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Is the propensity to emit alarm calls associated with health status?

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Abstract

The production and structure of animal signals may depend on an individual's health status and may provide more than one type of information to receivers. While alarm calls are not typically viewed as health condition dependent, recent studies have suggested that their structure, and possibly their propensity to be emitted, depends on an individual's health condition and state. We asked whether the propensity of yellow-bellied marmots (*Marmota flaviventer*) to emit calls is influenced by their immunological or parasite status, by quantifying both trap-elicited and natural calling rates as a function of their neutrophil-to-lymphocyte (NL) ratio, the presence of a blood borne trypanosome, and the presence of several intestinal parasites (*Eimeria* sp., *Entamoeba* sp., and *Ascaris* sp.). We fitted mixed effects models to determine if the health measures we collected were associated with the probability of calling in a trap and with annual rates of natural alarm calling. Marmots infected with a blood-borne trypanosome were marginally more likely to call naturally and when trapped, while those infected with the intestinal parasite *Ascaris* were less likely to call when trapped. NL ratio was not directly associated with in-trap calling probability, but males were more likely to call when they had higher NL ratios. Thus, health conditions, such as parasite infection and immune system activation, can modulate the production of alarm signals and potentially provide information to both predators and prey about the caller's condition. Playback experiments are required to confirm if receivers use such information.

Key words: alarm calling, animal communication, antipredator behavior, condition dependence, immune function, parasites

Animals emit alarm calls in response to detecting a predator to warn conspecifics or in an attempt to escape predation by signaling to a predator that it has been detected (Caro 2005). However, the propensity to emit calls is a function of multiple external and internal factors. External factors that can affect the propensity to emit alarm calls can include audience effects (Marler and Evans 1996; Ridley et al. 2007), the relative safety of an individual's position (Randall et al. 2000), an individual's perception of risk (Blumstein and Armitage 1997), and an individual's social connectedness (Fuong et al. 2015).

Audience effects are an example of an external factor that influences the propensity to call whereby whether an individual emits an alarm call depends on the presence of other conspecifics or heterospecifics (Marler and Evans 1996; Ridley et al. 2007). For instance, male Thomas's langurs, *Presbytis thomasi*, will only alarm call while fleeing from tigers if other Thomas's langurs are present (Wich and Sterck 2003). The production of calls may also depend on whether an individual is in a position of safety (Randall et al. 2000). Great gerbils, *Rhombomys optimus* (Randall et al. 2000), yellow-bellied marmots, *Marmota flaviventer* (Collier et al. 2010), and black-tailed

prairie dogs, *Cynomys ludovicianus* (Hoogland 1995) alarm call more frequently when they are near the safety provided by their burrows, thereby reducing any risks associated with emitting calls. Variation in an individual's perceived risk of different predators can also influence alarm calling propensity and structure. Marmots are more likely to alarm call to a predatory species that reflects a relatively higher risk of predation, such as coyotes, *Canis latrans*, compared with predators that are relatively less risky, such as smaller raptors, and as risk increases they call at higher rates (Blumstein and Armitage 1997). Moreover, inexperienced urban bonnet macaques, *Macaca radiata*, produced less noisy alarm calls to python models than their forest-dwelling conspecifics who had previously encountered the predator posing risk, pythons (Coss et al. 2007). An individual's position in its social network can also affect whether or not it calls and marmots that are socially connected to fewer individuals are more likely to alarm call, possibly because they can rely less on other marmots to detect and deter predators. Additionally, marmots with weaker relationships, which involve fewer interactions between individuals, are more likely to call, possibly to gain social status (Fuong et al. 2015). The context in which an individual is alarmed influences whether or not an individual calls and alarm call characteristics.

In addition to these external conditions, internal state, such as stress and parasite presence, can affect propensity to call and alarm call structure (Bercovitch et al. 1995; Blumstein et al. 2006; Nouri and Blumstein 2019). Rhesus macaques, *Macaca mulatta*, are more likely to alarm call with higher cortisol levels (Bercovitch et al. 1995) and marmots are more likely to emit alarm calls when they have higher fecal glucocorticoid levels (Blumstein et al. 2006). Additionally, while acutely stressed red-squirrels, *Tamiasciurus hudsonicus*, produce rattle calls with greater entropy (Sehrsweeney et al. 2019), yellow ground squirrels, *Spermophilus fulvus*, calls were identical when stressed by an approaching human and by a livetrapping event (Matrosova et al. 2010). Furthermore, the acoustic structure of calls can be influenced by health conditions; marmots with *Eimeria*, an intestinal parasite, produced noisier calls (quantified using Weiner entropy) than those without *Eimeria* (Nouri and Blumstein 2019).

Following on from Nouri and Blumstein's (2019) result, given that parasite status may influence call structure, we asked whether the presence of specific parasites, parasite richness, and/or immunological condition can influence the probability that a marmot will emit an alarm call. Some parasites present in marmots, such as *Eimeria* (Yun, 2000) can generate an immune response in hosts while others such as *Ascaris* (Faquim-Mauro and Macedo 1998), *Entamoeba* (Soboslay et al. 2006; Lejeune et al. 2009), and *Trypanosoma* (Hirokawa et al. 1981; Albright et al. 1990) are immunosuppressive. Neutrophil-to-lymphocyte (NL) ratios are an effective measure of stress and non-specific immune system activation because neutrophils are phagocytic and proliferate in response to infections and stress more than lymphocytes (Davis et al. 2008; Maceda-Veiga et al. 2015). Individuals with activated immune systems might be less likely to escape or avoid predation due to reduced energetic reserves (Martin et al. 2003; Navarro et al. 2004) or reduced energetic investment in vigilance (Chmura et al. 2016), leading to increased vulnerability.

It remains an open question as to whether this increased vulnerability influences the propensity to emit alarm calls. It is possible that more vulnerable animals may make themselves less conspicuous (Endler 1987; Hedrick 2000), particularly if calling is costly. Crickets, *Gryllus integer*, who had more conspicuous mating songs

behaviorally, compensated to this increased predation risk by waiting longer to call after interrupted by predator cues (Hedrick 2000). When predation risk was greatest, guppies, *Poecilia reticulata*, employed a less conspicuous copulation strategy instead of a visual courtship display (Endler 1987). Additionally, physical discomfort or pain from an infection could also reduce awareness and could cause an individual to be less likely to notice and call in response to the presence of a predator. Alternatively, increased vulnerability may make an individual more likely to discourage predators from attacking them by emitting defensive calls (Marler 1955; Tilson and Norton 1981; Digweed and Rendall 2009). Songbird mobbing calls, intended to threaten a predator, are easily localizable, while aerial predator calls, intended to warn others without detection, are much less conspicuous (Marler 1955). Reduced energetic reserves may also act directly to influence vocalizations. In white-crowned sparrows, *Zonotrichia leucophrys*, immune system activation modifies song structure by reducing the number of terminal notes (Munoz et al. 2010) and parasite infection is associated with reduced song production (Gilman et al. 2007). Immune system activation and parasite presence can compromise an individual's ability to call, possibly increasing vulnerability when those calls are for predator avoidance.

In addition to influencing an individual's vulnerability to predation, parasites may influence perception of predation risk as a mechanism to increase parasite fitness. For instance, laboratory rats infected with the parasite *Toxoplasma gondii* perceived less predation risk toward, and were sometimes even attracted to cats, *Felis catus*, the parasite's definitive host (Berdoy et al. 2000). In marmots, previous studies have found that antipredator vigilance is associated with parasite status; the presence of immunosuppressive parasites *Ascaris* and *Trypanosoma* are associated with less time spent vigilant (Chmura et al. 2016).

Thus, it is likely that a marmot's health status could influence the probability of alarm calling by either causing a reduction in calling due to reduced energetic reserves from mounting an immune response (Scheuber et al. 2003; Fedorka and Mousseau 2006), or an increase in calling due to greater perceived vulnerability (Blumstein and Armitage 1997; Nouri and Blumstein 2019). It is unclear exactly how these health conditions may affect alarm calling, but previous work allows for some predictions. Marmots with high glucocorticoid levels, a stress hormone, produce calls that had less Wiener entropy (Blumstein and Chi 2012), suggesting that perceived risk leads to greater energetic investment in producing well-articulated alarm calls. Additionally, *Eimeria* infection rates and overall parasite diversity have been shown to be positively associated with call structure—again measured with Wiener entropy—in yellow-bellied marmot alarm calls (Nouri and Blumstein 2019). These results suggest that sick marmots are less able to energetically invest in calls, thus generating "noisier," less structured calls, and would then be expected to be less likely to alarm call. If immune system activation is reducing available energetic reserves to call, *Eimeria* infection and an increased NL ratio would be associated with a decreased probability of alarm calling, while *Ascaris*, *Entamoeba*, and *Trypanosoma* could either positively associate or disassociate with alarm calling due to greater energy reserves. Alternatively, if immune activation increases perceived risk, then *Eimeria* infection and an increased NL ratio would be associated with an increased probability of alarm calling while *Ascaris*, *Entamoeba*, and *Trypanosoma* would generate the opposite effect.

We predicted that (1) increased NL ratios would be positively associated with probability and propensity of alarm calling in

yellow-bellied marmots by increasing perceived risk; (2) *Eimeria* infection and gut parasite richness would be positively associated with probability and propensity of alarm calling by increasing vulnerability through reduced energetic reserves; and (3) *Ascaris*, *Entamoeba*, and *Trypanosoma* infection would have a negative association with probability and propensity of alarm calling by reducing perceived risk through immunosuppression. By investigating these relationships, we will better understand the suite of factors that may or may not influence alarm calling.

Materials and Methods

Data collection

At the Rocky Mountain Biological Laboratory (RMBL) (38°77′ N, 106°59′ W), teams of trained observers have live-trapped and observed free-living yellow-bellied marmots since 1962 (Armitage 2010; Blumstein et al. 2013). Here, we focus on data collected at 11 geographically discrete colony sites from 2003 to 2016 where marmots were observed, health statuses were recorded, and alarm calls were quantified throughout the active season (April to mid-September) during times of greatest activity (0700 and 1100 h and 1630 and 1900 h MDT).

Trap data

We trapped marmots to collect blood and fecal samples, to affix permanent ear tags for individual identification (#3 Monel fingerling fish tags—National Band and Tag, Newport, KY, USA), and to mark individuals with Nyanzol fur dye (Albinal Dyestuff Inc., Jersey City, NJ, USA) for identification during behavioral observations. Marmots were trapped in Tomahawk live traps at known burrow entrances approximately every other week between late May (following snowmelt) and mid-September. We recorded in-trap behaviors, which included whether a marmot alarm called, before transferring marmots to a handling bag for processing whereupon we sexed individual marmots, collected morphometric data, and collected biological samples.

Blood samples were routinely collected when marmots were trapped. We collected up to 3 mL of blood from the femoral vein of marmots (no more than once every other week) that was then stored in a heparin or EDTA tube. Within 2 h of collection, we made a thin film blood smear (methods as in Chmura et al. 2016), which was later stained using the Hema 3 Stat Pack (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The presence, or absence, of *Trypanosoma lewisi* was based on a systematic examination of a slide for up to 30 min. Immune system activation in wild vertebrates can be reliably evaluated by NL ratios in blood samples (Davis et al. 2008), with an increase in the ratio of NL cells occurring due to stress or immune system activation (Maceda-Veiga et al. 2015). NL ratio was calculated by counting neutrophils, lymphocytes, basophils, and monocytes up to a maximum of 100 cells or for 30 min per slide, whichever came first (Nouri and Blumstein 2019).

We collected fecal samples from marmots that defecated while in trap, or during subsequent handling, and immediately stored these samples in formalin. Fecal samples were analyzed within 6 months of collection by performing fecal floats using Ova Float™ Zn 118 (zinc sulfate heptahydrate; Butler Animal Health Supply, Dublin, OH, USA). Fecal samples were processed by examining wet slides for the presence/absence of *Ascaris* (a nematode), *Eimeria* (a coccidian), and *Entamoeba* (a protozoan) (Lopez et al. 2013), 3 fecal–orally transmitted parasites (MacNeal 1904) and intestinal parasite richness was calculated by the sum of these 3 binary outcomes (Nouri and Blumstein 2019).

Observational data

Trained observers quantified all bouts of marmot alarm calling (a bout was defined as continuous alarm call utterances separated by at least 1 min) and the identity of the caller, when possible, using 15–45× spotting scopes and 10 × 40 binoculars from distances that did not obviously influence their behavior—which depended on the marmot's degree of habituation (Li et al. 2011). We used these data to calculate annual rates of natural alarm calling where (following Fuong et al. 2015) the number of observed bouts of calling was divided by the number of hours that an individual could have been observed (which was based on the number of hours a colony was watched on days when that subject was seen). To ensure adequate sampling, our analysis was restricted to colonies observed for ≥50 h in a given year.

Statistical analysis

We analyzed both in-trap calling probability and natural rates of alarm calling separately, to examine our hypotheses using complementary lines of evidence. Many individuals had in-trap calling and natural calling rate data for the same year. When animals called in a trap we were certain of their precise health status while natural rates of calling are calculated over a season during which time an individual's health status may change. However, natural calling rates reflect responses to natural stimuli as opposed to a trapping event. The use of rates is essential because while we know each time a person approached a trap; we were uncertain of each time a predator or other alarming stimulus could have elicited a bout of "natural" calling. Natural calling rate observations were paired with health data from all trapping events within year for individual marmots as natural calling rates were calculated for individuals across the year.

We used the lme4 1.1-18-1 (Bates et al. 2014) and lmerTest 3.1 (Kuznetsova et al. 2018) packages in R 3.5.1 (R Core Team 2018) to fit mixed effects models to explain variation in marmot alarm calling. To determine how parasite presence and NL ratio were associated with in-trap probability of calling, we fitted 6 generalized linear mixed models, dependent on the health condition of interest. Our fixed effects included one of the following health status terms: NL ratio, the presence of *Ascaris* (0,1), the presence of *Eimeria* (0,1), the presence of *Entamoeba* (0,1), the presence of *Trypanosoma* (0,1), total parasite diversity, and all other factors that can affect call propensity: predator index, age class, colony size, and sex. Predator index was calculated as a binary variable determined by whether the number of predators observed at that colony was above or below the median number of predator observations per colony across all colonies in that year. Age class was either yearling (1 year olds) or adult (≥2 year olds), as we excluded young of the year (i.e., 0 year olds) in these analyses because we have few samples from this cohort (we do not typically collect blood from them). Colony size is the number of marmots that were seen or trapped >4× per year at a given colony site and was standardized (Lopez et al. 2013). We modeled marmot identity and year as random effects to account for repeated observations of individuals within and between years. To determine how parasite presence and NL ratio were associated with natural rates of calling, we fitted linear mixed models. Our fixed effects included one health condition: the $\log_{10}$ transformation of the NL ratio $+ 1$, the presence of *Ascaris*, the presence of *Eimeria*, the presence of *Trypanosoma*, the

Table 1. Results from generalized linear mixed models and linear mixed effects models of trap calling as a function of *Ascaris* presence, in yellow-bellied marmots

Fixed effects

	Estimate	Std. error	z-Value	Pr(>|z|)
(Intercept)	−8.047	0.973	−8.27	2e−16
Ascaris	−2.596	0.873	−2.975	0.003
Sex (male)	0.529	0.895	0.591	0.555
Age class (yearling)	0.101	0.472	0.213	0.831
Predator index (low)	0.469	0.558	0.840	0.401
Scaled colony size	0.145	0.359	0.404	0.686

Random effects	Variance	Standard deviation
ID (intercept)	93.94	9.692

Quantified fixed effects include the presence of ascaris (0,1), predator index, age class, scaled colony size, and sex. Random effect is individual identity (ID).

presence of *Entamoeba*, or total parasite diversity, in addition to predator index, age class, colony size, and sex. Again, we modeled marmot identity and year as random effects. Some health measures are likely to interact with other individual traits, such as age class and parasite prevalence (Lopez et al. 2013). To represent these contingencies, we modeled interactions between the health condition being tested and either predator index, colony size, age class, or sex. If none of these interactions explained significant variation in the dependent variable, we fitted a new model without interactions. For the *Ascaris*, *Entamoeba*, *Eimeria*, and parasite richness trap-calling models only, year explained zero variance and was removed from the model. Results of the NL ratio trap-calling model were robust to the removal of apparent outliers in NL ratio from the dataset. We calculated correlation coefficients between predictor variables to ensure they were not collinear. To evaluate the importance of each significant health trait, we fitted the model without the health trait and compared the marginal R^2-values. We ensured these models were appropriate for the structure of our data by systematically examining residuals for normality using Q–Q plots and frequency histograms. Finally, marginal means were calculated using the emmeans package in R and were used to visualize how *T. lewisi* influenced natural calling rate while controlling for the influence of other independent variables (Searle et al. 1980).

Results

Our final dataset contained 5783 trapping events of 611 individuals, 925 annual natural calling rates from 248 individuals, 1907 blood samples from 525 individuals, and 745 fecal samples from 255 individuals from 14 years of observations and trapping. Significant model results are summarized in Tables 1–3 and summaries of the raw data for presence, absence in-trap models are provided in Supplementary Table S9. Multicollinearity was not an issue; correlations between all independent variables were <0.32. Both in-trap calling probability and rates of natural alarm calling were associated with some health status measures but not with others. *Ascaris* was negatively associated with the probability of calling when trapped (z=−2.975, P=0.003; Table 1) explaining 0.7% of the variation but was not associated with natural alarm calling rates (t=−0.057, df=326.028, P=0.955). Marmots infected with *Ascaris* were 55% less likely to call than those were not infected. Marmots infected

Table 2. (A) Results from generalized linear mixed models and linear mixed effects models of trap calling as a function of *Trypanosome* presence, in yellow-bellied marmots[a] and (B) Results from linear mixed effects models of natural alarm calling as a function of trypanosome presence[b]

A) Trap calling

Fixed effects

	Estimate	Std. error	z-Value	Pr(>|z|)
(Intercept)	−2.885	0.287	−10.049	<2e−16
Trypanosome	0.785	0.430	1.824	0.068
Sex (male)	0.402	0.272	1.478	0.139
Age class (yearling)	−0.158	0.199	−0.794	0.427
Predator index (low)	0.084	0.210	0.400	0.689
Scaled colony size	0.074	0.106	0.694	0.488

Random effects	Variance	Standard deviation
ID (Intercept)	3.966	1.992
Year (Intercept)	5.224e−14	2.286e−07

B) Natural calling

	Estimate	Std. Error	Df	t value	Pr(>|t|)
Fixed effects					
(Intercept)	5.138	0.385	21.692	13.360	6.08e-12
Trypanosome	0.688	0.402	890.653	1.713	0.087
Sex (male)	-0.118	0.299	238.144	-0.395	0.693
Age class (yearling)	1.307	0.176	914.215	7.442	2.28e-13
Predator index (low)	1.203	0.221	777.767	5.445	6.95e-08
Scaled colony size	0.499	0.121	628.156	4.127	4.17e-05

Random effects	Variance	Standard deviation
ID Intercept	3.757	1.938
Year (Intercept)	1.461	1.209

[a] Quantified-fixed effects include the presence of trypanosomes (0,1), predator index, age class, scaled colony size, and sex. Random effects include individual identity (ID) and year of observation (year)., [b] Fixed and random effects are identical to A.

with trypanosomes naturally called at marginally higher rates (t = 1.71, df=890.653, P = 0.087; Table 2A and Figure 1) and were marginally more likely to call when trapped (z = 1.824, P = 0.068; Table 2B). Trypanosome presence explained 2.6% of the variation in natural alarm calling. *Eimeria* (z = 1.020, P = 0.308; t=−0.715, df=297.727, P = 0.475), *Entamoeba* (z = 0.7821, P = 0.327; t=−0.606, df=289.146, P = 0.545), and intestinal parasite diversity (z = 0.406, P = 0.278; t=−0.704, df=309.686, P = 0.482) were not associated with either in-trap calling probability or natural alarm calling rates, respectively. NL ratio was not directly associated with in-trap calling probability (z=−0.543, P = 0.587) or natural alarm calling rates (t=−0.541, df=771.960, P = 0.588). However, there was a significant interaction between NL ratio and sex; males were more likely to call in-trap when they had higher NL ratios (z = 2.035, P = 0.042; Figure 2). This interaction explains 0.3% of the variation for in-trap calling probability. No other interactions were significantly associated with either in-trap probability or wild rate of calling. Marmot identity, modeled as a random effect, explained upward of 71% of the variation in alarm calling and most of the variation across all models. There were no annual effects on either calling measure. No other significant relationships were found (Supplementary Tables).
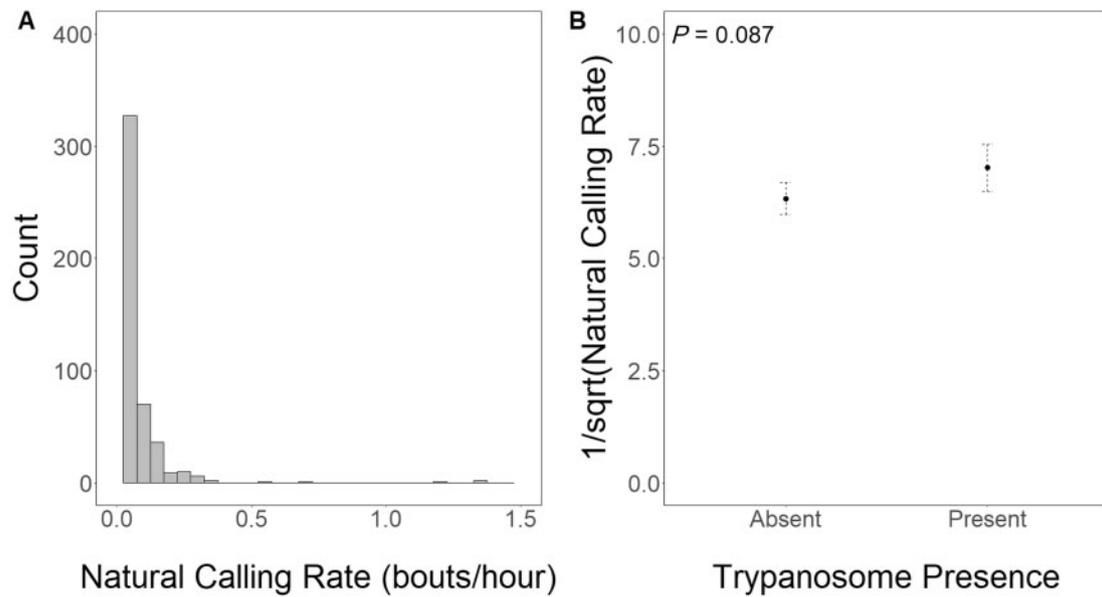
Figure 1. Relationship between the presence of the blood parasite *Trypanosoma lewisi* and the natural calling rate (bouts per hour) of yellow-bellied marmots. (**A**) The raw distribution of the data before transformation and (**B**) the marginal means±SE of 1/sqrt (natural alarm calling rate) for marmots whose fecal samples had *T. lewisi* present and those with *T. lewisi* absent. Significance (*P*-value) of the model, calculated from the transformed data, is included at the top of the figure.
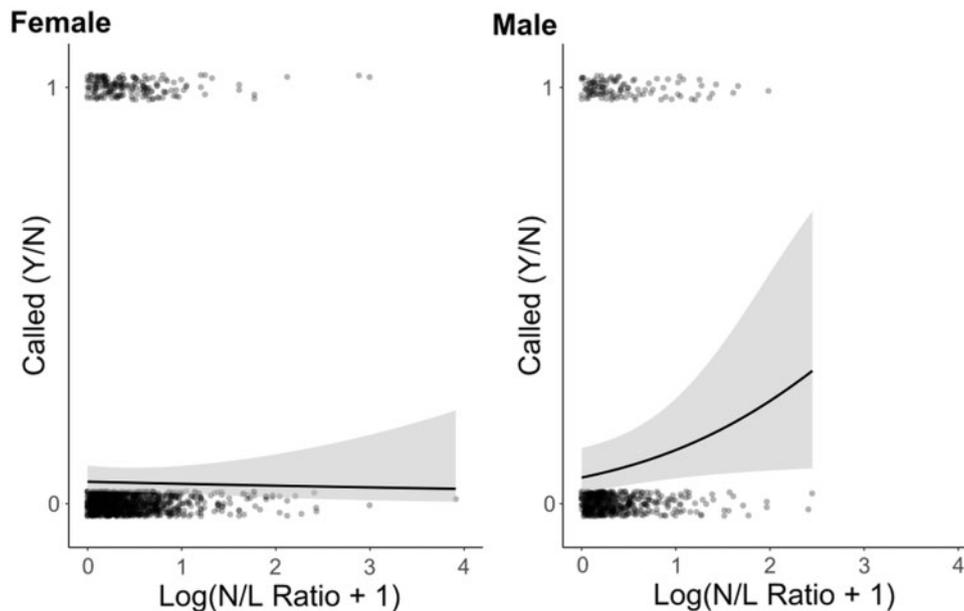
Figure 2. Relationships between $\log_{10}$(NL ratio+1) and probability of calling in trap for female and male marmots. NL ratio is positively associated with natural calling rate but only in male marmots. Black lines show the predicted effects from Table 3 (±SE), demonstrating the probability of calling as NL ratio changes. Points show the jittered raw data separated by called (1) or did not call (0) with darker shading showing a greater density of points at these values.

Discussion

While marmot identity explained most of the variation in alarm calling, some health condition measures were associated with variation in the propensity to emit alarm calls. We found that the effects of immune system activation and parasite presence on the rate of natural calling and probability of in-trap calling varied by parasite and calling measure. Marmots infected with *Ascaris*, an immunosuppressive parasite (Faquim-Mauro and Macedo 1998), were less likely to call when trapped (Table 1). In contrast, marmots infected with

trypanosomes, another immunosuppressive blood-borne parasite (Hirokawa et al. 1981; Albright et al. 1990), were marginally more likely to call naturally and when trapped (Figure 1 and Table 2A). Additionally, male marmots with greater immune system activation, as measured by NL ratio (Davis et al. 2008), were more likely to alarm call in trap (Figure 2 and Table 3) while, for females, there was no relationship between NL ratio and calling while trapped. The contradictory effects of *Ascaris* and trypanosomes on alarm calling propensity suggest that modeling just a binary activation/

Table 3. Results from generalized linear mixed models of in-trap alarm calling as a function of NL ratio interacting with sex

Fixed Effects

	Estimate	Std. Error	z value	Pr($>$|z|)
(Intercept)	−2.792	0.316	−8.841	2e−16
Log(NL ratio+1)	−0.165	0.304	−0.543	0.587
Sex (male)	0.008	0.322	0.025	0.980
Age class (yearling)	0.060	0.267	0.224	0.822
Predator index (low)	−0.208	0.279	−0.747	0.455
Scaled colony size	0.077	0.107	0.719	0.472
Log(NL ratio+1)×Age class (yearling)	−0.335	0.476	−0.704	0.481
Log(NL ratio+1)×Sex (male)	0.962	0.473	2.035	0.042
Log(NL ratio+1)×Predator index (low)	0.555	0.410	1.355	0.175

Random effects	Variance	Standard deviation
ID intercept	3.916	1.979
Year (Intercept)	2.775e−14	1.66e−07

Quantified fixed effects include the $\log_{10}$(NL ratio+1), predator index, age class, scaled colony size, and sex. Interactions include $\log_{10}$(NL ratio+1)×age class, $\log_{10}$(NL ratio+1)×sex, and $\log_{10}$(NL ratio+1)×predator index. Random effects include individual identity (ID) and year of observation (year).

suppression effect on the immune system is insufficient to determine how parasites modify an individual's perception of risk. This is compounded by potential difficulties of using parasite presence and NL ratios to determine the immune fitness of an individual without an experimental approach that directly links parasite presence/absence or high/low NL ratio to reproductive fitness (Davis et al. 2008), leaving room for interesting developments.

Nevertheless, these results illustrate that the propensity to call may be influenced by a host's internal condition. Additionally, separate pathogens may have different, species/genus-specific effects on animal vocalizations; a finding that is consistent with other studies (Laiolo et al. 2007; Gilman et al. 2007; Nouri and Blumstein 2019). For example, white-crowned sparrows infected with the parasites *Leucocytozoon* or *Plasmodium* had altered song behaviors while infection with *Haemoproteus* had no detectable effect on song (Gilman et al. 2007). Therefore, all pathogens are not expected to act uniformly and could influence alarm calling in opposing ways (Atkinson and van Riper 1991).

If energetic reserves were limited uniformly by parasitic infection so that alarm calls were less likely to be produced, we would expect parasite diversity (quantified as parasite richness) to be negatively associated with alarm call propensity. However, parasite diversity was not associated with alarm call propensity. Alternatively, the degree and severity of parasitic infection could affect individual alarm calling (Kennedy et al. 1987; Møller 1991). Parasites could vary in their ability to suppress/activate the immune system of their host and could vary in the extent they reduce the energetic reserves of their host. This may be due to variation in life history and the host tissues infected, which produce differing levels of virulence, as seen with hemosporidians and birds (Atkinson and van Riper 1991) and whether that tissue is part of the vocal apparatus. The diverse systems that parasitic species associate with, that is that *T. lewisi* is carried in the blood while *Ascaris* is carried in the gut, could help resolve their opposing associations with alarm call propensity (Albright et al. 1990; Faquim-Mauro and Macedo 1998). Additionally, parasite load and infection pattern may also interact with other individual characteristics, for example, exhibiting sex-specific effects when influencing alarm calling (Lopez et al. 2013). Consequently, future studies could expand upon our findings and examine how parasite load (which was unmeasured in our study)

and infection pattern, not just presence or richness, is associated with calling.

NL ratio was positively associated with in-trap alarm calling but only in males. Immune system activation may act in conjunction with the additional stress that male marmots experience to increase perceived risk and elicit alarm calls. Interestingly, prior work has shown that male marmots have higher baseline glucocorticoid levels than females (Smith et al. 2012). Prior work demonstrating that female marmot calls are noisier than male calls supports increased stress levels in males, as more stressed marmots produce less noisy calls (Blumstein and Chi 2012). This may be due to differing social strategies, as males use aggression to gain social position while females do not (Wey and Blumstein 2012). Additionally, male's increased calling propensity could make them more conspicuous to predators, influencing fitness through increased mortality from predation.

However, it is important to note that the magnitudes of these condition-dependent effects are modest and these health factors are likely not the main drivers of alarm call propensity and probability. Marmot identity explained most of the variation in wild rates of calling and more variation than all of our fixed effects for in-trap calling. Other factors that vary individually, such as social status (Fuong et al. 2015) or temperament (Couchoux et al. 2017), play a more substantial role in generating variation in alarm calling propensity and probability. Social status and health characteristics could act additively to affect alarm calling propensity. Animals that are infected with trypanosomes and in lower social standing could call even more (Hare and Atkins 2001). Regardless of the effects of other external factors, the numerous documented effects of parasites, pathogens, and internal state on alarm calling strongly suggest that alarm calling is condition dependent.

Additionally, there are health factors other than parasite infection that could potentially affect alarm calling, such as metabolic rates, mass, and testosterone levels. Testosterone has been demonstrated to positively correlate with the sexually selected "rusty gate call" of the gray partridge, *Perdix perdix* (Fusani et al. 1994) and increases the production of aerial alarm calls in male domestic fowl, *Gallus gallus* (Gyger et al. 1988). Testosterone focused analyses are a logical next step in examining how internal factors contribute to alarm calling in marmots. Nevertheless, these results have a number

of exciting implications for receivers of these condition-dependent alarm calls.

One such implication is the varying effect of information in alarm calls based on who receives the call. Most condition-dependent vocal signals that have been studied are sexually selected (Fusani et al. 1994; Beani and Dessi-Fulgheri 1995). However, in the case of alarm calls, information is not specifically broadcast to a potential sexual partner, but rather to many different types of receivers, including conspecifics, heterospecifics, and predators. In marmots, alarm calls not only encode a signal of danger but also information on the condition of the caller through call characteristics (Nouri and Blumstein 2019) and the propensity of an individual to alarm call. This additional information could have different implications depending on the receiver. Conspecifics could increase their estimates of predation risk and caller reliability. Information about health contained in calls could increase a receiver's ability to distinguish the risk given a caller's health status or may reduce the certainty about the true predation risk. Heterospecifics that eavesdrop may suffer greater predation pressure when a caller population is sick and calling less, or they may lose foraging opportunities if a sick caller population calls more. Predators may be able to clue in on and target individuals who are in poorer health, or they might be distracted by sick marmots bluffing about their health (Pettorelli et al. 2011).

Condition-dependent alarm signals may provide information about health status in addition to predation risk, and how this information is perceived and used by different receivers deserves further attention. Understanding what information is broadcast not only to other conspecifics, but to the entire acoustic community, offers an exciting new frontier in animal communication. Further study should conduct playback experiments to determine if these, admittedly modest, health-driven differences in propensity are used by receivers.

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Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

Conflict of Interest statement

The authors report no conflicts of interest.

References

Albright JW, Holmes KL, Albright JF, 1990. Fluctuations in subsets of splenocytes and isotypes of Ig in young adult and aged mice resulting from Trypanosoma musculi infections. *J Immunol* **144**:3970–3979.

Armitage KB, 2010. Individual fitness, social behavior, and population dynamics of yellow-bellied marmots. In: Billick I, Price MV, editors. *The Ecology of Place: Contributions of Place-Based Researcher to Ecological Understanding*. Chicago (IL): University of Chicago Press. 134–154.

Atkinson CT, van Riper C III, 1991. Pathogenicity and epizootiology of avian haematozoa: *Plasmodium*, *Leucocytozoon*, and *Haemoproteus*. In: Loye JE, Zuk M, editors. *Bird–Parasite Interactions: Ecology, Evolution, and Behavior*. Oxford: Oxford University Press. 19–48.

Bates D, Maechler M, Bolker B, Walker S, 2014. lme4: linear mixed-effects models using Eigen and S4. R package version 1.1.7 [cited 2019 August 20]. Available from: http://www.r-project.org/.

Beani L, Dessi-Fulgheri F, 1995. Mate choice in the grey partridge *Perdix perdix*: role of physical and behavioural male traits. *Anim Behav* **49**:347–356.

Bercovitch FB, Hauser MD, Jones JH, 1995. The endocrine stress response and alarm vocalizations in rhesus macaques. *Anim Behav* **49**:1703–1706.

Berdoy M, Webster JP, Macdonald DW, 2000. Fatal attraction in rats infected with *Toxoplasma gondii*. *Proc R Soc B* **267**:1591–1594.

Blumstein DT, Armitage KB, 1997. Alarm calling in yellow-bellied marmots: i. The meaning of situationally variable alarm calls. *Anim Behav* **53**: 143–171.

Blumstein DT, Chi YY, 2012. Scared and less noisy: glucocorticoids are associated with alarm call entropy. *Biol Lett* **8**:189–192.

Blumstein DT, Patton ML, Saltzman W, 2006. Faecal glucocorticoid metabolites and alarm calling in free-living yellow-bellied marmots. *Biol Lett* **2**: 29–32.

Blumstein DT, Nguyen KT, Martin JGA, 2013. Ontogenetic variation of heritability and maternal effects in yellow-bellied marmot alarm calls. *Proc R Soc B* **280**:20130176.

Caro T, 2005. *Antipredator Defenses in Birds and Mammals*. Chicago (IL): University of Chicago Press.

Chmura HE, Wey TW, Blumstein DT, 2016. Assessing the sensitivity of foraging and vigilance to internal state and environmental variables in yellow-bellied marmots (*Marmota flaviventris*). *Behav Ecol Sociobiol* **70**: 1901–1910.

Collier TC, Blumstein DT, Girod L, Taylor CE, 2010. Is alarm calling risky? Marmots avoid calling from risky places. *Ethology* **116**:1171–1178.

Coss RG, McCowan B, Ramakrishnan U, 2007. Threat-related acoustical differences in alarm calls by wild bonnet macaques (*Macaca radiata*) elicited by python and leopard models. *Ethology* **113**:352–367.

Couchoux C, Clermont J, Garant D, Réale D, 2017. Signaler and receiver boldness influence response to alarm calls in eastern chipmunks. *Behav Ecol* **29**:212–220.

Davis AK, Maney DL, Maerz JC, 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. *Func Ecol* **22**: 760–772.

Digweed SM, Rendall D, 2009. Predator-associated vocalizations in North American red squirrels, *Tamiasciurus hudsonicus*: are alarm calls predator specific?. *Anim Behav* **78**:1135–1144.

Endler JA, 1987. Predation, light intensity and courtship behaviour in *Poecilia reticulata* (Pisces: Poeciliidae). *Anim Behav* **35**:1376–1385.

Faquim-Mauro EL, Macedo MS, 1998. The immunosuppressive activity of Ascaris suum is due to high molecular weight components. *Clinical and Experimental Immunology* **114**:245–251.

Fedorka KM, Mousseau TA, 2006. Immune system activation affects male sexual signal and reproductive potential in crickets. *Behav Ecol* **18**: 231–235.

Fusani L, Beani L, Dessi-Fulgheri F, 1994. Testosterone affects the acoustic structure of male call in the grey partridge *Perdix perdix*. *Behaviour* **128**: 301–310.

Fuong H, Maldonado-Chaparro A, Blumstein DT, 2015. Are social attributes associated with alarm calling propensity?. *Behav Ecol* 26:587–592.

Gilman S, Blumstein DT, Foufopoulos J, 2007. The effect of hemosporidian infections on white-crowned sparrow singing behavior. *Ethology* 113:437.

Gyger M, Karakashian SJ, Dufty AM Jr, Marler P, 1988. Alarm signals in birds: the role of testosterone. *Horm Behav* 22:305–314.

Hare JF, Atkins BA, 2001. The squirrel that cried wolf: reliability detection by juvenile Richardson's ground squirrels (*Spermophilus richardsonii*). *Behav Ecol Sociobiol* 51:108–112.

Hedrick AV, 2000. Crickets with extravagant mating songs compensate for predation risk with extra caution. *Proc R Soc B* 267:671–675.

Hirokawa K, Eishi Y, Albright JW, Albright JF, 1981. Histopathological and immunocytochemical studies of Trypanosoma musculi infection of mice. *Infection and Immunity* 34:1008–1017.

Hoogland JL, 1995. *The Black-Tailed Prairie Dog: Social Life of a Burrowing Mammal*. Chicago (IL): University of Chicago Press.

Kennedy CEJ, Endler JA, Poynton SL, McMinn H, 1987. Parasite load predicts mate choice in guppies. *Behav Ecol Sociobiol* 21:291–295.

Kuznetsova A, Brockhoff PB, Christensen RHB, 2018. *lmerTest: Tests in Linear Mixed Effects Models*. R package version 3.0–1.

Laiolo P, Serrano D, Tella JL, Carrete M, Lopez G, Navarro C, 2007. Distress calls reflect poxvirus infection in lesser short-toed lark *Calandrella rufescens*. *Behav Ecol* 18:507–512.

Lejeune M, Rybicka JM, Chadee K, 2009. Recent discoveries in the pathogenesis and immune response toward Entamoeba histolytica. *Future Microbiology* 4:105–118.

Li C, Monclús R, Maul TL, Jiang Z, Blumstein DT, 2011. Quantifying human disturbance on antipredator behavior and flush initiation distance in yellow-bellied marmots. *Applied Animal Behaviour Science* 129:146–152.

Lopez J, Wey TW, Blumstein DT, 2013. Patterns of parasite prevalence and individual infection in yellow-bellied marmots. *J Zool* 291:296–303.

Maceda-Veiga A, Figuerola J, Martínez-Silvestre A, Viscor G, Ferrari N, Pacheco M, 2015. Inside the Redbox: applications of haematology in wildlife monitoring and ecosystem health assessment. *Sci Total Environ* 514: 322–332.

MacNeal WJ, 1904. The life-history of *Trypanosoma lewisi* and *Trypanosoma brucei*. *J Infect Dis* 1:517–543.

Marler P, 1955. Characteristics of some animal calls. *Nature* 176:6–8.

Marler P, Evans C, 1996. Bird calls: just emotional displays or something more? *Ibis* 138:26–33.

Martin LB, Scheuerlein A, Wikelski M, 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proc R Soc B* 270:153–158.

Matrosova VA, Volodin IA, Volodina EV, Vasilieva NA, 2010. Stability of acoustic individuality in the alarm calls of wild yellow ground squirrels *Spermophilus fulvus* and contrasting calls from trapped and free-ranging callers. *Naturwissenschaften* 97:707–715.

Møller AP, 1991. Parasite load reduces song output in a passerine bird. *Anim Behav* 41:723–730.

Munoz NE, Blumstein DT, Foufopoulos J, 2010. Immune system activation affects song and territorial defense. *Behav Ecol* 21:788–793.

Navarro C, De Lope F, Marzal A, Møller AP, 2004. Predation risk, host immune response, and parasitism. *Behav Ecol* 15:629–635.

Nouri K, Blumstein DT, 2019. Parasites are associated with noisy alarm calls. *Front Ecol Evol* 7:1–6.

Pettorelli N, Coulson T, Durant SM, Gaillard JM, 2011. Predation, individual variability and vertebrate population dynamics. *Oecologia* 167: 305–314.

R Core Team, 2018. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. Available online at: https://www.R-project.org/.

Randall JA, Rogovin KA, Shier DM, 2000. Antipredator behavior of a social desert rodent: foot-drumming and alarm calling in the great gerbil, *Rhombomys opiums*. *Behav Ecol Sociobiol* 48:110–118.

Ridley AR, Child MF, Bell M, 2007. Interspecific audience effects on the alarm-calling behaviour of a kleptoparasitic bird. *Biol Lett* 6:589–591.

Scheuber H, Jacot A, Brinkhof MWG, 2003. Condition dependence of a multi-component sexual signal in the field cricket *Gryllus campestris*. *Anim Behav* 65:721–727.

Searle SR, Speed FM, Milliken GA, 1980. Population marginal means in the linear model: an alternative to least squares means. *Am Stat* 34:216–221.

Sehrsweeney M, Wilson DR, Bain M, Boutin S, Lane JE et al, 2019. The effects of stress and glucocorticoids on vocalizations: a test in North American red squirrels. *Behav Ecol* 30:1030–1040.

Smith JE, Monclús R, Wantuck D, Florant GL, Blumstein DT, 2012. Fecal glucocorticoid metabolites in wild yellow-bellied marmots: Experimental validation, individual differences and ecological correlates. *General and Comparative Endocrinology* 178:417–426.

Soboslay PT, Hamm DM, Pfäfflin F, Fendt J, Banla M, Schulz-Key H, 2006. Cytokine and chemokine responses in patients co-infected with Entamoeba histolytica/dispar, Necator americanus and Mansonella perstans and changes after anti-parasite treatment. *Microbes and Infection* 8:238–247.

Tilson RL, Norton PM, 1981. Alarm duetting and pursuit deterrence in an African antelope. *Am Nat* 118:455–462.

Wey TW, Blumstein DT, 2012. Social attributes and associated performance measures in marmots: bigger male bullies and weakly affiliating females have higher annual reproductive success. *Behav Ecol Sociobiol* 66: 1075–1085.

Wich SA, Sterck EHM, 2003. Possible audience effect in Thomas langurs (primates; *Presbytis thomasi*): an experimental study on male loud calls in response to a tiger model. *Am J Primitol* 60:155–159.

Yun C, 2000. Intestinal immune responses to coccidiosis. *Developmental & Comparative Immunology* 24:303–324.