* at UCLA laboratories. Fecal samples were analyzed using fecal floatation and scored for presence of intestinal parasite oocytes such as *Eimeria* and *Entamoeba*.

Foraging behavior was observed and quantified during two-minute foraging focal animal samples. During the focal, each instance of the following seven behaviors was dictated into a microcassette recorder: stand forage, rear forage, stand look, rear look, walk, run, and other. Stand look and rear look were the vigilance behaviors of interest. A marmot exhibited stand look behavior when it stood on all four feet and lifted its head to examine its surroundings. A marmot exhibited rear look behavior when it stood on its rear feet and lifted its head similarly. Focals were collected in the early morning (0630-1100) and in the late afternoon (1600-2000) when marmots are most active. During the focal substrate and incline of local environment were recorded. Our goal was to collect a foraging focal from each marmot active in the RMBL study area during each season. Behavior was scored and analyzed using JWatcher 1.0 software (Blumstein and Daniel, 2007) and the proportion of time allocated to each behavior, behavior rate, and average behavior duration were calculated.

A single focal with corresponding parasite record was selected for each marmot in each year of analysis. Given that an individual's body condition and parasite status may vary from year to year, assuming independence between data collected from individuals between years is acceptable for this system (Blumstein *et al.*, 2004). The focals used in the dataset were selected to minimize the interval between a focal observation and parasite sampling.

Data were analyzed using a general linear model using SPSS- 11.04 (SPSS, Inc, 2007). We focused on stand look vigilance behavior because marmots rarely reared and looked especially early in the season when vegetation was low. We examined three different attributes of stand look vigilance behavior. The total proportion of time that marmots were vigilant and within sight during the focal was calculated and angularly transformed to meet assumptions of normality. We also used the rate of vigilance behavior, calculated by dividing the number of bouts of stand look behavior by the total time marmots were in sight during the focal and the average bout duration of stand look behavior. The primary independent variables for analysis were presence/ absence of *Entamoeba*, *Eimeria*, and *Trypanosoma* as well as marmot condition. Because marmot mass varies with many factors including age, sex, colony, year and time of year (Armitage *et al.*, 1976) we estimated marmot condition using residuals from a general

linear model with mass as the dependent variable and these factors as independent variables. Positive residuals reflected marmots in better condition. Using residuals to approximate condition allowed us to factor out known sources of mass variation such as age and sex and focus on date-relative condition for each marmot in our sample. Finally our model included several potentially confounding or obscuring variables which prior research has suggested explain variation in marmot vigilance behavior while foraging (Blumstein *et al*, 2004). These independent variables included substrate (Dirt/ Stones, Low Vegetation, High Vegetation) slope (0-10°, 10-30°, >30°) time in season (early or late), year, age class (adult or yearling) and sex. Even though exact focal dates were available for all records, date was scored as the dichotomous variable “time in season” because focals in our sample had a bimodal distribution by date. Focals collected before June 5th, the inflection point in the distribution histogram, were classified as early season, and those collected on or after June 5th were classified as late season

Results:

Given that parasite scoring of samples from 2007 and 2008 was not completed at the time of analysis and that many focal observations from 2003 did not have complete records of parasites at the time of focal, of the 1113 focal observations in our database, only 119 individuals representing data from years 2004-2006 were included in our initial analysis. Further analysis representing a larger dataset will be completed in Fall 2008.

Marmots in our final dataset were observed at 5 different colonies throughout the East River Valley: River, Bench, Gothic Townsite, Marmot Meadow, and Picnic. Our dataset consisted of 74 adult marmots and 45 yearlings. Juveniles were excluded from analysis. Eighty-five subjects were female and 34 were male. Fifty- eight of the female subjects were adults (27 were yearlings) whereas as 16 of the male subjects were adults (18 were yearlings).

Our model significantly ($P < 0.001$) explained 21.1% of the variation in the proportion of time standing and looking (Table 2). Substrate and age were significant factors in explaining proportion of time allocated vigilance behavior. Adult marmots were less vigilant than yearling marmots and marmots foraging on dirt/ stones or in high vegetation were less vigilant than those foraging in low vegetation (Table 2). Marmot condition and presence of *Entamoeba* exhibited non-significant trends affecting marmot vigilance. Increases in marmot condition decreased marmot vigilance behavior. Marmots with *Entamoeba* tended to look more than those without (Table 2; Figure 1).

Similarly, our model significantly ($P < 0.001$) explained 19.8% of the variation in rate of stand look behavior (Table 3). Substrate and age were significant factors explaining rate of standing and looking. Adult marmots stood and looked at a lower rate than yearling marmots and marmots foraging on dirt/ stones or in high vegetation stood and looked at lower rates than those foraging in low vegetation (Table 3). Marmot condition and *Entamoeba* infection exhibited non-significant trends affecting rate of marmot vigilance behavior. Marmots in better condition stood and looked at lower rates. Marmots with *Entamoeba* tended to stand and looked at higher rates than those without (Table 3; Figure 2).

Finally, our model significantly ($P < 0.001$) explained 23.9% of variation in the average

bout of stand look behavior (Table 4). Time of season, age, condition, and presence of *Eimeria* were significant factors in explaining the duration of stand look vigilance behavior. The average bout duration of stand look behavior was significantly shorter early in the season than late in the season. Adult marmots exhibited average bouts of vigilance behavior that were significantly shorter than those exhibited by yearling marmots. Duration of stand look behavior decreased with increases in condition and decreased with *Eimeria* infection. *Trypanosoma* infection exhibited a non-significant trend affecting average duration of marmot stand look behavior. Marmots with *Trypanosoma* tended to have shorter bouts of vigilance behavior than those without (Table 4; Figure 3).

Discussion:

Results are not only consistent with prior research which suggest that environmental factors affect marmot vigilance behavior, but more importantly suggest that marmot vigilance behavior is affected by the internal factors of body condition and parasite infection. Moreover, partial eta squared values for body condition and parasite infection were comparable to that given by substrate, suggesting that internal state is at least as important as external environment in explaining variation in marmot vigilance behavior.

In our general linear models explaining variation in proportion of time allocated to vigilance behavior and rate of vigilance behavior, substrate significantly explained variation in marmot vigilance behavior (Tables 2 and 3 Figures 1 and 2). Marmots allocated a lower proportion of time to vigilance and exhibited vigilance behavior at a lower rate when foraging in dirt/ stones and in high vegetation than when foraging in high vegetation. This is expected given that previous studies have found that substrate explains variation in marmot wariness as measured by flight initiation distance (Blumstein *et al.*, 2004). Surprisingly, in our study, incline does not explain variation in vigilance behavior in my model, whereas it was highly significant in the study by Blumstein *et al.* (2004). This may have been because they selected cases so as to increase variation in incline to measure the effect of incline on marmot behavior, while we selected cases to minimize the time interval between focal and parasite sampling. Regardless, extrinsic factors clearly affect vigilance behavior.

In all three of our models, yearling marmots exhibited significantly different vigilance behavior than adult marmots. Adult marmots are less vigilant than yearling marmots allocating a smaller proportion of time to vigilance behavior, displaying vigilance behavior at a lower rate, and exhibiting shorter bouts of duration than yearling marmots (Tables 2, 3, and 4). This is consistent with prior research that indicates that age affects marmot time budget (Armitage *et al.*, 1996) and consistent with our hypothesis that differences in the internal state of marmots (yearling marmots have not yet reached adult mass and do not typically reproduce (Armitage *et al.*, 1976)) explains variation in the marmot vigilance while foraging.

Marmot body condition explained variation in marmot vigilance behavior in each of our models, providing further evidence that marmot internal state is important for marmot anti-predator vigilance (Tables 2, 3, and 4, Figures 1,2, and 3). Increases in marmot condition corresponded with decreases in all three measures of marmot vigilance

behavior. This is unsurprising given evidence suggesting that animals in weaker condition are more susceptible to predators (Temple, 1986). However, this is the first evidence that marmot condition may explain marmot anti-predator vigilance.

Similarly, our models suggest that parasitism by *Entamoeba*, *Eimeria*, and *Trypanosoma* all affect marmot vigilance while foraging, however each parasite affects marmot vigilance behavior in different ways. Infection with *Entamoeba* resulted in a non-significant trend towards increase in proportion of time allocated to vigilance and rate of vigilance behavior in our models (Tables 2 and 3; Figures 1 and 2). This is consistent with our result suggesting that marmots in worse body condition exhibit increased vigilance behavior. Interestingly, infection with *Entamoeba* does not affect the average duration of marmot vigilance behavior. Thus, marmots with *Entamoeba* increase vigilance by looking more frequently, not by increasing the vigilance bout duration.

Evidence supporting the hypotheses that *Eimeria* and *Trypanosoma* affect marmot vigilance behavior is much less conclusive. *Eimeria* and *Trypanosoma* infection do not significantly affect proportion of time allocated to vigilance or rate of vigilance behavior. However, *Eimeria* infection significantly decreases average vigilance bout duration. Similarly, *Trypanosoma* infection corresponds with a non-significant trend towards decreased vigilance bout duration (Table 4; Figure 3). This is consistent with research that suggests rats (*Rattus norvegicus*) infected with *Toxoplasma gondii* and mice infected with *Eimeria vermiformis* exhibit less fear of cat odor (Berdoy *et al.*, 2000) (Moore, 2002). This suggests the effect of parasitism on marmot vigilance behavior depends on the type of parasite infection.

Parasite infection may affect marmot vigilance behavior directly through parasite-host manipulation or indirectly through host-physiological response to parasite infection (Thomas *et al.*, 2005). *Toxoplasma gondii* may decrease rat aversion to cats directly through the formation of cysts in the brain (Berdoy *et al.*, 2000) whereas other parasites may affect host behavior indirectly by draining energy reserves (Alzaga *et al.*, 2008). It is possible that the different effects of parasitism by the three parasites examined in this study can be explained by differences in parasite ability to manipulate host behavior. Further research on the effect of *Eimeria*, *Entamoeba*, and *Trypanosoma* infection on marmot behavior and physiology must be done.

Ultimately, it is unclear how parasitism by *Eimeria*, *Entamoeba*, and *Trypanosoma* affect marmots' risk of predation. Whereas parasitism may increase marmots risk to predation by making them easier targets for predators such as foxes and coyotes, it is also possible that parasitized marmots make less desirable prey due to risk of parasite transmission to predators. Understanding the affect of parasitism on marmot vigilance behavior may involve understanding the risk of parasite transmission from marmots to common marmot predators.

Though our models suggest that internal factors including marmot body condition and parasite infection explain variation in marmot anti-predator vigilance behavior, our analysis also indicates that anti-predator vigilance is a complex behavior which cannot be explained solely by the internal and environmental factors examined in this study. Adjusted R^2 values for our models of marmot vigilance behavior ranged between 19-

23%. Clearly, much about anti-predator vigilance remains to be explained.

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Table 1: GPS coordinates for observation sites in East River Valley, CO, USA. Each colony (with the exception of River) is represented by a central coordinate and the approximate radius of the study site. * River colony is represented by five coordinates representing the vertices of a polygon encompassing the study site.

Colony	Central Coordinates		Radius (m)
Up Valley			
Picnic	325811.130	4316838.902	177
Marmot Meadow	326767.098	436275.412	133
Down Valley			
Town	327918.798	4314106.816	277
Horse mound	327561.275	4313195.399	23
Bench	32810.557	4313163.982	110
River *	327638.450	4313199.770	
	327585.179	4313166.988	
	327637.084	4313049.518	
	327768.896	4312930.682	
	327846.754	4313001.710	

Table 2: Results of general linear model explaining variation in the angularly transformed proportion of time exhibiting stand look vigilance behavior (n= 119 subjects). Independent variables that significantly ($P < 0.05$) explained variation in vigilance are highlighted in bold, those for which $0.05 < P < 0.10$, are italicized.

	B	P-value	Partial Eta Squared
Intercept	0.504	0	0.274
<i>Year</i>		0.053	0.054
2004	0.0252	0.531	0.004
2005	-0.0706	0.075	0.03
2006	0		
Time of Season		0.236	0.013
Early	-0.0423	0.236	0.013
Late	0		
Incline		0.658	0.008
0-10	0		
10-30°	0.0212	0.532	0.004
> 30°	-0.0189	0.673	0.002
Substrate		0.038	0.061
Dirt/ Stones	-0.139	0.066	0.032
Low Vegetation	0		
High Vegetation	-0.09	0.05	0.036
Age		0.021	0.049
Adult	-0.0796	0.021	0.049
Yearling	0		
Sex		0.121	0.023
Female	0.0559	0.121	0.023
Male	0		
<i>Entamoeba</i>		0.065	0.032
Absence	-0.0953	0.065	0.032
Presence	0		
Eimeria		0.256	0.012
Absence	0.0371	0.256	0.012
Presence	0		
Trypanosoma		0.121	0.023
Absence	0.109	0.121	0.023
Presence	0		
<i>Condition</i>	-0.0291	0.061	0.033

The model significantly explained 21.1% of the variation.

Table 3. Results of the general linear model explaining variation in the rate of stand look behavior (n= 119 subjects).

	B	Sig.	Partial Eta Squared
Intercept	0.24	0.001	0.096
Year		0.063	0.051
2004	0.0246	0.493	0.004
2005	-0.0591	0.095	0.026
2006	0		
Time of Season		0.199	0.016
Early	-0.0410	0.199	0.016
Late	0		
Incline		0.732	0.006
0-10	0		
10-30	0.0161	0.595	0.003
> 30	-0.0150	0.708	0.001
Substrate		0.067	0.05
Dirt/Stones	-0.12	0.076	0.03
Low Vegetation	0		
High Vegetation	-0.0682	0.096	0.026
Age		0.024	0.047
Adult	-0.0695	0.024	0.047
Yearling	0		
Sex		0.144	0.02
Female	0.0469	0.144	0.02
Male	0		
Entamoeba		0.074	0.03
Absence	-0.0821	0.074	0.03
Presence	0		
Eimeria		0.182	0.017
Absence	0.039	0.182	0.017
Presence	0		
Trypanosoma		0.122	0.023
Absence	0.0972	0.122	0.023
Presence	0		
Condition	-0.025	0.071	0.031

The model significantly explained 19.8 % of the variation in the rate of stand look behavior.

Table 4. Results of general linear model explaining variation in the average bout duration of stand look vigilance behavior (n= 119 subjects).

	B	P Value	Partial Eta Squared
Intercept	27.127	0	0.13
Year		0.679	0.007
2004	2.994	0.382	0.007
2005	1.607	0.632	0.002
2006	0		
Time of Season		0.015	0.055
Early	-7.468	0.015	0.055
Late	0		
Incline		0.379	0.018
0-10	0		
10-30	-1.32	0.648	0.002
> 30	4.184	0.275	0.011
Substrate		0.374	0.019
Dirt/ Stones	-3.997	0.533	0.004
Low Vegetation	0		
High Vegetation	-5.091	0.192	0.016
Age		0.004	0.077
Adult	-8.589	0.004	0.077
Yearling	0		
Sex		0.123	0.023
Female	4.744	0.123	0.023
Male	0		
Entamoeba		0.176	0.017
Absence	-5.93	0.176	0.017
Presence	0		
Eimeria		0.028	0.045
Absence	6.181	0.028	0.045
Presence	0		
<i>Trypanosoma</i>		0.051	0.036
Absence	11.775	0.051	0.036
Presence	0		
<i>Condition</i>	-3.933	0.003	0.079

This model significantly explained 23.9% of the variation.

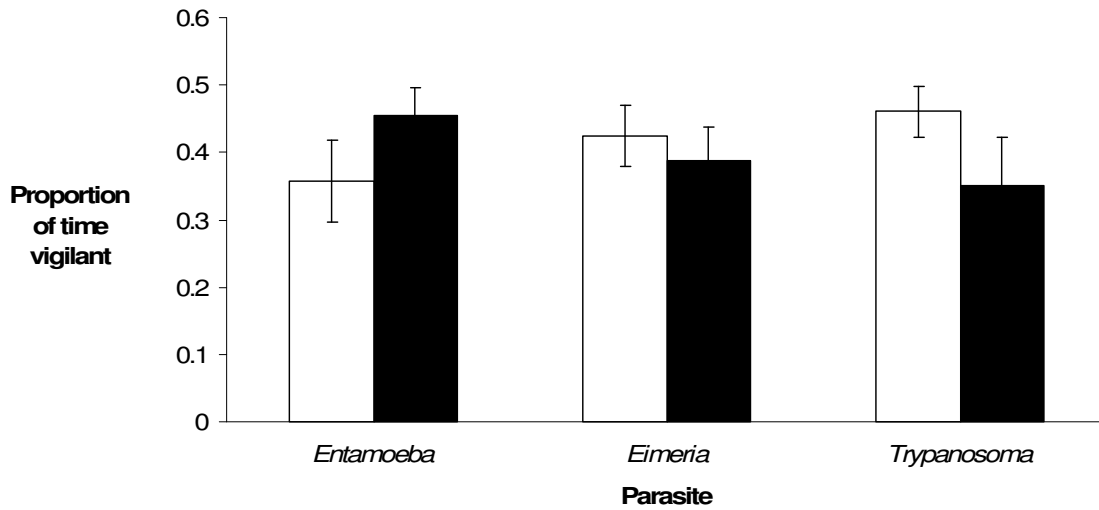


Figure 1. The effect of parasite infection on the proportion of time marmots allocate to stand look vigilance behavior. Marmots infected with *Entamoeba* showed a non-significant trend towards increased time allocated to vigilance ($P=0.065$). Empty bars represent non-parasitized marmots and filled bars represent parasitized marmots. Error bars represent standard error of the marginal mean.

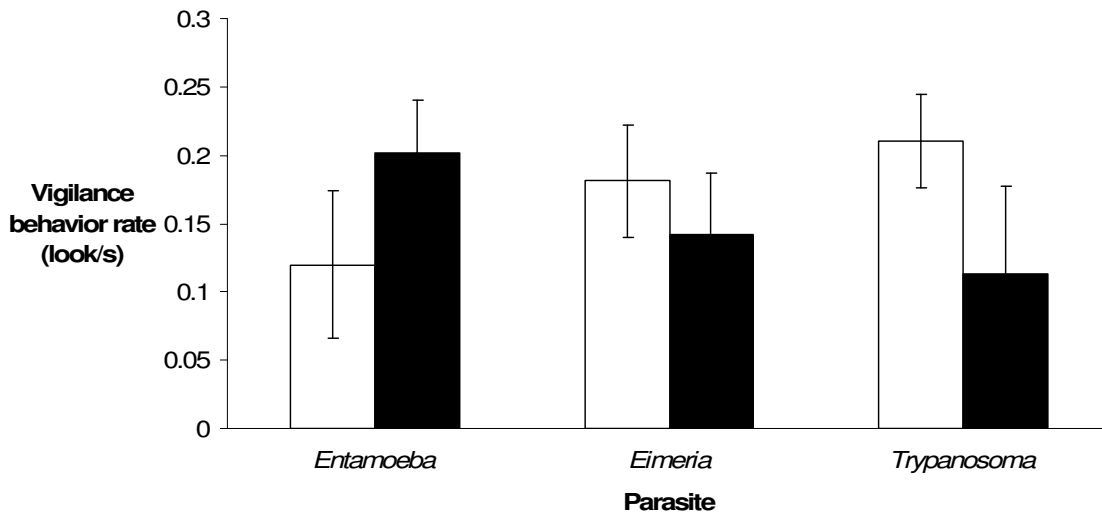


Figure 2. The effect of parasite infection on rate of stand look vigilance behavior. Marmots infected with *Entamoeba* exhibit a non-significant trend towards increased vigilance behavior rate ($P=0.074$). Empty bars represent healthy marmots and filled bars represent parasitized. Error bars represent standard error of the marginal mean.

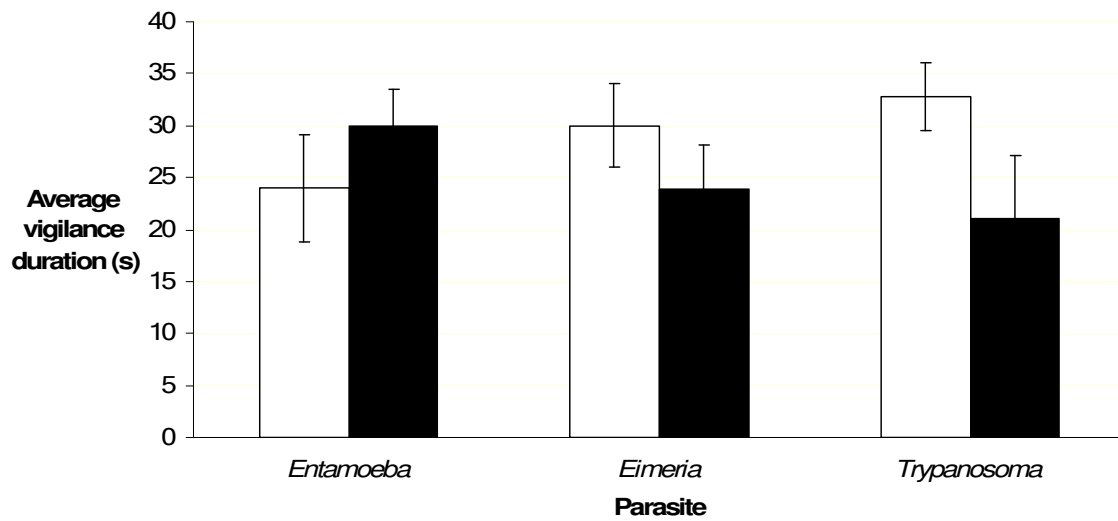


Figure 3. The affect of parasite infection on the average bout duration of stand look vigilance behavior. Marmots infected with *Eimeria* exhibited significantly shorter bouts of stand look behavior than those without ($P= 0.028$). Marmots infected with *Trypanosoma* exhibited a non-significant trend towards decreased bout duration of stand look behavior ($P= 0.051$). Empty bars represent uninfected marmots and filled bars represent parasitized marmots. Error bars represent stand error of the marginal mean.

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