

**Correlating Host Metapopulation Size and Parasite Species Richness at Multiple Spatial
Scales in an Amphibian Host-Parasite System**

Joseph R. Mihaljevic

Washington University in St. Louis
Environmental Studies Program
Senior Honors Thesis
April 2010

ABSTRACT

With emerging evidence for the importance of parasite communities in shaping free-living biodiversity patterns and even ecosystem health, a rigorous understanding of the mechanisms driving parasite community structure is necessary. Parasite richness was historically studied as a function of host characteristics and immunity, but recent evidence suggests that landscape characteristics in which host populations are embedded can greatly influence parasite richness patterns. Because the spatial dynamics of hosts influence the movement of their parasites, host metapopulation characteristics can influence parasite species richness. In this study, I collected metamorphs of the pond-dwelling frog, *Rana clamitans*, from ponds throughout southeast Missouri, surveyed their endoparasite communities, and correlated the parasite species richness with *R. clamitans* at within-frog, within-pond, and within-site spatial scales across regions that varied in the number of ponds present (i.e., metapopulation size). I found that parasite species richness was strongly positively correlated with host metapopulation size at the within-frog and within-pond scale, and marginally so at the within-site scale. Additionally, within a subset of common parasite species, individual parasite species' prevalence (presence/absence in a host) was also positively correlated with host metapopulation size. Through exploring the effects of host metapopulation size in this study, I show that both host- and parasite-relevant landscape dynamics are integral to explaining parasite richness patterns at multiple spatial scales.

INTRODUCTION

Contemporary community ecology's goal is to discern how multiple species interact with each other and with their environment to shape organismal diversity and community patterns (Elton 1966; Whittaker 1975; Hairston 1989; Ricklefs 1990). Interestingly, most studies on diversity relationships ignore parasites, even though parasitism is the most common animal life-strategy on the planet (de Meeûs and Renaud 2002). Only recently have ecologists begun to consider the implications of interactions between parasites and their hosts as potential drivers of diversity patterns observed in nature. For example, recent food-web studies that include parasites differ dramatically in food-web architecture compared to similar studies that only consider free-living life forms (Marcogliese and Cone 1997; Lafferty et al. 2006). Furthermore, emerging evidence suggests parasites to be drivers of free-living biodiversity and show that more diverse parasite communities may benefit overall ecosystem health (Hudson et al. 2006). In light of these important findings, much ecological research is required to understand the factors influencing parasite community dynamics themselves.

Classically, parasitologists have approached parasite community structure as being more or less dependent on their hosts' characteristics and ecology (see reviews in Poulin 1997; Morand 2000; Poulin and Morand 2000). Recently, however, researchers have criticized the lack of understanding of parasite ecology from a community ecology perspective (Pedersen and Fenton 2006). As a result of such critiques, ecologists have begun to rigorously test whether parasite communities fit the mold of classic community ecological theories. Indeed, certain parasite communities respond in a similar manner as free-living organisms in terms of diversity-productivity relationships (Poulin et al. 2003), species area relationships (Dove and Cribb 2006), and, in some instances, null models of co-occurrence (Krasnov et al. 2009). Still, even as these

patterns have emerged, community ecologists struggle to explain variation in parasite richness patterns due to parasites' complex relationships with their hosts, and, potentially, with their host's environment (Poulin 2007).

As might be expected, many classic parasite species diversity patterns are reliant on host characteristics and host-mediated epidemiology, such as host body size and host population density (reviewed in Poulin 1997). However, in some parasite community systems, spatial correlates of richness are beginning to emerge, helping to explain large amounts of variation in parasite richness patterns. Indeed, along with host identity, Krasnov et al. (2008) discovered that environmental variables, particularly local temperature and precipitation, were significant predictors of mite parasite species richness across mammal host populations. Lindenfors et al. (2007) showed that latitude was a strong predictor of parasite richness in carnivore hosts. Additionally, distance decay in similarity has been observed in helminth communities of mammals (Poulin 2003) and fish (Poulin 2003; Oliva and González 2005), showing that parasite communities often respond to landscape-level spatial variation. Notwithstanding these findings, parasite community ecologists have often neglected to consider landscape-level characteristics relevant to host movement and parasite transmission as potential correlates of parasite community structure.

This knowledge gap may at least partially emerge from semantic and conceptual issues related to spatial hierarchy in parasite communities versus free-living species communities. From a parasitological perspective, Bush et al. (1997) established a framework for describing levels of spatial structuring in parasite communities. At the most local scale, the infracommunity defines all parasite species living within a single host individual. Scaling upwards, the component community encompasses all parasite species found within all populations of one host

species. From a free-living community ecology stance, however, this is a large spatial leap, as even populations of one host species are often dispersed among vast areas of continuous landscape or even between barriers, such as continents. Defining a regional scale of parasite communities, even from a parasitological viewpoint, has become a topic of dispute, precisely due to the spatial ambiguity of the component community definition (Krasnov et al. 2006).

Additionally, neither the infracommunity nor the component community acknowledges parasite communities of multiple host species that may interact within the same spatial boundaries, as defined from the hosts' perspective. In other words, while multiple host species may be interacting with each other within a habitat, parasite communities are only defined by a single host species' individuals and populations. Thus, these definitions ignore host-level landscape characteristics that may be highly relevant to parasite transmission and, therefore, to parasite community diversity patterns.

Similar semantic and conceptual problems arise in defining free-living species' communities' spatial structuring. For example, that which defines a host species' population or host community, in a spatial context, varies depending on the natural history of the species considered, as well as the spatial scope of any given study. A free-living species' population or community may be more continuous in space, or multiple populations or communities may be spatially fragmented, separated by physical barriers or gradients (such as resource or climate gradients). Ecologists often consider the latter case of patchy landscapes to be most appropriate for many free-living species (Hanski 1982).

These patchy populations and communities are conceptually modeled by metapopulations (Hanski and Gilpin 1997) and metacommunities (Leibold et al. 2004), which define populations and communities in a spatially explicit manner. For lack of confusion, I will only consider

metapopulations hereinafter. A metapopulation is defined as a variable number of geographically bounded patches of inhabitable environment embedded within a matrix of uninhabitable space. Thus metapopulation theory considers the implications of immigration and emigration to and from these separated patches on population dynamics through time and space.

Studies considering parasite communities in a host-metapopulation framework are incipient but nonetheless informative. Bush and Kennedy (1994) showed that host population fragmentation was of little relevance for local parasite extinction. They speculate that certain host populations may act as reservoirs for parasite species, that repopulate neighboring localities – essentially source-sink dynamics. Gillespie and Chapman (2006) found that variations among patches in a red colobus (*Ptilocolobus tephrosceles*) metapopulation alter the prevalence of parasitic nematodes. Therefore, a closer inspection of host metapopulation characteristics may reveal host metapopulation-mediated patterns of parasite community structure.

A host's metapopulation size (as defined by patches, not host individuals; see Hanski and Gilpin 1997 and Methods) may be relevant to parasite community dynamics for a variety of reasons, stemming from both epidemiological and ecological predictions. A larger host metapopulation may be able to sustain more host individuals (Hanski and Gilpin 1997), leading to higher probability of single parasite species being able to persist. For example, in a larger metapopulation some patches may be spatially isolated from others, such as border patches on opposite sides of a site, between which host individuals are unlikely to migrate. This may hinder the transmission of some parasites to all individuals or patches of a large metapopulation, allowing more parasite species to persist in the overall metapopulation. On the other hand, in smaller metapopulations, encounters among host individuals may be more frequent, leading to

the spread of the same suite of parasite species among individuals in that metapopulation (i.e. less parasite species turnover between hosts compared to that of larger metapopulations).

In this study, I consider how spatial characteristics of a host metapopulation may influence species richness of its parasite community at various spatial scales. To more synthetically bridge free-living community ecology with parasite community ecology, I use a hybrid spatial hierarchy to define parasite communities, taking into account variables relevant to both parasite species and host species. The spatial scales considered are as follows: within-host (i.e. infracommunity), within-patch, and within-site. I define a site as a collection of patches that is separated from other similar collections of patches in terms of host migration. Specifically, at each scale, I ask how parasite species richness varies with host metapopulation size using an amphibian study system, *Rana clamitans* and its suite of endoparasite species. I additionally explore if individual parasite species within an infracommunity respond differentially to metapopulation size. I predict that parasite species richness will be positively correlated with host metapopulation size, though perhaps not at each spatial scale considered.

I found that parasite species richness responds strongly to metapopulation size at multiple spatial scales. Most individual parasite species' prevalence responded similarly, with only few exceptions. Following these results, I discuss their significance as it relates to scale of study in host-parasite systems. I also discuss the epidemiological implications of these results for *R. clamitans*, and amphibian host-parasite relationships in general.

METHODS

Study System

In Missouri, *R. clamitans* is a common amphibian species found in most permanent aquatic habitats (Collins 1993). This species' natural history makes it a prime model system for the questions addressed in this study. Throughout their range, adult frogs lay their eggs in ponds, where the larvae develop, usually for a period of at least one year (Smith 1961). During this time, the frog larvae ingest or are otherwise infected by parasites from the pond in which they develop. By capturing metamorphs - larvae that have recently developed into adults - from ponds, one is able to accurately presume that each observed endoparasite species in a metamorph was acquired from its pond of origin during development.

Additionally, anuran metamorphs house a diverse suite of endoparasite species, spanning a diverse phylogeny (Buller et al. 2009), allowing maximum power for analyses involving species richness. In amphibian adults and larvae, endoparasites vary greatly in host pathology, from benign commensalisms to virulent pathogens that can lead to host deformity and/or mortality. Most species have complex life cycles, each cycle relying on intermediate hosts. In these instances, juvenile life stages infect and develop (encyst) in intermediate hosts (e.g. Table 1). In many instances, these intermediate hosts are consumed by secondary intermediate or definite hosts, in which the juvenile parasites either develop further or reproduce, respectively. Thus, many parasite species are reliant on multiple hosts' ecologies to persist. *R. clamitans* is both a common intermediate and definitive host (Buller et al. 2009).

Sampling Methods

Ponds and sites in Southeast Missouri were selected prior to sampling in a strategic manner in order to sample from a range of host metapopulation sizes (Fig 1). Sites (usually conservation units; Table 2) were selected based on: maximum number of ponds at a site, site area, and density of ponds in a site. Sites were scouted for ponds via Google Earth image

searching, communication with site managers, and prior on-the-ground exploration. The maximum number of ponds per site was determined and included ponds that were deemed ‘temporary’ (i.e. ephemeral) and permanent. Site area (km²) was calculated using Google Earth, based on the boundaries of the site (Table 2).

At each site (n=9), six ponds were visited (a sub-sample of the total number of ponds at the site), though *R. clamitans* was not present - or could not be captured - at all ponds. Ponds were visited between 15 June 2009 and 8 July 2009. Approximately one hour was spent using dip nets to stealthily capture *R. clamitans* metamorphs at each pond. The number of metamorphs captured depended on variability in ease of capture as well as numbers of metamorphs available at each pond. Metamorphs were placed in coolers after capture, transported back to Tyson Research Center, Eureka, MO and placed in separate terraria for no more than one night before shipment to University of Colorado at Boulder.

Metamorphs were overnight-priority shipped to UC-Boulder in coolers with freezer packs. All metamorphs arrived to UC-Boulder alive and in good condition (PTJ Johnson, personal communication). On the day of arrival, the metamorphs’ SVL measurement, developmental stage, and sex were noted. The metamorphs were dissected and endoparasites were counted and identified to species or taxonomic unit, following standard operating protocol (unpublished protocol, PTJ Johnson). All handlers and members of the necropsy team were trained by and followed standards of the Institutional Animal Care and Use Committee (IACUC).

Determining Metapopulation Size

Metapopulation size was defined by landscape variables of the nine sites, specifically maximum number of ponds and site area, with each site considered a different metapopulation.

This seemed biologically realistic considering the home range of *R. clamitans* and the distance between sites. Simply defining metapopulation size in terms of pond density at a site would not be useful. Pond density is quite spatially implicit, meaning that values of density do not allow one to speculate on the area in consideration. Density values could be very similar for very dissimilar sites. Instead, I conducted a linear regression of the maximum number of ponds at a site versus the site area (km²), and took the residuals from this regression. Thus, larger residual values imply a site with higher pond density, correcting for differences in site area (Table 2).

Testing whether these metapopulations (sites) housed truly separate populations of *R. clamitans* was outside the scope of this study. No genetic analyses of relatedness were performed, and no measure of adult amphibian migration was obtained. While some sites were closer to their neighboring sites than others, the shortest distance between sites was ~8 km (center to center), though potential corridors between sites were not ruled out in this study. The home range of the average *R. clamitans* adult has been estimated at no more than 110m (Martof 1953), though sub-adults may move from 200m–4.8 km (3 mi) during their first season following transformation (Schroeder 1968). From this information, the assumption of separate host metapopulations is rational.

Analyses

Parasite Richness and Metapopulation Size

Within-Host (Infracommunity). Since the observed parasite richness within each frog followed a poisson distribution, a poisson regression was used to determine if infracommunity richness responded to metacommunity size (i.e. do metamorphs embedded in different sized metapopulations harbor a different number of parasite species). In order to account for any parasite richness variation due to an individual's body size or sex, these variables were added to

the general linear model. I also determined how host-scale parasite abundance varied with metapopulation size. I used a zero-inflated poisson regression, including metapopulation size, body size (snout-vent-length) and sex in the model.

Within-Pond (Patch). In order to determine the parasite richness per pond, all metamorphs captured from a pond were pooled, and presence/absence of parasite species was determined. Since a variable number of metamorphs were captured from each pond, I did not attempt to scale up abundance data. Only parasite species richness data was used. Again, due to the differences in the number of metamorphs captured from each pond, I adjusted parasite richness incorporating sampling effort. I first square-root transformed richness data in order that the data meet assumptions of normality. A linear regression of the transformed parasite richness observed per pond versus the number of metamorphs collected from each pond was marginally significant ($F=3.60$, $p=0.066$). Since this was marginally significant, I took the residuals of each data point on this regression and substituted these values for values of parasite species richness for the subsequent analyses, in order to remove any differences to sampling effort that may exist. I conducted a linear regression of adjusted parasite richness per pond versus metapopulation size to determine if pond-level parasite species richness correlated with host metapopulation size.

Within-Site. Similarly to the pond scale, I pooled all pond-level data at each site in order to determine presence/absence of each parasite species. Again, since a variable number of ponds were sampled at each site, I performed a regression of parasite richness per site versus number of ponds sampled per site to determine if a sampling effort effect existed. The initial regression was insignificant ($F=2.55$, $p=0.154$); however, this insignificance seemed to be driven by an

outlier (Forest 44_TRC; Fig 6). Therefore, I performed a robust linear regression on the same variables, which corrects for outliers that are not due to data entry errors and that have no compelling reason for deleting from the analysis. This regression revealed a significant result ($t_7=2.97$, $p=0.022$). Thus, I again took the residuals from the regression to substitute for parasite richness per site for the remainder of the analyses, in order to correct for this apparent sampling effort. As before, I conducted a linear regression of adjusted parasite richness per site versus metapopulation size to determine if site-level parasite species richness correlated with host metapopulation size. Analyses were performed in R (2009). Graphics were also created in R (2009).

Individual Parasite Species' Responses to Metapopulation Size

I used separate general linear models with host metapopulation size, body size (snout-length) and sex as variables function to test how each individual endoparasite species or taxon responded to host metapopulation size. In these models I used a binomial error distribution with a logit link function with presence/absence data from all captured metamorphs. Analyses were performed in R (2009).

RESULTS

A total of 261 *R. clamitans* metamorphs were collected and dissected from 37 ponds and 9 sites (Table 2; Fig 1). Sites varied considerably in area and maximum number of ponds present, offering an excellent variation for regression analyses. Sixteen endoparasite species/taxonomic groups (hereinafter referred to as species) were included in these analyses, comprising of cestodes, nematodes, and trematodes. (Table 1; Fig 8). These species range in pathology and lifecycle. Ectoparasites, such as leeches, were not included in the analyses. In some instances, unknown endoparasite species were discovered. These species are pending

genetic analyses, and were, therefore, not included in the analyses. Parasite species richness varied from 0-6 per frog (mean \pm SE: 1.24 ± 0.08), 0-7 per pond (mean \pm SE: 3.16 ± 0.35), and 2-10 per site (mean \pm SE: 6.89 ± 1.01). Abundance of parasites per frog ranged from 0-1164 individuals (mean \pm SE: 45.44 ± 8.87).

Parasite Richness and Metapopulation Size

Parasite species richness was strongly positively correlated with metapopulation size at both the infracommunity and pond scales, but marginally correlated at the site scale (Tables 3, 6-7; Fig. 2, 5 and 7). At the infracommunity scale, parasite abundances were also strongly positively correlated with metapopulation size (Table 4; Fig. 3). Also at the infracommunity scale, parasite richness and parasite abundance were highly correlated (Table 5, Fig 4).

Unfortunately, neither poisson nor zero-inflated regressions return values of r , the proportion of variance explained by the model. Therefore, I cannot speculate as to how much variance is explained by metapopulation size at the infracommunity scale. However, at the pond scale and the site scale, host metapopulation size explains over 20% and nearly 30% of the variation in the data, respectively.

Body size (snout-vent-length) was also a significant predictor of parasite richness at the infracommunity scale; however, sex was not (Table 3). In the case of parasite abundance at the infracommunity scale, neither body size (snout-vent-length) nor sex was a significant predictor (Table 4).

Individual Parasite Species' Responses to Metapopulation Size

Seven out of sixteen species were not present frequently enough to justify the application of these models, and were thus not included. Six of the nine remaining species' presence in an individual exhibited significantly positive correlations with host metapopulation size (Table 8).

Only *Glypthelmins* sp. was significantly negatively correlated with host metapopulation size.

Two species, *Megalodiscus temperatus* and Tapeworm Cyst showed no response to host metapopulation size. Interestingly, host body size was also a significant predictor in many of the parasite species.

DISCUSSION

In this study I found that the endoparasite species richness of *R. clamitans* metapopulations significantly increased with increasing host metapopulation size at the infracommunity and pond (patch) scales, and marginally so at the site scale. Additionally, most observed endoparasite species' prevalence (in terms of presence/absence per amphibian) increased with increasing host metapopulation size, with few exceptions. These results all emphasize the importance of host-relevant landscape characteristics in the structuring of parasite communities across spatial scales.

Firstly, host body size was a significant predictor of parasite richness on an individual host scale. This pattern makes sense since larger hosts have more resources on which, potentially, a more diverse suite of parasite species can capitalize. Indeed, this same pattern has been observed on numerous occasions in fish (reviewed in Poulin 2003). Since this species of frog is not particularly sexually dimorphic, lack of sex as a significant predictor of parasite richness or abundance in any of the analyses in this study was expected.

I found that metamorphs embedded in larger metapopulations harbored greater abundances of endoparasites. However, not only was the parasite abundance greater per frog, but the richness per frog also increased with increasing host metapopulation size. One mechanism that could explain these results is source-sink dynamics of patchy communities.

Some of the parasites found in this study have direct horizontal transmission, meaning they are transmitted via direct contact with an infected host. Other species have mobile juvenile stages that enter the pond water via definitive host feces. Applying metacommunity theory to this system, an individual amphibian larvae host can be considered a patch in which a suite of parasite species resides, and the pond water is the matrix that separates patches. In this context, transmission occurs via immigration of parasites to a new patch - either via chance-events of contact between patches or via juvenile parasites emigrating from the matrix. A larger host metapopulation - in this case a site with more ponds or a denser array of ponds - may harbor more patches for the parasites (i.e. more hosts) in a given area, compared to a smaller host metapopulation. Using the theory of metacommunity source-sink dynamics (Hanski and Gaggiotti 2004), more available patches allow for more rare species (or species that prefer less commonly encountered environments) to persist in the overall system. It is more likely that these species will sustain source populations, since the probability of finding a suitable patch increases with increasing patch abundance. Parasites within these source patches can then disperse to neighboring patches. In terms of parasitology and epidemiology, this explanation suggests that more rare parasite species (or parasites that prefer conditions that appear less frequently) will persist in larger host metapopulations.

The fact that infracommunity parasite abundance and richness are positively correlated suggests that the amphibian hosts utilized in this study are not saturated with parasites. In other words, as a new parasite species is added to a host, total abundance of parasites goes up, suggesting that no “cap” on parasite abundance or richness per host was encountered in this study. In a review, Poulin (2006) measured saturation based on how infracommunity parasite

richness scales with component community parasite richness, finding that saturation and non-saturation are equally likely in parasite systems.

Often the lack of species saturation is interpreted as suggesting little or no negative species interactions (e.g. competition) among parasites in the host. In other words, the addition of a parasite species to the parasite community does not cause the decline of another parasite species' abundance. Interestingly, in this study, most individual parasite species that occurred frequently enough to be statistically analyzed showed strong positive correlations with host metapopulation size. Indeed, one might interpret these results as showing that parasites in this system are not negatively interacting, since most show similar patterns of increase. However, I suggest that these results concur, not with the latter hypothesis, but with epidemiological theory. All of the endoparasites observed in this amphibian system have a horizontal mode of transmission. Parasites transmitted horizontally that are embedded in denser or more numerous host populations are expected to have higher population growth rates (Diekmann and Heesterbeek 2000), meaning that the proportion of susceptible hosts with infection should be higher. In this study, I defined a larger host metapopulation as a site with a greater number of ponds, or a site with a denser network of ponds. Thus, in these larger host metapopulations, the commonness of individual parasite species should be higher, following parasite/pathogen transmission dynamic theory.

Future studies could utilize null models of co-occurrence to more thoroughly disentangle questions of parasite species interactivity in this system. For example, Jones et al. (2009) found that bacterial assemblages within individual fleas of prairie dogs had extensive interactions between and among dominant taxa, as measured by the null model approach. Further, in a null model analysis involving ectoparasites of rodent hosts spanning three continents, Krasnov et al.

(2010) discovered that, while random and non-random patterns of parasite species co-occurrence were present, non-random positive co-occurrence predominated (i.e. positive species interactions). Thus, it is possible that the correlation observed between parasite richness and abundance in this study reveals either negligible interactions or positive interactions among parasites.

Nonetheless, what is important to the results of this study is that the average parasite richness and abundance per host increased with host metapopulation size. This suggests that whether or not *R. clamitans* metamorphs saturate at some species pool-level of parasite richness, landscape-level characteristics and phenomena are highly influential in determining the potential/observed parasite species pool from region to region. Furthermore, these landscape features result in similar patterns across spatial scales. Even at the within-site spatial scale, parasite richness marginally ($P=0.077$) increases with host metapopulation size. Although this result is not statistically significant, I argue that this linear relationship is highly biologically significant, capturing nearly 30% of the variation in the data. The lack of statistical significance at this spatial scale may have to do with the lack of power as the data scales upwards. There were only nine sites sampled versus a total of 261 individual frogs sampled. This reduces the power of the analysis. Additionally, the relationship between the number of ponds sampled per site and the parasite richness observed per site was highly linear ($P=0.022$), suggesting that if more ponds were sampled per site (especially in the larger metapopulations), that more parasite species would be discovered per site. This may have caused the linear relationship between parasite richness per site and host metapopulation size to be less pronounced.

The observed patterns of parasite richness at the various spatial scales could also be related to effects of landscape-level characteristics on other host species relevant to the parasites

found in this study. The vast majority of the parasites species herein have complex lifecycles with multiple life-stages. In particular, most of the species are reliant on aquatic intermediate hosts (arthropods, amphibians) and terrestrial definitive host species (birds, mammals, reptiles) to complete their lifecycle (Table 1) and are, thus, dependent on the presence of multiple host species to persist in a given area. Hechinger et al. (2006) found that a more diverse assemblage of birds is correlated with a higher local diversity of helminth parasites in aquatic systems. Therefore, it is clear that definitive and intermediate host identity and diversity are significant drivers of parasite community richness. Furthermore, Allan et al. (2003) found that landscape characteristics, namely the level of fragmentation, altered the available suite of host species for ectoparasites (ticks) to feed on. These findings, combined with those of Hechinger et al. (2006), offer further evidence that landscape-level characteristics are important correlates for parasite species richness. In the present study, a larger metapopulation of *R. clamitans* may correlate with a more abundant or diverse definitive or intermediate host communities, since metapopulation size is directly related to site area (e.g. there may be higher diversity of birds within a larger site).

Many of the hypotheses used to generate the predictions of this study, and to explain the results are based on host population sizes and densities and how they respond to metapopulation size. Unfortunately, direct measurements of adult or metamorph abundances were not taken at either the pond or site scale. However, Ebert and colleagues (2001) showed similar patterns of host-relevant patch dynamics influencing parasite richness in invertebrate host (*Daphnia* sp.) metapopulations. These authors studied *Daphnia* sp. assemblages in island pools, finding that the number of pools on an island and the number of ponds within a 10m radius were partial predictors of the parasite richness found per island pool. These invertebrate hosts may have

more barriers to migration than the vertebrate hosts studied herein. It seems logical that in an amphibian system, patch dynamics should be more important compared to *Daphnia* sp., as these organisms are presumably more mobile and more easily disperse among patches. Thus, host migration, patch dynamics, and the landscape-level characteristics that influence these dynamics may also be of greater importance to this anuran host's parasite community structure.

For amphibians in particular, the fact that the majority of individual parasite species respond positively to host metapopulation size has epidemiological implications beyond explaining parasite community richness patterns. Certain helminth parasite species not only wreak havoc on individual amphibian hosts, but also on entire amphibian populations. For example, Johnson et al. (2003 and 2004) have strongly linked *Ribeiroia ondatrae* – a parasite species observed in many of the frogs in this study – with severe limb malformations. These deformities are suspected to make the diseased frogs more susceptible to bird (definitive host) predation, and may be implicated in the decline of at least some amphibian species (Johnson et al. 2005). My results suggest that landscape characteristics more suitable for sustaining larger populations of amphibian host species, may also be the same characteristics suitable to harbor and sustain their virulent pathogens.

Through exploring the effects of host metapopulation size in this study, I show that both host- and parasite-relevant landscape dynamics are significant to explaining parasite richness patterns at multiple spatial scales. The two potential explanatory mechanisms discussed – metacommunity source-sink theory and the effects of definitive/intermediate host diversity – may act independently or in concert to lead to similar patterns of host metapopulation size on parasite species richness at various spatial scales. Future studies that incorporate parasite communities from multiple hosts that interact within the same space could help to disentangle

how landscape and host diversity separately and/or interactively explain variation in parasite infracommunities and parasite community dynamics at multiple spatial scales. Indeed, linking metacommunity theory to the study of parasite communities may be a useful way to move toward a more explanatory – and, potentially, a predictive – conceptual framework of parasite community ecology.

ACKNOWLEDGEMENTS

I am extremely grateful for the guidance of my mentor, Jon Chase, in the process of designing and implementing the experiment, as well as for commenting on early drafts of this thesis. Lauren Woods did a wonderful job of designating which sites to survey and organizing the whole sampling endeavor. This was a collaborative project with Piet Johnson's lab at UC-Boulder. Without the hard work of the necropsy team – namely Sarah Orlofske, Ian Buller and Don Larson – this project would not have been possible. Additionally, the staff and scientists at Tyson Research Center were extremely helpful in completing this project, especially Elizabeth Biro, Kevin Smith, and Meghan Kelly. I would like to thank the Chase lab for comments on data analyses and the overall framework of the project. I also thank the secondary readers of this thesis, Tiffany Knight and John Orrock for their valuable input. Finally, funding for this project came from Tyson Research Center in Eureka, Missouri, the Arnold and Mabel Beckman Scholars Program, and Washington University's Environmental Studies Program Research Grant.

REFERENCES

- Allan, B. F., Keesing, F. and Ostfeld, R. S. (2003). Effects of forest fragmentation on Lyme Disease risk. *Conservation Biology* 17, 267–272.
- Bush, A. O., Lafferty, K. D., Lotz, J. M. and Shostack, A. W. (1997). Parasitology meets ecology on its own terms: Margolis et al. revisited. *Journal of Parasitology* 83, 575–583.

- Bush, A. O., and C. R. Kennedy. 1994. Host fragmentation and helminth parasites: Hedging your bets against extinction. *International Journal for* 24: 1333-1343.
- Buller, I.D., Orlofske, S.A., & Johnson, P.T.J. (2009). North American Amphibian Parasite List. Retrieved March 1, 2010 http://www.colorado.edu/eeb/facultysites/pieter/Parasite_Database/Home.html
- Ebert, D., J. W. Hottinger, and I. Pajunen. 2001. Temporal and spatial dynamics of parasite richness in a *Daphnia* metapopulation. *Ecology* **82**:3417–3434.
- Elton, C.S. 1966. *The Pattern of Animal Communities*. Chapman & Hall, London.
- Collins, J.T. 1993. *Amphibians and reptiles in Kansas*. Third edition, revised. Natural History Museum, University of Kansas, Lawrence, Kansas.
- de Meeûs, T. and F. Renaud. 2002. Parasites within the new phylogeny of eukaryotes. *Trends Parasitol.* **18**: 247–251.
- Diekmann and J. A. P. Heesterbeek. 2000. *Mathematical Epidemiology of Infectious Diseases*, Wiley, New York.
- Dove ADM and Cribb TH. 2006. Species accumulation curves and their applications in parasite ecology. *Trends Parasitol* 22:568–574
- Gillespie, T. R. and Chapman, C. A. 2006. Prediction of parasite infection dynamics in primate metapopulations based on attributes of forest fragmentation. *Conservation Biology* 20, 441–448.
- Hanski, I. 1982. Dynamics of regional distribution: The core and satellite species hypothesis. *Oikos* 38: 210-221.
- Hanski, I., and O. E. Gaggiotti. 2004. *Ecology, genetics, and evolution of metapopulations*. Elsevier, San Diego, California, USA.
- Hanski, I. and Gilpin, M. E. 1997. *Metapopulation Biology: Ecology, Genetics and Evolution*. Academic Press, London.
- Hairston, N.G. 1989. *Ecological Experiments*. Cambridge University Press, Cambridge.
- Hechinger RF, Lafferty KD (2005) Host diversity begets parasite diversity: bird final hosts and trematodes in snail intermediate hosts. *Proc Biol Sci* 272:1059–1066
- Hudson, P. J., Dobson, A. P. and Lafferty, K. D. 2006. Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology and Evolution* 21, 381–385.

- Johnson, P. T.J., & Lunde, K.B. (2005). Parasite infection and limb malformations: A growing problem in amphibian conservation. Pp. 124-138 in *Amphibian Declines: the Conservation Status of United States Species*, M. J. Lannoo (ed). University of California Press.
- Johnson, P.T.J., & Sutherland, D.R. (2003). Amphibian deformities and *Ribeiroia* infection: an emerging helminthiasis. *Trends in Parasitology*, 19(8), 332-335.
- Johnson, P.T.J., Sutherland, D.R., Kinsella, J.M., & Lunde, K.B. (2004). Review of the trematode genus *Ribeiroia* (Psilostomidae): ecology, life history and pathogenesis with special emphasis on the amphibian malformation problem. *Advances in Parasitology*, 57, 191-253.
- Jones, R. T., R. Knight, and A. P. Martin. 2009. Bacterial communities of disease vectors sampled across time, space, and species. *ISME Journal*
- Krasnov, B. R. et al. 2006. Are ectoparasite communities structured? Species co-occurrence, temporal variation and null models. *J. Anim. Ecol.* 75: 1330-1339.
- Krasnov, B. R., Korallo-Vinarskaya, N. P., Vinarsky, M. V., Shenbrot, G. I., Mouillot, D. and Poulin, R. 2008. Searching for general patterns in parasite ecology: host identity versus environmental influence on gamasid mite assemblages in small mammals. *Parasitology* 135, 229–242.
- Krasnov, BR, S Matthee, M Lareschi, N. P. Korallo-Vinarskaya and M. V. Vinarski 2009. Co-occurrence of ectoparasites on rodent hosts: null model analyses of data from three continents. *Oikos* 119: 210-128.
- Lafferty, K. D., A. P. Dobson, and A. M. Kuris. 2006. Parasites dominate food web links. *Proceedings of the National Academy of Sciences (USA)* 103:11211–11216.
- Leibold, M. A., M. Holyoak, N. Mouquet, P. Amarasekare, J. M. Chase, M. F. Hoopes, R. D. Holt, J. B. Shurin, R. Law, D. Tilman, M. Loreau, and A. Gonzalez. 2004. The metacommunity concept: a framework for multi-scale community ecology. *Ecology Letters* 7:601–613.
- Lindfors, P., Nunn, C. L., Jones, K. E., Cunningham, A. A., Sechrest, W. and Gittleman, J. L. (2007). Parasite species richness in carnivores: effects of host body mass, latitude, geographical range and population density. *Global Ecology and Biogeography* 1, 1–14.
- Marcogliese DJ, Cone DK. 1997. Food webs: a plea for parasites. *Trends Ecol Evol* 12:320–325.

- Martof, B. S. 1953. Home range and movements of the green frog, *Rana clamitans*. *Ecology* 34:529-543.
- Morand S. and Poulin R. 1998. Density, body mass and parasite species richness of terrestrial mammals. *Evol Ecol* 12:717–727.
- Oliva, M. E. and Gonza'lez, M. T. (2005). The decay of similarity over geographical distance in parasite communities of marine fishes. *Journal of Biogeography* 32, 1327–1332.
- Pedersen, A. B. and Fenton, A. 2006. Emphasizing the ecology in parasite community ecology. *Trends in Ecology and Evolution* 22, 133–139.
- Poulin R. 1997. Species richness of parasite assemblages: evolution and patterns. *Annu Rev Ecol Syst* 28:341–358.
- Poulin, R., Mouillot, D. & George-Nascimento, M. (2003) The relationship between species richness and productivity in parasite communities. *Oecologia*, 137, 277–285.
- Poulin R. 2007. Are there general laws in parasite ecology? *Parasitology* 134: 763-776.
- Poulin R. and Morand S. 2000. The diversity of parasites. *Q Rev Biol* 75:277–293.
- R Development Core Team (2009). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL <http://www.R-project.org>.
- Ricklefs, R.E. 1990. *Ecology*, 3rd Edition. W.H. Freeman, New York.
- Schroeder, E.E. 1968. Movements of subadult green frogs, *Rana clamitans*. *Journal of Herpetology* 1:119.
- Smith, P.W. 1961. The amphibians and reptiles of Illinois. *Bulletin of the Illinois Natural History Survey*, Number 28, Urbana, Illinois
- Whittaker, R.H. 1975. *Communities and Ecosystems*, 2nd Edition. MacMillan, New York.

TABLES AND FIGURES

Table 1. Endoparasites (symbionts) observed, their pathologies and transmission cycles, if known.

Symbiont Taxonomy (Class Headings)	Pathology	Lifecycle/Transmission
Cestoda		
Cestoda Cyst	Usually no damage to host; largely unknown	Largely unknown; multiple intermediate hosts
Secernentea		
Encysted Nematode	Cyst stage (unknown)	Cyst stage (unknown)
<i>Oswaldocruzia sp.</i>	Unknown	Unknown
<i>Rhabdias ranae</i>	High prevalence can lead to host mortality	Amphibian hosts; horizontal infection
Spirurida (Order)	Variable	Arthropod intermediate host
Trematoda (Subclass: Digenea)		
<i>Alaria sp.</i>	No effect on amphibian	Mammal → Snail → Amphibian → Mammal
<i>Apharyngostrigea pipientis</i>	Unknown	Birds as definitive hosts; otherwise unknown
<i>Clinostomum attenuatum</i>	No effect on amphibian	Heron → Snail → Amphibian → Heron
<i>Echinostoma sp.</i>	No effect on amphibian	Mammal/Bird/Reptile → Snail → Amphibian → Mammal/Bird/Reptile
<i>Fibricola sp.</i>	Unknown	Mammal → Snail → Amphibian → Mammal
<i>Glypthelmins sp.</i>	Unknown	Frog larvae skin → Frog larvae
<i>Gorgoderid sp.</i>	Unknown	Clam (<i>Sphaerium occidentale</i>) → Snail → Frog
<i>Manodistomum syntomenteran</i>	Minor hind limb malformations	Snake → Snail → Amphibian → Snake
<i>Megalodiscus temperatus</i>	No effect on amphibian	Snail → Frog larvae skin → Frog larvae
<i>Ribeiroia ondatrae</i>	May cause mortality, severe limb deformities	Mammal/Bird → Snail → Amphibian → Mammal/Bird
Unknown immature metacercariae	Immature (unknown)	Immature (unknown)

Taxonomy, pathology and lifecycles provided by Buller et al. (2009).

Table 2. Sites used in this study, as well as their characteristics.

Site	Maximum Pond Number	Site Area (km ²)	Metapopulation Size (Residuals)	Number of Ponds Sampled
Daniel Boone	31	8.75	13.02	6
Huzzah	40	28.35	-4.16	6
Indian Creek	13	11.25	-8.32	3
Long Ridge	26	15.00	-0.33	3
Meramec CA	22	17.10	-7.13	4
Pea Ridge CA	7	4.00	-4.64	2
Shaw Nature Reserve	17	3.75	5.69	5
Tyson Research Center Area	48	24.00	9.65	2
USFS	6	2.60	-3.77	6

Table 3. Test (Poisson regression) for associations between parasite species richness per frog and multiple predictors.

	Estimate	Std. Error	z-value	Pr(> z)
(Intercept)	1.445	0.186	7.763	<0.001
Metapopulation Size	0.033	0.005	7.086	<0.001
Body Size (SVL)	-0.013	0.006	-2.166	0.030
Sex	0.052	0.062	0.841	0.401

Table 4. Test (Zero-inflated Poisson regression) for associations between parasite abundance per frog and multiple predictors.

	Estimate	Std. Error	z-value	Pr(> z)
(Intercept)	-0.655	0.825	-0.793	0.428
Metapopulation Size	-0.086	0.023	-3.757	<0.001
Body Size (SVL)	-0.001	0.027	-0.021	0.984
Sex	-0.024	0.272	-0.087	0.931

Table 5. Test (OLS regression) for associations between parasite abundance per pond and parasite richness per frog.

	Estimate	Std. Error	t-value	Pr(> t)
(Intercept)	-23.892	10.5	-2.275	0.0237
Richness Per Frog	56.027	5.829	9.612	<0.001

Table 6. Test (OLS regression) for associations between adjusted parasite richness per pond and host metapopulation size.

	Estimate	Std. Error	t-value	Pr(> t)
(Intercept)	-0.016	0.094	-0.175	0.8623
Metapopulation Size	0.042	0.013	3.286	0.0023

Table 7. Test (OLS regression) for associations between adjusted parasite richness per site and host metapopulation size.

	Estimate	Std. Error	t-value	Pr(> t)
(Intercept)	<0.001	0.725	<0.001	1.000
Metapopulation Size	0.208	0.101	2.071	0.0771

Table 8. Test (Logistic Regression) for associations between individual parasite species and multiple predictors.

	Alaria sp.	Apharyngostrigea pipientis	Clinostomum attenuatum	Echinostoma sp.	Encysted Nematode	Fibricola sp.	Glypthelmins sp.	Gorgoderid sp.
Metapopulation Size	NA	NA	+0.047*	+0.086***	NA	+0.223***	-0.215***	NA
Body Size (SVL)	NA	NA	-0.002	-0.092*	NA	-0.148**	+0.073	NA
Sex	NA	NA	+0.108	+0.419	NA	+0.288	-0.793*	NA
	Manodistomum syntomenteran	Megalodiscus temperatus	Oswaldocruzia sp.	Rhabdias ranae	Ribeiroia ondatrae	<i>Spirurida</i>	Tapeworm Cyst	Unknown immature metacercariae
Metapopulation Size	+0.219***	+0.054	NA	NA	+0.131***	NA	+0.080	+0.294***
Body Size (SVL)	-0.098*	-0.010	NA	NA	-0.097*	NA	-0.146*	-0.272***
Sex	+0.167	+0.675	NA	NA	+0.402	NA	-0.210	-0.616

The values in the table represent estimates of slope for each predictor. ***P<0.001; **P<0.01; *P<0.05

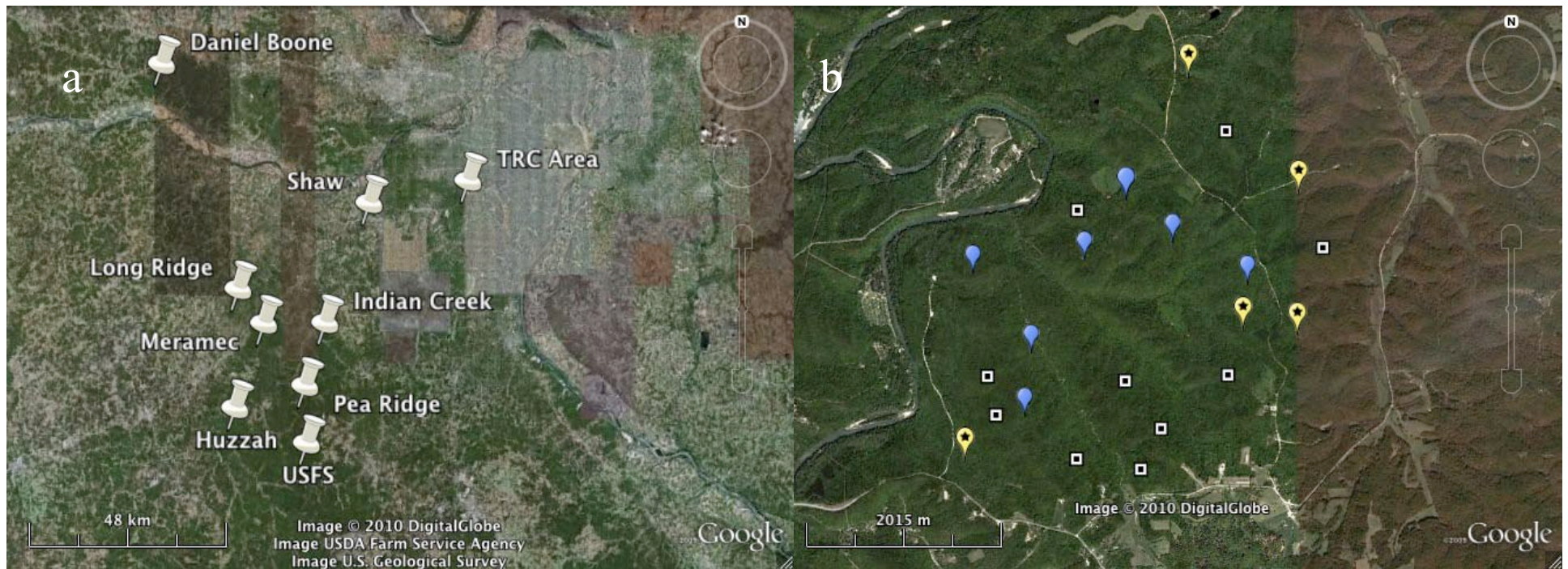


Figure 1. (a) Map of all sites. Markers represent sites; (b) Example of one site, Meramec Conservation Area. Markers represent ponds. (Maps were generated in Google Earth).

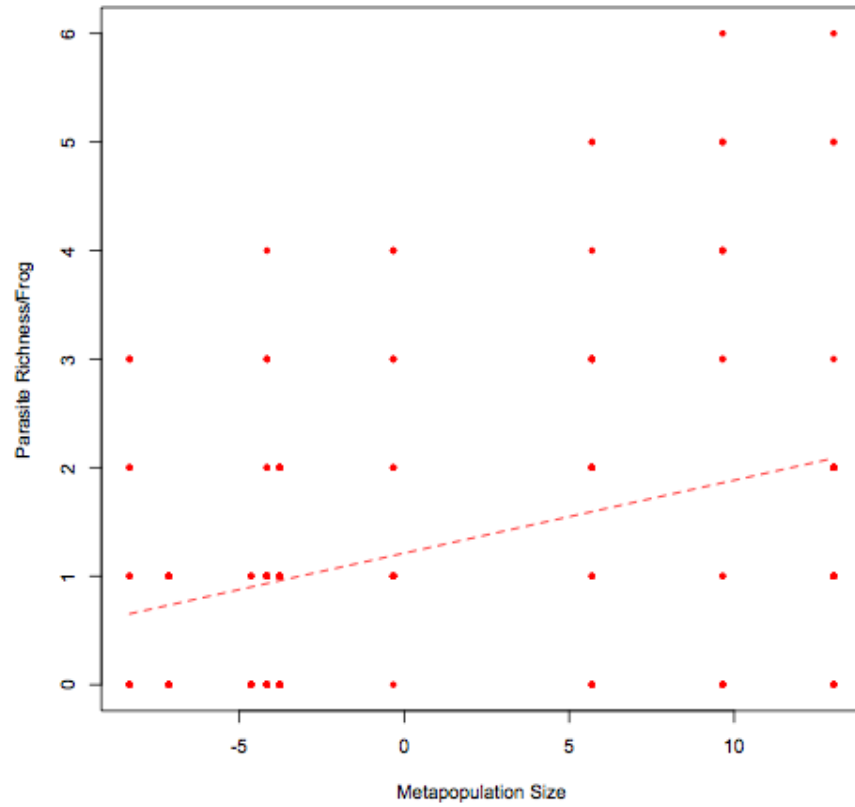


Figure 2. The relationship between parasite species richness per frog and host metapopulation size.

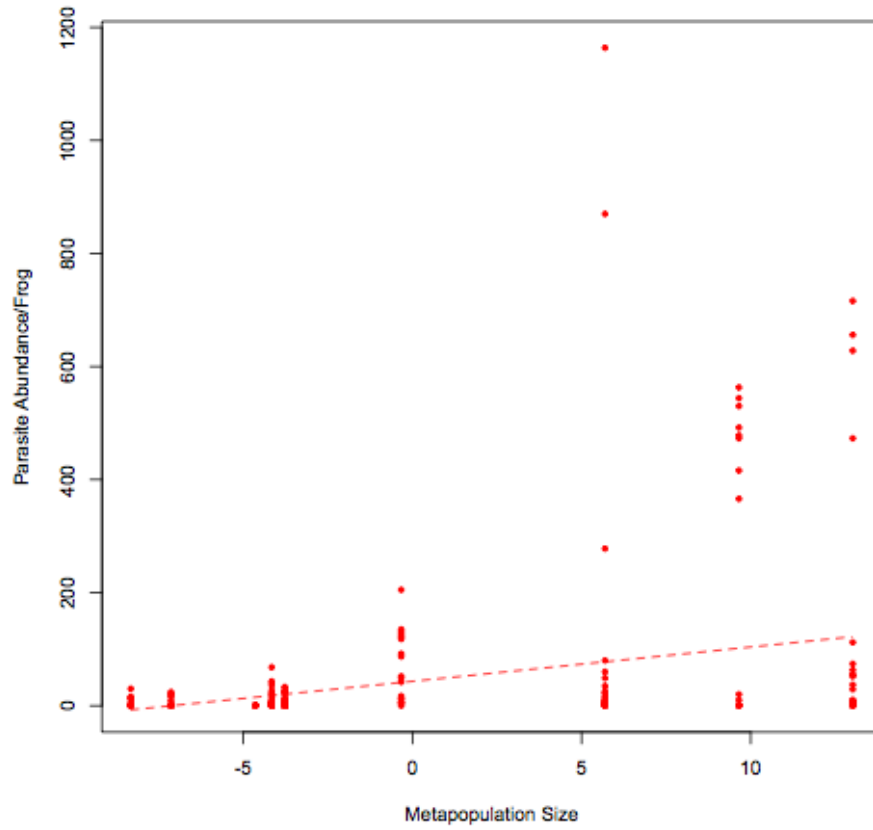


Figure 3. The relationship between parasite abundance per frog and host metapopulation size.

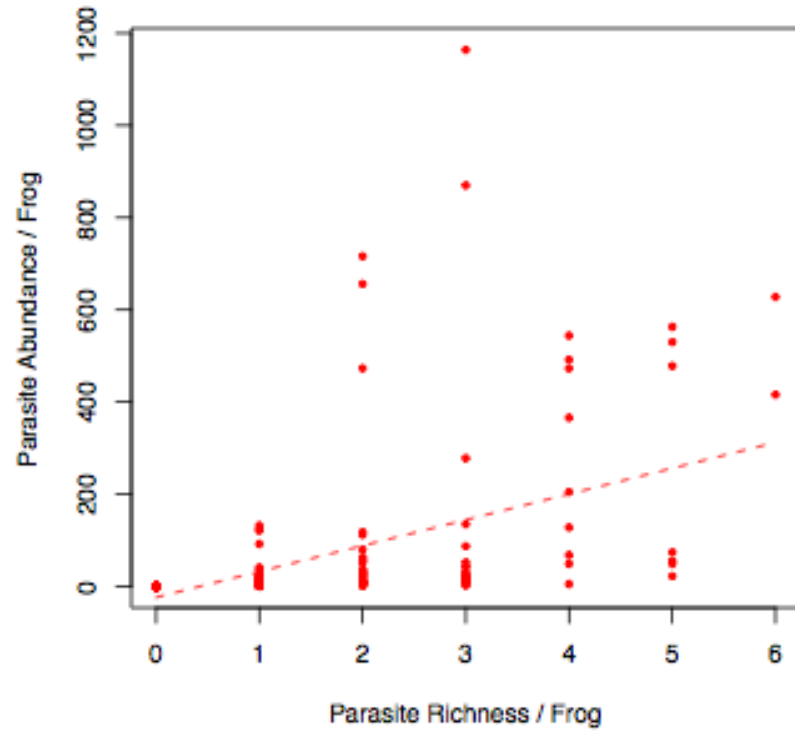


Figure 4. The relationship between parasite abundance per frog and parasite richness per frog (Multiple R^2 : 0.263; Adjusted R^2 : 0.260).

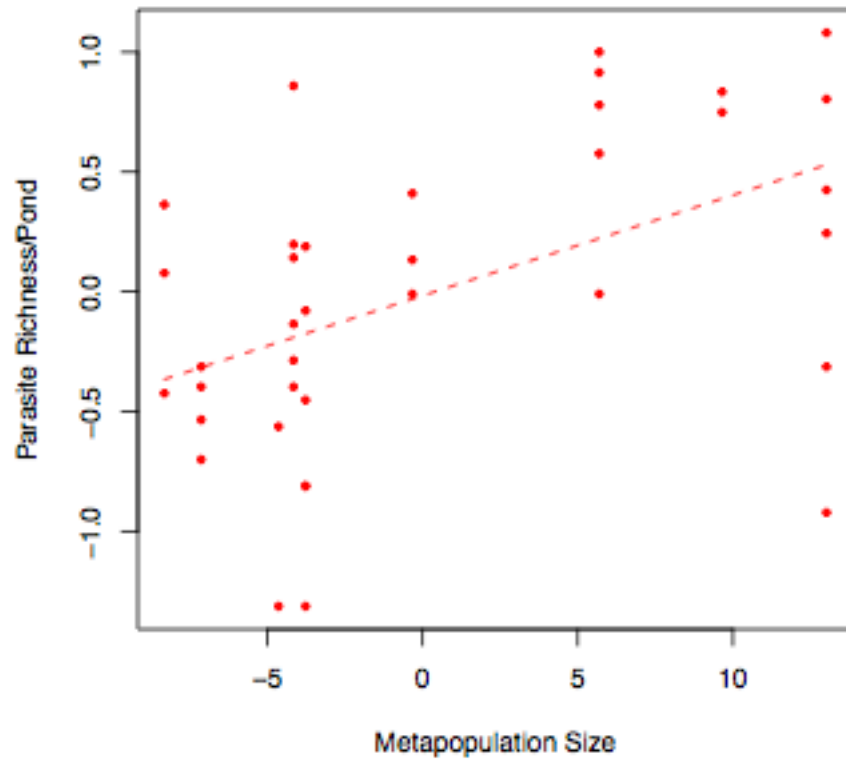


Figure 5. The relationship between adjusted parasite richness per pond and host metapopulation size (Multiple R^2 : 0.235; Adjusted R^2 : 0.214).

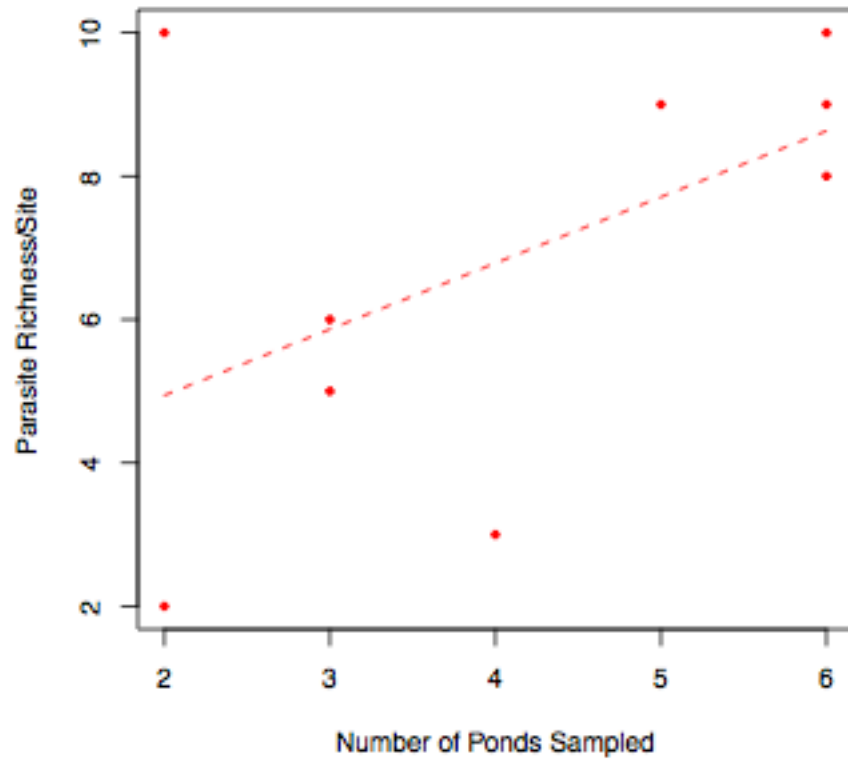


Figure 6. The relationship between raw parasite richness per site and number of ponds sampled per site.

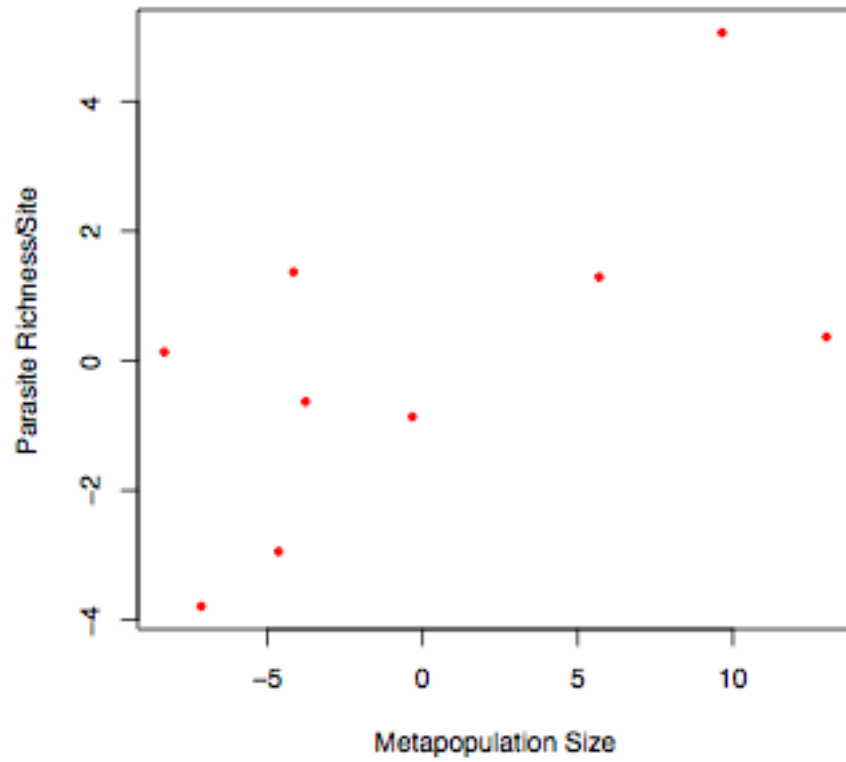


Figure 7. The relationship between adjusted parasite richness per site and host metapopulation size (Multiple R^2 : 0.380; Adjusted R^2 : 0.291).

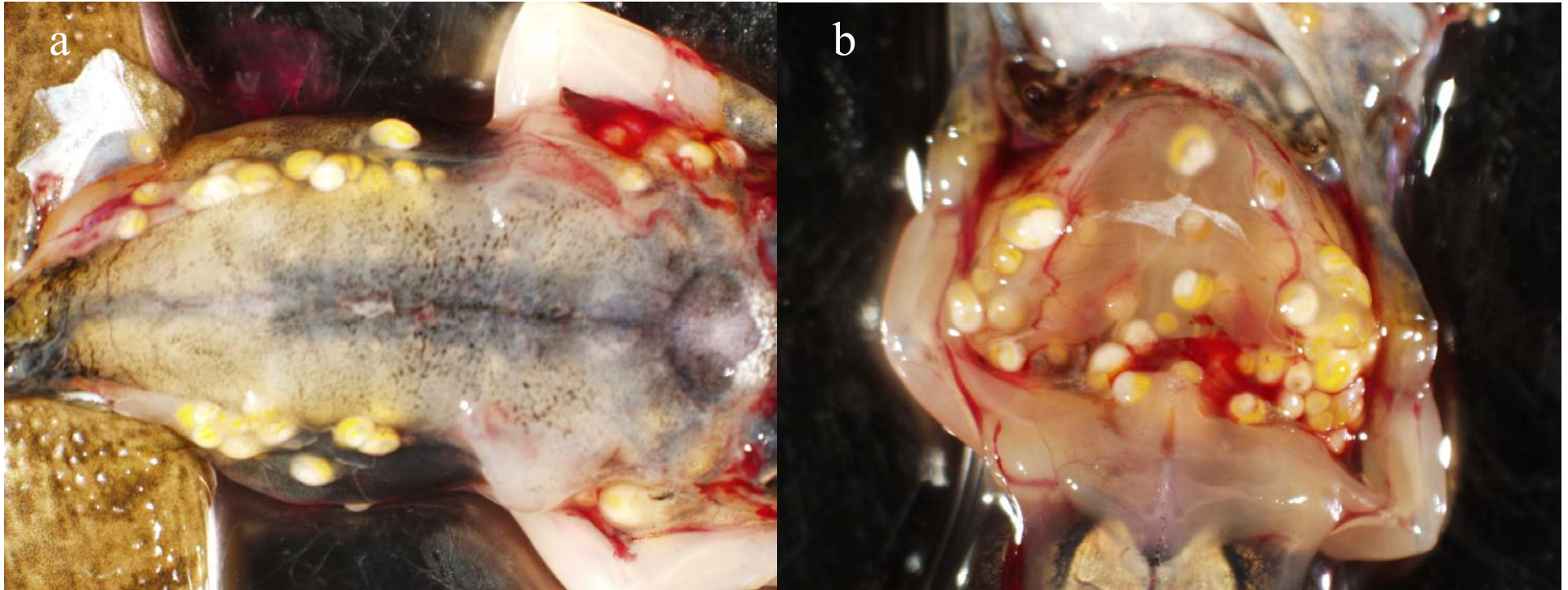


Figure 8. *Clinostomum attenuatum* cysts. (a) Dorsal and (b) jaw views. Photo courtesy of S. Orlofske.