Substrates & Parasitism: The effect of soil type and moisture on the incidence on endo- and ecto-parasitism of wild-caught deer mice (*Peromyscus maniculatus*)

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Dominic J. Acri[1]

Advisor: Dr. David G. Flagel[1]

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[1]University of Notre Dame Environmental Research Center, Department of Biology, University of Notre Dame, IN 46556

Abstract

Parasites on reservoir species can prove to be a threat to human health and research in the external factors can inform management efforts when needed. Previous research suggests there is a relationship between environmental factors and incidence of parasitism on small mammals. This study explores effect of substrate type and soil moisture on the incidence of ecto- and endo-parasites of two deer mice subspecies, *Peromyscus maniculatus osgoodi* and *P. m. artemisiae*, on the National Bison Range in Western Montana. My research suggests soil moisture variation affects flea density per body mass and helminthe density in the spleen per organ mass. These relationships between ecto- and endo-parasitism and soil moisture supports the hypothesis that external factors can differentially affect parasites by type and by organ.

Introduction

Human health and small mammal ecology have often intersected throughout history, from the spread of the black plague to more recent concerns involving Lyme disease and hantavirus (Mills & Childs 1998). The prevalence of parasites in small mammals is also observable evidence for the direct human health application of mammal research; when the presence of parasites varies by spatial/ecological distribution, they can inform researchers on potential health risks in the area and guide health-related policy (Ostfeld & Holt 2004; Van Buskirk & Ostfeld 1995).

Variation in habitat conditions could explain some of the differences in parasite distributions for mammalian hosts (Hotez et al. 2006). Environmental conditions that are conducive for both parasite and host may work in addition to genetic predisposition to increase prevalence (Cooper et al. 2012; Wolinska & King 2009). For example, researchers have found that longevity and prevalence of co-evolved parasitism in primates and ungulates are related to environmental conditions such as range and social group size (Cooper et al. 2012). In the Arctic, differential mammalian host-pathogen interactions with biotic factors affected by changing climate have been documented (Hueffer et al. 2011). However, these relationships need to be more precisely studied in order to control the spread of diseases.

Small wild rodent species in particular are well-documented as major reservoirs for various parasites and diseases which also affect humans (Mills & Childs 1998; Ostfeld & Holt 2004). The deer mouse (*Peromyscus maniculatus)* is an abundant rodent host found throughout much of North America, where it is thought to be a major reservoir for both hantavirus and lyme disease. Lyme disease in particular has gained much attention as its prevalence has increased in the United States (Mills & Childs 1998; Ostfeld & Holt 2004; Van Buskirk & Ostfeld 1995). The health and presence of parasites on such a wide spread mammalian host, integral to forest communities across the continent, has been the topic of much research for the last eight decades.

However, in spite of the attention given to Lyme disease, the study of other forms of disease/parasitism in wild-caught mice has implications for human health such as water-borne helminthes and trematode-related GI infections (Ogunniyi et al. 2014; Schwanz 2006). Elton et al. (1931) set the precedent for the study of rodent disease ecology in the field, indexing rodent health and parasite incidence. Their research yielded instructive tables that can inform of the health implication associated with each parasite, a practice that others soon followed (Erickson 1938). Expanding this research to the deer mouse population in Western Montana could further inform the plausible risks that parasite-borne diseases such as Lyme disease and countless others have for human health.

Much of the current research being done on small mammal parasites focuses upon the landscape distribution of parasites and hosts in specific areas (Malkova and

Tantsev 2011; Guerra et al. 2016) or the effects of factors such as sexual dimorphism on prevalence of parasites (Morand 2015). By studying factors of the surrounding environment and their effect on the prevalence of parasites on mice on the National Bison Range, this study goes beyond distribution/species differences to incorporate environmental factors integral to host-parasite interactions that can be found in the literature. Helminthes that are transmitted via soil are particularly sensitive to soil moisture and relative humidity of an area; of note, egg populations of intestinal roundworms and whipworms will not develop under low soil moisture and humidity (Mascarini-Serra 2011). Therefore, the study of the potential effect of substrates on the type or incidence of parasitism focused on what could prove to be one of the limiting factors of certain parasite populations.

This study explored distribution and type of parasites present on deer mice (*Peromyscus maniculatus)* caused by variation in substrate type. This study will investigate the variations of those substrate types on presence of parasites that may spread disease. Given the effect of soil moisture on ectoparasites and endoparasites (Robbins & Barker 1974; Ryckman 1971), I hypothesized that substrates of increased moisture would correspond with an increase in the presence of parasites differentially in separate organs.

Methodology

Study Site

Trapping grids were haphazardly selected on 0.3-0.5 acre plots, of relatively similar elevation (Table 1) with differing substrate type: Sandy Loam, Loam, and Silt Loam. The soils of the National Bison Range are low in clay, which limits the variety of substrate types (Figure 1). Moisture content (%) was measured via probe at approximately 10 cm below the soil (Omega Engineering, Stamford, CT, USA) and soil samples were taken at each site periodically before and throughout trapping to confirm the substrate type (Figure 2, Table 1). Substrate type was identified using a flowchart (Thien 1979; USDA 2014). Each plot was assessed for size (via Google Maps; Google Inc., Santa Clara, CA) and approximate population density. The time of trapping and the sample size at each location was used to compare the relative densities so that the analysis can include discussion of any confounding effects.

Trapping grids of 3 x 3 squares with 5 meters between were placed with the center of the grid at least 50 meters from the road. After three weeks, some of these grids were expanded to 5 x 5 in order to contend with low trapping rates, as per permission given by National Bison Range officials (A. Lisk, personal communications). Five sites were selected based on existing differential moistures and substrate types (Figure 2). After the initial round of trapping, one unsuccessful site (no *Peromyscus* caught after 3 nights of trapping) was replaced with an alternate.

The populations surveyed at the five sites were dominated by the subspecies *P.m. osgoodi*, although *P. m. artemisiae* was also present. These subspecies vary considerably in morphological characteristics, so identification was done based on tail length, foot length, tail length, and color consistent with local averages (Glazier 1971). Although pseudoreplication would be a concern to accurately describe the factors that affect species differences and the following parasitic analysis, study sites were limited by IACUC protocol; however, as is the case with other animal behavior studies, the concept of pseudoreplication overlooks the appropriate depth and statistical independence and is therefore not a concern (Schank et al. 2009).

Animal Trapping

P. maniculatus specimens were trapped using Victor snap traps (wooden base 4.5 × 9.7 cm; Woodstream Corporation, Lititz, PA, USA) baited with peanut butter. All captured animals were sexed, weighed, aged (juvenile, lactating female, pregnant female, adult), and identified by ear length, tail length and hind leg length (Smith & Carpenter 2009; Glazier 1971). Mice were analyzed for presence of parasites directly after trapping. The specimens were assessed for non-specific health concerns, presence of ectoparasites, and presences of helminthes. Non-specific health was assessed and recorded when non-parasitic illness was observed; which included recording organ mass (Elton et al. 1931). All animal work was done under University of Notre Dame IACUC protocol 17-04-3820 and under permit from the National Bison Range, the Montana Department of Fish Wildlife and Parks, and the Confederated Salish and Kootenai Tribes.

Ectoparasite and Helminthe identification

Each ectoparasite was identified to order. The presence of ticks (Acari), fleas (Siphonaptera), and mites (Phthiraptera) were recorded under a dissecting microscope, consistent the methods described in Mize et al. (2011). Dissections were done for helminthes using a standard dissecting kit and dissecting microscope. The heart, kidney, large intestine, liver, lung, small intestine, spleen, and stomach of each mouse was removed, massed, and cut lengthwise to look for helminthes (Smith & Carpenter

2009). All endoparasites were counted and standardized by calculating the density of all endoparasites in each organ.

Statistical analyses

Parasite analysis with regards to substrate type and moisture was recorded by indexing the density of parasites per organ in animals found at each site in order to normalize parasitism against size of the animal. Due to a large number of zeroes in the parasite data, the Theil-Sen estimator, a nonparametric regression designed to deal with datasets dominated by zeroes, was performed to test the interaction between density and moisture (Theil 1950; Sen 1968). ANOVA was used to analyze differences in site, moisture, substrate type, animal weight (as a proxy for age), and total parasites found to eliminate any confounding factors.

Results

A total of 43 (33 male and 10 female) mice were caught on trapping sites (Figure 1) and assessed for overall health and parasitism from June to July 2016. Two non-targets, both *Microtus spp.*, were trapped and disposed of properly as per protocol at the National Bison Range (A. lisk, personal communications). The weights of the mice caught at each site, as a proxy for age, were normally distributed ($W = 0.9740$, $p > 0.1$), an ANOVA showed no significant differences ($F_{4,38} = 1.673$, $p > 0.1$). Two separate ANOVA tests of moisture at each site and substrate type showed that significant differences between moisture at each site ($F_{4,25} = 23.43$, $p < 0.001$) and for each substrate type ($F_{2,27} = 5.267$, $p < 0.01$). Tukey HSD post-hoc analysis shows a relationship between site, substrate, and moisture illustrated in Figure 2 and Table 1.

The presence of ectoparasites failed the Shapiro-Wilk normality test for each class (flea, W = 0.3724, $p < 0.1$; mite, W = 0.2834, $p < 0.1$; tick, W = 0.5961, $p < 0.1$) and could not be normalized. Nonparametric analysis via Theil-Sen estimator reported a significant ($p < 0.1$) relationship between soil moisture and density of ectoparasites (flea, V = 3, $p = 0.0719$; mite, V = 33, $p > 0.1$; tick, V = 39, $p > 0.1$; intercept, V = 26, $p = 0.119$, Figure 3).

The presence of helminthic parasites failed the Shapiro-Wilk normality test for each organ (heart, W = 0.1644, $p < 0.1$; kidney, W = 0.1397, $p < 0.1$; large intestine, W = 0.5258, $p < 0.1$; liver, W = 0.3432, $p < 0.1$; small intestine, W = 0.2650, $p < 0.1$; spleen, W = 0.3675, $p < 0.1$; stomach, W = 0.2627, $p < 0.1$) and could not be normalized. Nonparametric analysis via Theil-Sen estimator reported a significant relationship between soil moisture and density of helminthes in the spleen (V = 4, $p = 0.0164$; Figure 4a), but not for any other organ (heart, V = 6, $p > 0.1$; kidney, V = 1, $p > 0.1$; large intestine, V = 46, $p > 0.1$; liver, V = 3, $p > 0.1$; small intestine, V = 48, $p > 0.1$; stomach, V = 3, $p > 0.1$) (Figure 4b-g). No statistics were reported for the density of lung parasites as no helminthes were found in any of the 43 sets of lungs dissected. The number of total parasites failed the Shapiro-Wilk normality test and did not show a significant relationship with respect to moisture (W = 0.5258, $p < 0.1$; V = 318, $p > 0.1$; Figure 4h).

Shapiro-Wilk normality tests on the plausible confounding site-, species-, and age-specific factors showed that all these factors were normally distributed (weight, W = 0.9744, $p > 0.1$). An ANOVA run between the total number of parasites and the plausible confounding factors showed no significant differences (weight, $F_{1,41} = 1.174$, $p > 0.1$; site, $F_{4,38} = 0.329$, $p > 0.1$; species, $F_{1,41} = 0.390$, $p > 0.1$).

Discussion

Significant differences were found between moisture and sites, indicating that despite the lack of variation the moisture varied beyond chance and could be used as an independent variable in the study. Similar studies of parasitism have accounted for soil type to a lesser extent: studies of flea parasitism on small mammals in Central Kenya differentiated soil type by sand:silt ratio and productivity (Young et al. 2015). In place of a standard description of substrate type in similar studies, soil moisture and type were the factors named and compared in this study. The low moisture observed in the study could be a function of lack of rain and the lack of a gradient could be a function of soil type homogeneity. Studies of soil type show a relationship between soil type, penetration, particle size and capability to hold moisture (Khan et al. 2011). No significant differences were found between site and weight, indicating that any confounding factors of age did not affect the relationships explored in this study.

Analysis of ectoparasites showed a significant relationship between soil moisture and number of fleas per gram body weight ($p < 0.1$), but showed no such relationship for ticks or mites (Figure 2). The trends for each of the ectoparasites surprisingly seem to be either unchanging or downward. This is contrary to the hypothesis that ectoparasites will vary proportionally with soil moisture. Previous studies have shown that ectoparasites, specifically fleas, vary by soil type, but site other factors not focused upon in this study—such as vegetation—as a reason for the variation (Young et al. 2015). Other studies show that the prevalence of all ectoparasites is correlational (Hoffmann et al. 2016), which provides some explanation for the similar trends. The spread of ectoparasites has direct implications for human health, specifically ticks and the spread of Lyme disease. As researchers continue to investigate the factors that affect incidence of parasitism on disease reservoirs, substrate type can not be ruled out as a major component.

Analysis of helminthe parasitism by organ revealed a relationship between soil moisture and density of parasites in the spleen (p = 0.0164), but showed no relationship in any of the organs or to total parasites. Previous researchers have found that parasite richness can differentially affect organs and make a particular impact on spleen parasite prevalence and size (Watermann et al 2013). This phenomenon of enlarged gastrointestinal, liver, and spleen mass with incidence of parasitism is also noted in other studies (Shwanz 2006); this may have confounded with this study's calculation of density and contributed to the lack of power in the data. Observationally, when the organs are organized by system (cardiovascular, circulatory, digestive, and lymphatic) trends can be seen, but more data would be needed to demonstrate significantly. Although this study did not specify the species of parasite, it is worth noting that parasites are organ specific and the study of external factors which affect each species prevalence need to be studied further (Ogunniyi et al. 2014).

Analysis did not show a relationship between total number of parasites and soil moisture, but incidence of non-specific health suggests that there may have been factors outside the scope of the study that affect the data. Of note, one individual was found with extreme liver cirrhosis, which along with the literature about parasitism's affect on the liver and gastrointestinal system obfuscates the data (Schwanz 2006). Where this study's question of parasitism focused on density of certain organs with respect to soil moisture, the appearance of such non-specific health effects reveals the need for behavioral, longitudal studies focusing on parasitism in organ systems.

The main limiting factor of this study is the lack of variation between study sites; due to low clay content at the study location and lack of homogeneity in the topography of different soil types, only three of twelve substrate types could be described and moisture content varied considerably with topography (grassland prairie, forest boarder, and dry creek bed). While the density of helminthic parasites per organ was researched with respect to soil moisture, factors beyond the scope of this study—such as food availability, physiological stress, pollution, and the presence of other parasite hosts—may have resulted in parasite variability or the lack thereof (Osted 2008). The study sites on which the specimens were trapped did vary in a describable way (Figure 2).

The results of this study partially supported the existence of a relationship between substrate type and incidence of parasitism. Despite limiting factors of study site, a relationship between soil moisture, flea parasitism, and parasitism in the spleen of affected mice was observed. These relationships support the hypothesis that substrate type, specifically increase soil moisture, has a correlative relationship to parasitism in *P. maniculatus.*

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Appendix

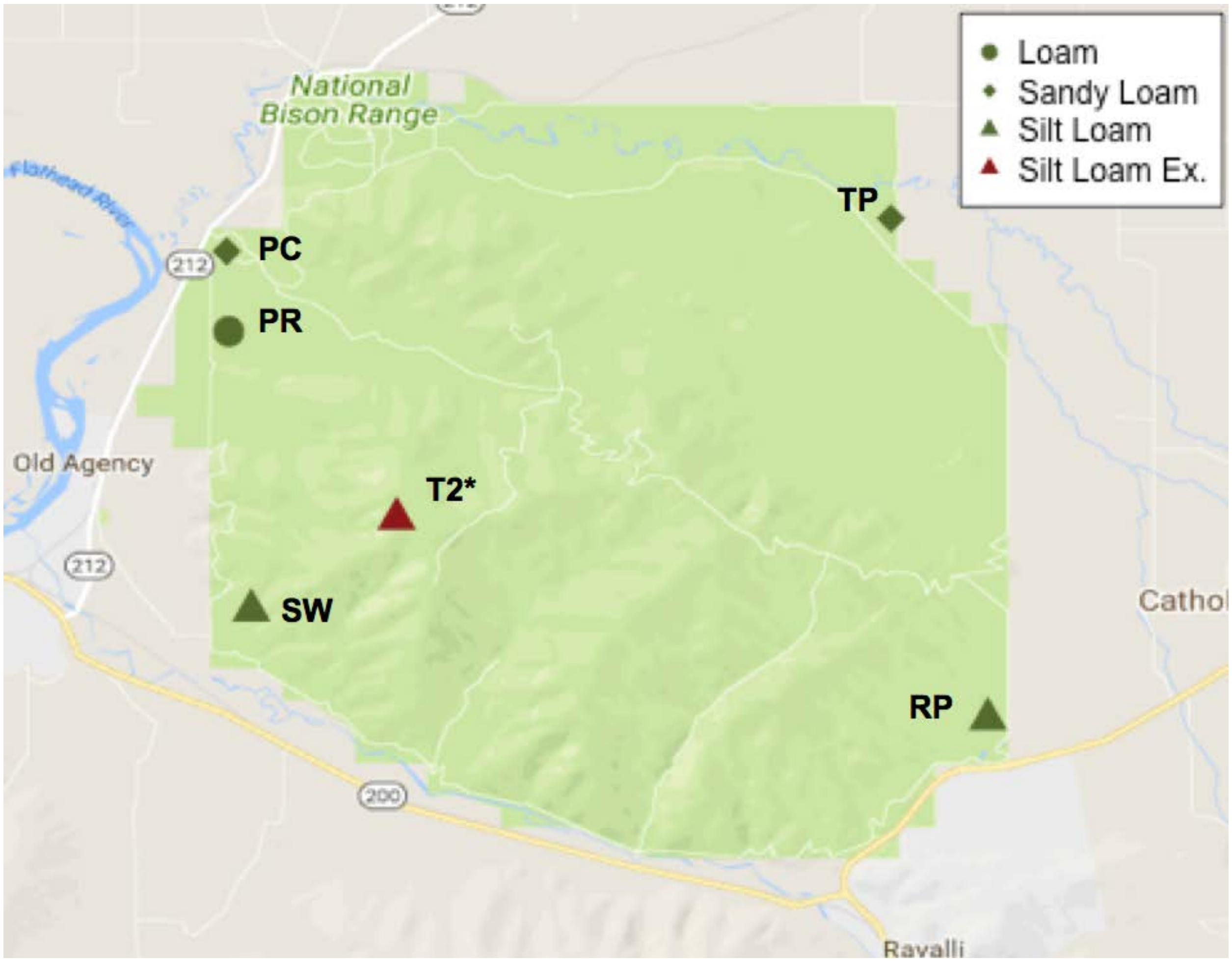

Figure 1 ***Trapping Sites on the National Bison Range.*** From June 2017 – July 2016, trapping occurred at six grassland sites characterized by soil type (Loam, Sandy Loam, Silt Loam). One site (*) was excluded after three consecutive nights without catching a target species.

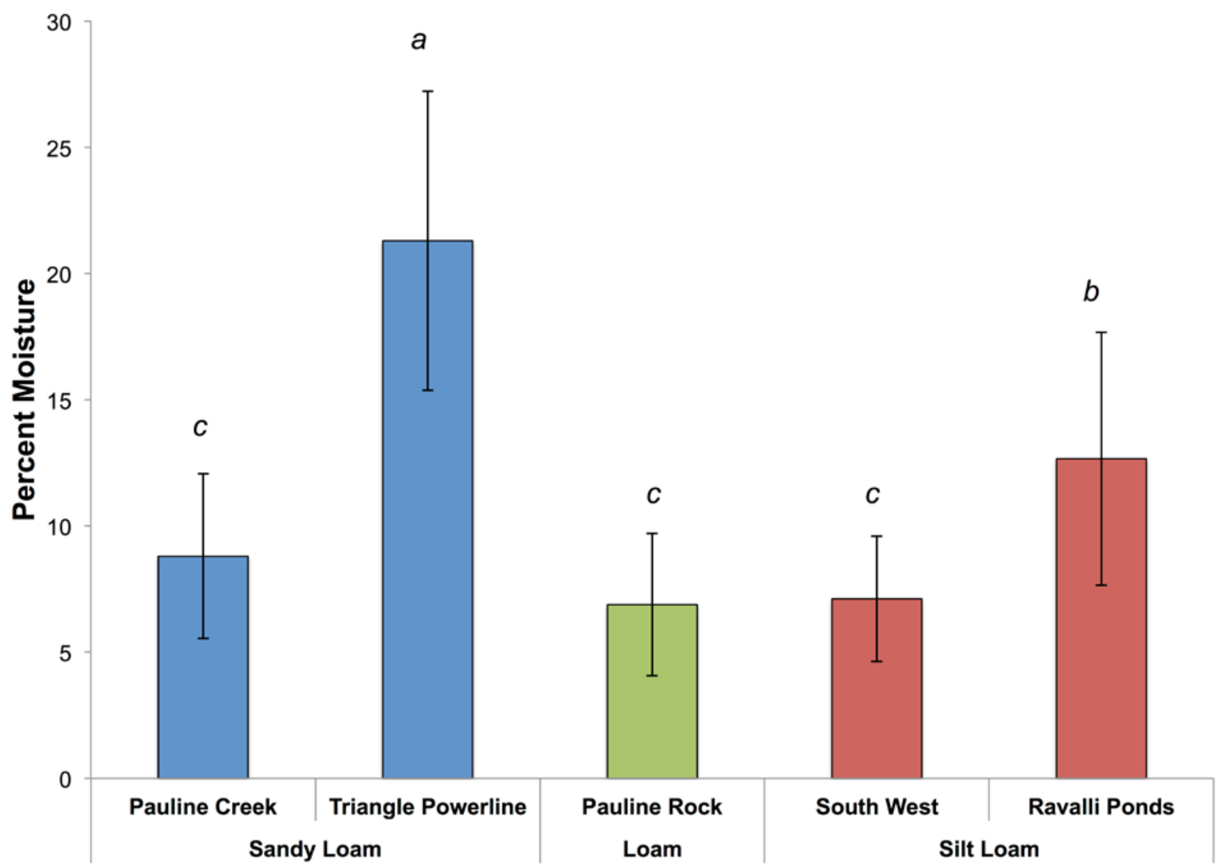

Figure 2 ***Average Percent Soil Moisture at Trapping Sites by Soil Type.*** The average percent moisture (mean ± sd) varied significantly between sites ($F_{4,25} = 23.43$, $p < 0.001$) and substrate types ($F_{2,27} = 5.267$, $p < 0.01$). Tukey HSD post-hoc analysis on sites revealed that Triangle Powerline (*a*) and Ravalli Ponds (*b*) varied significantly from the other sites; Tukey HSD post-hoc analysis on substrate type revealed that Silt Loam (red) and Sandy Loam (blue) varied significantly from the intermediary type (Loam; green) but not from each other.

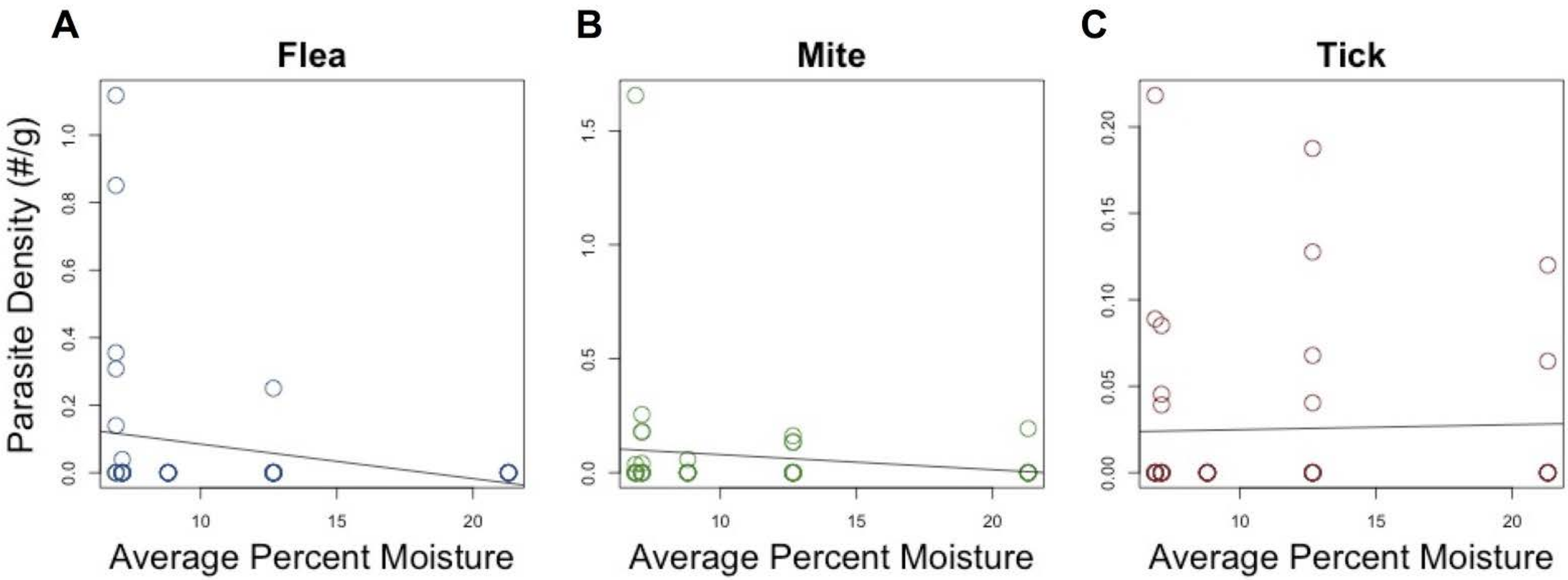

Fig. 3 ***Linear Model of Soil Moisture versus Parasite Density of Ectoparasites.*** Plot of difference in average percent moisture against ectoparasite density with linear regression line for each identified ectoparasitic class show a nearly significant relationship for fleas (A, flea, V = 3, $p = 0.0719$; B, mite, V = 33, $p < 0.1$; C, tick, V = 39, $p < 0.1$; intercept, V = 26, $p = 0.0519$).

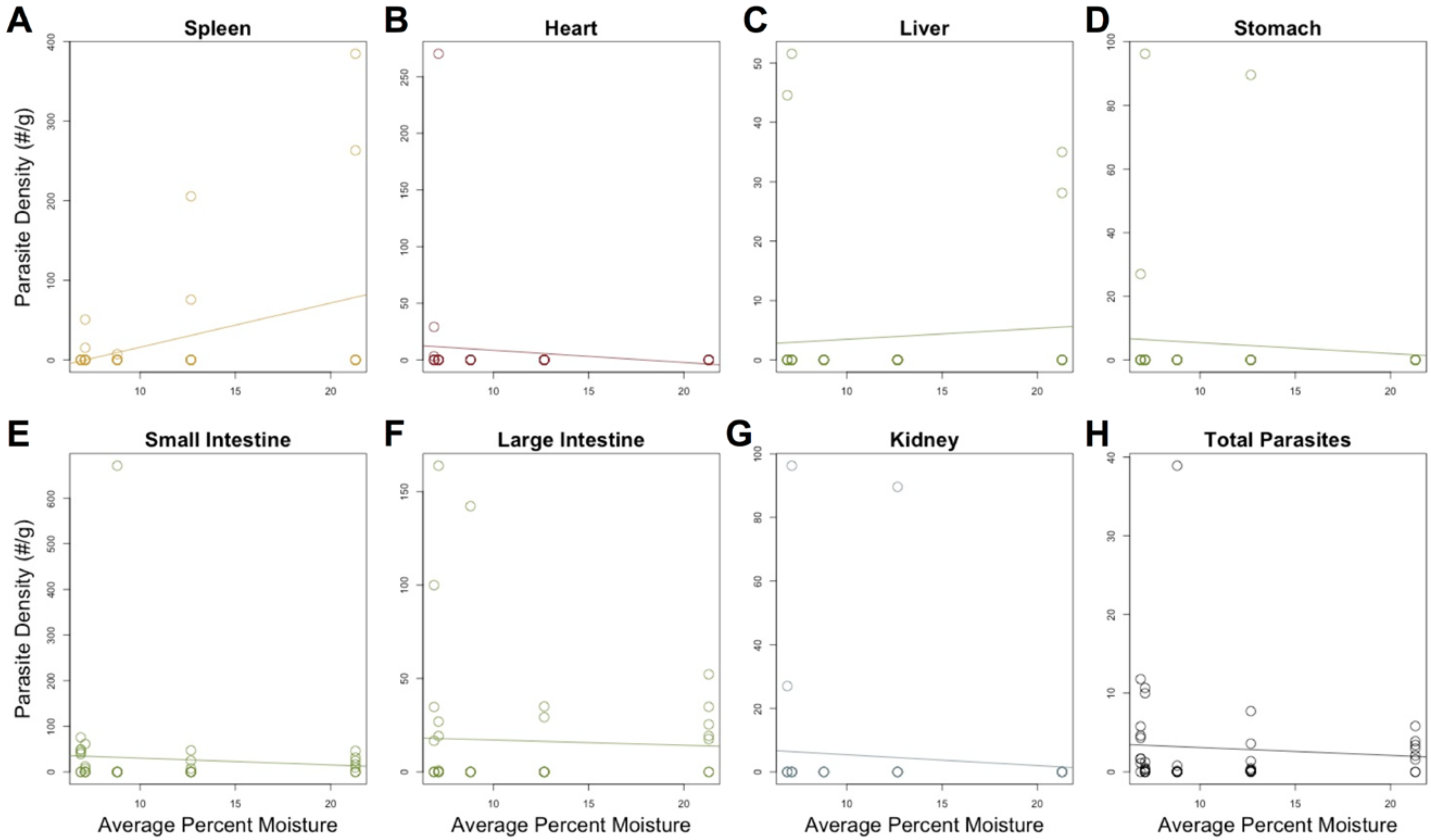

Fig. 4 ***Linear Model of Soil Moisture versus Parasite Density of Endoparasites by Organ.*** Plot of difference in average percent moisture against endoparasite density by organ with linear regression line for each identified ectoparasitic class show a nearly significant relationship for the spleen (A, spleen, V = 4, $p = 0.0164$; B, heart, V = 6, $p > 0.05$; C, liver, V = 3, $p > 0.05$; D, stomach, V = 3, $p > 0.05$; E, small intestine, V = 48, $p > 0.05$; F, large intestine, V = 46, $p > 0.05$; G, kidney, V = 1, $p > 0.05$; H, total parasites, V = 318, $p > 0.05$). Color corresponds to organ system: cardiovascular (red, heart), digestive (green, liver, stomach, small intestine, large intestine), lymphatic (yellow, spleen), circulatory (blue, kidney), total parasites (black, general).

Table 1 Data for study sites substrate type, moisture, elevation, and coordinate location.

Abbr	Name	Substrate Type	Moisture	Elev.	Location
PPR	Pauline Pasteur	Loam	6.883333333	2620	47 20 42 N, 114 17 8 W
PCB	Pauline Creek Bed	Sandy Loam	8.888888888	2590	47 21 26 N, 114 16 33 W
TP	Triangle Power line	Sandy Loam	21.28333333	2700	47 21 32 N, 114 10 37 W
SW	South West	Silt Loam	7.933333333	2680	47 18 43 N, 114 16 38 W
RP	Ravalli Ponds	Silt Loam	13.16666667	2920	47 17 58 N, 114 09 39 W