Is the Propensity to Emit Alarm Calls Associated with Health Status in Yellow-Bellied Marmots,

Marmota flaviventer?

By

Austin Nash

Ecology and Evolutionary Biology, University of Colorado at Boulder

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Thesis Advisor:

Dr. Christy McCain, Ecology and Evolutionary Biology

Defense Committee:

Dr. Christy McCain, Ecology and Evolutionary Biology

Dr. Jaelyn Eberle, Geological Sciences

Dr. Pieter Johnson, Ecology and Evolutionary Biology

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.

Introduction

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 to 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

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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 et al. 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 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; Maceida-Vega 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 2007), 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 1987; 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 towards, 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 neutrophil to lymphocyte (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 neutrophil to lymphocyte ratio would be associated with an increased probability of alarm calling while *Ascaris*, *Entamoeba*, and *Trypanosoma* would generate the opposite effect.

Hypotheses

We predicted that: (1) increased neutrophil to lymphocyte 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), and to mark individuals with Nyanzol fur dye (Albinal Dyestuff Inc., Jersey City, NJ) 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 two hours 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 neutrophil to lymphocyte ratios in blood samples (Davis et al. 2008), with an increase in the ratio of neutrophil to lymphocyte cells occurring due to stress or immune system activation (Maceida-Vega et al. 2015). Neutrophil to lymphocyte (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 six months of collection by performing fecal floats using Ova FloatTM 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), three fecal-orally transmitted parasites (MacNeal 1904) and intestinal parasite richness was calculated by the sum of these three 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 one minute) and the identity of the caller, when possible, using 15–45x 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. 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 in that year for that individual.

We used the lme4 1.1-18-1 (Bates and Maechler 2018) 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: neutrophil to lymphocyte 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 yr olds) or adult (≥2 yr olds), as we excluded young of the year (i.e., 0 yr 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 > 4x 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 neutrophil to lymphocyte ratio + 1, the presence of *Ascaris,* the presence of *Eimeria,* the presence of *Trypanosoma,* the 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. 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 *Trypanosoma lewisi* influenced

natural calling rate while controlling for the influence of other independent variables (Searle et

al. 1979).

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, 2, and 3 and summaries of the raw data for presence, absence in-trap

models are provided in the supplementary material (Supplementary Table 9). 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 with

trypanosomes naturally called at marginally higher rates (t = 1.71, df = 890.653, *p* = 0.087; Table

2A; 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 upwards 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).

Discussion

While marmot identity explained most of the variation in alarm calling, some health conditions 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, 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, Table 3) whilst, 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/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 therefore 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 haemosporidians 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; Wey and Blumstein 2007). 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 grey 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) and is 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.

Acknowledgements

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Tables and Figures

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. 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).

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	Std. Dev.
ID (Intercept)	93.94	9.692

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. 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) results from linear mixed effects models of natural alarm calling as a function of trypanosome presence. Fixed and random effects are identical to A.

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	0.4879

Random Effects

	Variance	Std. Dev.
ID (Intercept)	3.966	1.992
Year (Intercept)	5.224e-14	2.286e-07

B) Natural Calling
Fixed Effects

	Estimate	Std. Error	df	t value	Pr(>|t|)
(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	Std. Dev.
ID Intercept	3.757	1.938
Year (Intercept)	1.461	1.209

Table 3. Results from generalized linear mixed models of in-trap alarm calling as a function of NL ratio interacting with sex. 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) X age class, $\log_{10}$ (NL ratio + 1) X sex, and $\log_{10}$ (NL ratio + 1) X predator index. Random effects include individual identity (ID) and year of observation (year).

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) X Age class (yearling)	-0.335	0.476	-0.704	0.481
Log(NL ratio + 1) X Sex (male)	0.962	0.473	2.035	0.042
Log(NL ratio + 1) X Predator index (low)	0.555	0.410	1.355	0.175

Random Effects

	Variance	Std. Dev.
ID Intercept	3.916	1.979
Year (Intercept)	2.775e-14	1.66e-07

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) shows the raw distribution of the data before transformation and (B) the marginal means ± SE of 1/sqrt(natural alarm calling rate) for marmots who were infected (present) and uninfected (absent) with *T. lewisi*. Significance (p value) of the model, calculated from the transformed data, is included at the top of the figure.
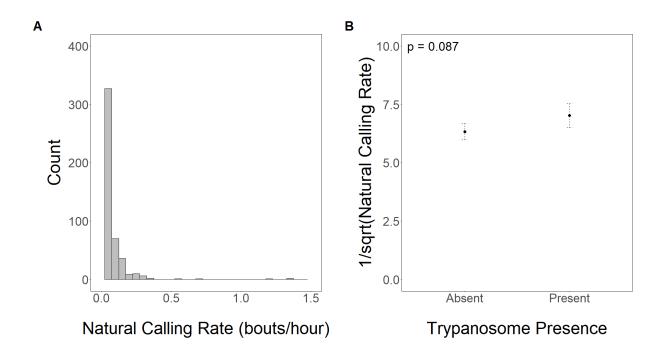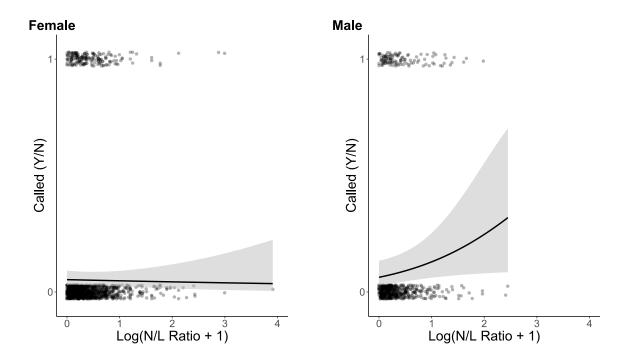
Figure 2. Relationships between $\log_{10}$ (NL ratio + 1) and probability of calling in trap for female and male marmots. NL ratio is positively associated with natural calling rate but only in male marmots. Black lines show the predicted effects from Table 3 (±SE), demonstrating the probability of 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.

Supplementary Materials

Supplementary Table 1. Results from linear mixed effects models of natural alarm calling as a function of *Ascaris* presence. Quantified fixed effects include the presence of *Ascaris* (0,1), predator index, age class, scaled colony size, and sex. Random effects include individual identity (ID) and year of observation (year).

Fixed Effects

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	5.935	0.400	12.375	15.005	2.61e-09
Ascaris	-0.022	0.388	326.028	-0.057	0.955
Sex (male)	0.470	0.423	101.693	1.112	0.269
Age class (yearling)	0.651	0.310	298.422	2.103	0.036
Predator index (low)	0.664	0.398	223.707	1.668	0.097
Scaled colony size	0.972	0.185	133.556	5.250	5.85e-07

Random Effects

	Variance	Std. Dev.
ID Intercept	2.520	1.587
Year (Intercept)	0.445	0.667

Supplementary Table 2. Results from linear mixed effects models of natural alarm calling as a function of NL ratio. Quantified fixed effects include the log(NL ratio + 1), predator index, age class, scaled colony size, and sex. Random effects include individual identity (ID) and year of observation (year).

Fixed Effects

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	5.210	0.391	24.166	13.322	1.25e-12
log(NL ratio + 1)	-0.091	0.168	771.960	-0.541	0.588
Sex (male)	-0.117	0.300	237.111	-0.391	0.696
Age class (yearling)	1.311	0.179	914.786	7.324	5.29e-13
Predator index (low)	1.202	0.221	776.729	5.435	7.33e-08
Scaled colony size	0.507	0.121	625.130	4.180	3.33e-05

Random Effects

	Variance	Std. Dev.
ID Intercept	3.763	1.940
Year (Intercept)	1.433	1.197

Supplementary Table 3. Results from generalized linear mixed models and linear mixed effects models of trap calling as a function of *Eimeria* presence, in yellow-bellied marmots. Quantified fixed effects include the presence of Eimeria (0,1), predator index, age class, scaled colony size, and sex. Random effect is individual identity (ID).

Fixed Effects

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-8.713	1.297	-6.718	1.84e-11
Eimeria	0.857	0.840	1.020	0.308
Sex (male)	0.464	0.857	0.541	0.588
Age class (yearling)	0.124	0.459	0.271	0.786
Predator index (low)	0.260	0.541	0.480	0.631
Scaled colony size	0.193	0.344	0.562	0.574

Random Effects

	Variance	Std. Dev.
ID (Intercept)	81.22	9.012

Supplementary Table 4. Results from generalized linear mixed models and linear mixed effects models of trap calling as a function of *Entamoeba* presence, in yellow-bellied marmots. Quantified fixed effects include the presence of Entamoeba (0,1), predator index, age class, scaled colony size, and sex. Random effect is individual identity (ID).

Fixed Effects

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-8.565	1.245	-6.879	6.02e-12
Entamoeba	0.766	0.782	0.980	0.327
Sex (male)	0.420	0.856	0.491	0.624
Age class (yearling)	0.159	0.459	0.346	0.729
Predator index (low)	0.380	0.536	0.710	0.478
Scaled colony size	0.178	0.344	0.519	0.604

Random Effects

	Variance	Std. Dev.
ID (Intercept)	79.1	8.894

Supplementary Table 5. Results from generalized linear mixed models and linear mixed effects models of trap calling as a function of parasite richness, in yellow-bellied marmots. Quantified fixed effects include the parasite richness, predator index, age class, scaled colony size, and sex. Random effect is individual identity (ID).

Fixed Effects

	Estimate	Std. Error	z value	Pr(>|z|)
(Intercept)	-7.059	1.192	-5.924	3.14e-09
Parasite Richness	-0.441	0.406	-1.084	0.278
Sex (male)	0.493	0.863	0.571	0.568
Age class (yearling)	0.065	0.459	0.143	0.887
Predator index (low)	0.375	0.538	0.697	0.486
Scaled colony size	0.137	0.343	0.401	0.689

Random Effects

	Variance	Std. Dev.
ID (Intercept)	82.06	9.059

Supplementary Table 6. Results from linear mixed effects models of natural alarm calling as a function of *Eimeria* presence. Quantified fixed effects include the presence of *Eimeria* (0,1), predator index, age class, scaled colony size, and sex. Random effects include individual identity (ID) and year of observation (year).

Fixed Effects

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	6.271	0.619	57.488	10.131	2.11e-14
Eimeria	-0.367	0.514	297.727	-0.715	0.475
Sex (male)	0.466	0.421	101.756	1.106	0.271
Age class (yearling)	0.665	0.310	296.094	2.146	0.033
Predator index (low)	0.660	0.398	230.474	1.658	0.099
Scaled colony size	0.973	0.184	132.584	5.279	5.16e-07

Random Effects

	Variance	Std. Dev.
ID Intercept	2.486	1.577
Year (Intercept)	0.472	0.687

Supplementary Table 7. Results from linear mixed effects models of natural alarm calling as a function of *Entamoeba* presence. Quantified fixed effects include the presence of *Entamoeba* (0,1), predator index, age class, scaled colony size, and sex. Random effects include individual identity (ID) and year of observation (year).

Fixed Effects

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	6.150	0.535	40.479	11.487	2.59e-14
Entamoeba	-0.252	0.416	289.146	-0.606	0.545
Sex (male)	0.472	0.423	101.541	1.116	0.267
Age class (yearling)	0.650	0.310	298.719	2.100	0.037
Predator index (low)	0.654	0.398	227.648	1.641	0.102
Scaled colony size	0.967	0.185	133.587	5.217	6.77e-07

Random Effects

	Variance	Std. Dev.
ID Intercept	2.524	1.588
Year (Intercept)	0.459	0.677

Supplementary Table 8. Results from linear mixed effects models of natural alarm calling as a function of parasite diversity. Quantified fixed effects include parasite diversity, predator index, age class, scaled colony size, and sex. Random effects include individual identity (ID) and year of observation (year).

Fixed Effects

	Estimate	Std. Error	df	t value	Pr(>|t|)
(Intercept)	6.250	0.600	61.086	10.417	3.58e-15
Parasite Diversity	-0.167	0.236	309.686	-0.704	0.482
Sex (male)	0.465	0.422	102.127	1.103	0.273
Age class (yearling)	0.662	0.310	296.166	2.137	0.033
Predator index (low)	0.658	0.398	225.948	1.655	0.099
Scaled colony size	0.973	0.184	133.010	5.277	5.20e-07

Random Effects

	Variance	Std. Dev.
ID Intercept	2.495	1.579
Year (Intercept)	0.454	0.674

Supplementary Table 9. Proportional and raw (n) data for the four parasite classes (*Trypanosoma lewisi, Ascaris sp, Eimeria sp.* and *Entamoeba sp.*) recorded as present/absent for in-trap calling measures (called/did not call).

Parasite	Presence	Proportion Called (n)	Proportion Did Not Call (n)
Trypanosome	Present	0.21 (16)	0.79 (60)
	Absent	0.16 (294)	0.84 (1537)
Ascaris	Present	0.09 (8)	0.91 (81)
	Absent	0.16 (106)	0.84 (550)
Eimeria	Present	0.15 (106)	0.85 (595)
	Absent	0.18 (8)	0.82 (36)
Entamoeba	Present	0.16 (107)	0.84 (566)
	Absent	0.10 (7)	0.90 (65)