# Dissecting the Relationship Between Free-Living Biodiversity and Disease Risk Using Experimental Manipulations:

An example from freshwater ponds of California

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Defense Date: March 14, 2016

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#### Abstract

How changes in biodiversity affect infectious diseases remains largely unknown. As biodiversity on Earth continues to change, due both to invasive species introductions and native species losses, understanding this relationship will become increasingly important in the study of disease outbreak. Trematodes, which are parasitic flatworms with complex life cycles, offer a useful system in which to study the effects of change in biodiversity on rates of disease prevalence. Here, we used a field experiment to evaluate the effects of change in bird diversity and abundance on patterns of infections by trematode in California freshwater ecosystems. We deployed large-scale environmental manipulations designed to alter visitation rates by definitive avian hosts, either by attracting birds, deterring birds, or leaving bird behavior unchanged. We then measured the resulting bird visitation patterns through time-lapse photography. At each site, we also estimated the diversity and prevalence of trematode infections within freshwater snails, which function as the first intermediate hosts for trematodes. Our manipulations were highly effective at attracting birds, and "attractant" sites exhibited significantly higher bird abundance and richness relative to other treatments. Deterrent manipulations did not significantly reduce bird abundance and richness. Across all sites, bird richness correlated strongly with bird abundance, suggesting low levels of competition among host species, with implications for the potential of dilution in this ecosystem. Host abundance/richness were unrelated to infection prevalence, possibly owing to drought conditions at many study sites. These results suggest that large-scale manipulations can effectively alter patterns of host abundance and diversity with potential relevance for management.

Key Words: Definitive host, parasite, trematode, avian host, aquatic, California, dilution

#### Introduction

Biodiversity loss is widely recognized as a threat to Earth's ecosystems and natural processes, including the physical formation of habitats, biogeochemical cycles, and ecosystem productivity (Cardinale et al. 2012). Growing evidence also suggests that changes in biodiversity could play an important role in the emergence and spread of infectious diseases, including those that affect humans (Myers et al. 2012). For instance, based on a meta-analysis of 61 parasite species of human and non-human hosts, Civitello et al. (2015) reported that host richness often has a negative effect on parasite abundance. Such findings support a hypothesis known as the "dilution effect", which suggests that increased biodiversity – including that of both host and non-host species – reduces host to host parasite transmission (Keesing et al. 2006; Johnson & Thieltges 2010). Conversely, however, other studies find an increase in infection and disease as a response to higher biodiversity, which is known as the "amplification effect". For example, Dunn et al. (2010) suggests that increased richness in mammal and bird species leads to increased pathogen richness. Furthermore, in a study of fish biodiversity and parasites, Wood et al. (2014) found that fishing-driven biodiversity loss was associated with both increased abundance of some parasite species (dilution) and decreased abundance of other parasite species (amplification). Such observations suggest that more research is necessary to understand the mechanistic relationships between these two dynamic factors.

Although the richness and abundance of hosts often correlate with fluctuations in parasite transmission (Johnson et al. 2015), the mechanisms through which biodiversity affects disease risk remains a source of debate (Ostfeld & Keesing, 2013; Wood & Lafferty 2013; Johnson et al. 2015; Salkeld et al. 2015). A higher overall number of hosts and hosts species indicates a greater

number of individuals and species that can potentially become infected and continue the parasite lifecycle, effectively generating more opportunity for parasite transmission and increasing infection rates (amplification). By the same token, if an ecosystem supports very few potential parasite hosts, there is less opportunity for parasite transmission. However, the mechanisms of parasite transmission may look very different when focusing on diversity in an ecosystem. If an ecosystem supports a high variation among species (richness), this indicates that less competent hosts will be sharing that parasite load with more competent hosts, which can lead to an overall decrease in parasite prevalence in an ecosystem (dilution). Pathogen transmission depends on both host richness and host abundance (Mihajievic et al. 2014), and therefore analyzing the two factors in conjunction can illuminate what is driving hosts' impacts on community composition in ecosystems that support several host species.

Multi-host parasites with complex life cycles are a useful tool for investigating relationships between biodiversity and disease. Not only are such parasites a natural component of many ecosystems, but they also represent nearly half of the animal species on Earth (Dobson et al. 2008) and are especially sensitive to changes in biodiversity (Lafferty 2012; Wood et al. 2014). Parasites with complex life cycles, such as *Ribeiroia ondatrae, Clinostomum* spp., and *Schostosoma mansoni*, depend on several different host species to complete their life cycles and reproduce successfully. As a result, these parasites can establish and transmit if their required hosts are also present, often leading to a positive relationship between the diversity of hosts in a system and the number of parasite species. For instance, Hechinger & Lafferty (2005) found that a higher diversity of bird species, which act as definitive hosts for many trematode parasites, correlated with the presence of more trematode species in estuarine snail hosts. But diversity can also inhibit the transmission of trematodes between their different host species. Organisms vary

in their competence as trematode hosts, therefore if there are many potential hosts in an ecosystem with varying levels of competence, less competent hosts may become infected with the trematode. This in turn leads to less effective dispersal of the parasite and an overall decrease in parasite prevalence and infection (Johnson et al. 2008).

To date, most studies on trematodes and biodiversity have used correlational analysis to explore the effects of diversity on infection, often with a relatively small number of study sites. While a number of studies have been done, such as Johnson et al. (2013) amphibian field surveys and laboratory and mesocosm experiments as well as Hechinger and Lafferty's (2005) fieldbased study, most field-based studies are correlational, which can obscure mechanism, whereas most experimental studies are done at small spatial scales or in the lab. Previous studies employing large-scale environmental manipulations to investigate pathogen diversity and abundance have focused on plant communities (Mitchell et al. 2002; Rottstock et al. 2014) and did not look into systems with mobile hosts. Understanding whether the trematode-host relationship is causal requires field experiments that manipulate host behavior to effectively control bird biodiversity and abundance.

Pond ecosystems in California offer a tractable system in which to experimentally investigate the link between biodiversity and parasite transmission. These ponds support a diverse range of trematodes, which are parasitic flatworms with complex life cycles, some of which cause severe pathology in their hosts. For example, *Ribeiroia ondatrae* is a trematode that cycles throughfreshwater snails, amphibians, and birds.Infections in amphibians often cause severe malformations, such as additional, missing, or malformed limbs and digits, which are hypothesized to increase transmission to definitive bird hosts by decreasing the amphibians ability to avoid predators (Johnson et al. 2002). However, freshwater trematode species differ

widely in their use of definitive hosts, which can affect their ability to disperse across the landscape. Ecosystems supporting parasites that infect definitive hosts with a larger dispersal range, such as birds, demonstrate increased parasite prevalence, whereas parasites that are geographically restricted have lower rates of parasitism (Perez-Tris and Bensch 2005). Small, freshwater habitats can support parasite species that infect avian definitive hosts and are relatively easy to manipulate, lending themselves well to experimental approaches to determine how increased and decreased host visitation affects parasite prevalence and diversity. Focusing on the definitive avian host in the trematode life cycle can illuminate how parasite prevalence and diversity is influenced by a host with profound impact on the dispersal and infection success of parasites. Birds have been established as key players in parasite transmission and are, for many trematode species, the source of the life stage that is infectious to snails (Smith 2000; Hechinger & Lafferty 2005).

Here, we test how experimental manipulations of bird abundance and species richness affect the abundance and richness of trematode parasites in small pond ecosystems. Specifically, we developed treatments to increase, decrease or leave bird activity unchanged, and implemented these treatments across 24 ponds in California. We monitored bird activity at the ponds using time-lapse photography and analyzed the photos to quantify bird abundance and diversity. We then linked these data with patterns of trematode infection in freshwater snails at each of the study sites. This project had two primary aims. First, we sought to test whether our experimental deterrents and attractants were effective in changing patterns of bird visitation to ponds. Second, we aimed to test the relationships among avian biodiversity, avian abundance, and trematode parasite infection, with the latter measured in first intermediate snail hosts. By using an experimental approach to explore the links between host diversity and parasite infection within

natural ecosystems, this study helps to bridge the gap between small-scale experimental studies and field-based correlational investigations.

#### **Materials and Methods**

#### Pond manipulation and avian data collection

We selected 24 small ponds located on two adjoining properties in the East Bay area of central California (Figure 2). This area is located on the Pacific flyway, which serves as one of four major migration routes for birds in North America and provides naturally high levels of bird activity (Migratory Bird Program 2012). We selected 12 ponds at Joseph D. Grant County Park and another 12 San Felipe Ranch (Table 1), based on accessibility, existence of prior data, and past presence of pathogenic trematodes. All ponds were then randomly assigned to one of three treatment types: deterrent, attractant, or control (eight ponds per treatment, four on each property; Table 1).

#### **Deterrent manipulations**

We designed each treatment to manipulate the frequency of bird visitation to ponds. To deter birds, we used a variety of commercial bird deterrents, including scare-eye balloons, owl decoys, and mylar reflective tape, and we removed perching habitat in and around the pond to the greatest extent that was acceptable to property managers. Mylar strips of 60-cm length were tied (and reinforced with cable ties) to rebar posts that ringed the pond at 1-m intervals. Two rings of rebar posts were installed, one around the predicted perimeter of the pond at maximum water level and one around the predicted perimeter of the pond at minimum water level, to ensure that at least one ring of mylar would be visible and close to the pond edge, even as the

pond water level changes with the passage of seasons. This method ensured that our manipulations were durable and would be effective throughout the year. The mylar tape (Flash Tape, Bird-B-Gone, Inc., Mission Viejo, CA), was affixed in a manner that enabled it to move freely in the wind - glinting, rustling, and shaking in even light breezes. We suspended one owl decoy (Prowler Owl, Bird-X, Inc., Chicago, IL) from a tree within approximately 20 meters of each deterrent pond in such a way that the model could move freely in the wind. Such decoys, if sufficiently life-like, have been demonstrated to effectively deter certain bird species (Marsh et al. 1992). Lastly, we employed two "octopus scare flag" deterrents at each deterrent pond by hanging them from nearby trees. These deterrents consist of a large yellow circular head with large black eyes and red and mylar streamers (Octopus Bird Scare Flag, Bird-X, Inc., Chicago, IL) (Figure 10).

The initial deployment of the manipulations for this project took place in October of 2014. This original round of deterrent installations included the placement of the inner ring of rebar posts at each pond with mylar tape zip tied to ends, as wells as the hanging of owl decoys, scare-eye balloons, and octopus scare flags and removal of perching habitat. At some ponds, such as Yerba Buena, fallen trees were removed from the ponds to decrease perching habitat. In February 2015, the outer round of rebar posts was installed at each pond, with mylar tape zip tied to ends. Maintenance of the manipulations was required to maximize effectiveness of deterring birds and to accommodate wear-and-tear. Due to the dynamic nature of the environments in which these large-scale manipulations were installed, all deterrents were subject to various stresses such as high winds, rain, human and animal interference, and temperature fluctuations.

In the summer of 2015, we updated the deterrent manipulations at all 8 deterrent ponds to combat deterioration, maintain manipulation effectiveness, and eliminate trash that the

manipulations might have produced. This maintenance included replacing bent or dislodged rebar posts from the perimeter of ponds (often displaced by land mammals or curious hikers), replacing all mylar on posts, re-hanging or replacing fallen or torn scare-eye balloons and raptor decoys, removing large branches from shorelines, and removing any and all garbage from the sites. This maintenance was completed by June 10<sup>th</sup>, 2015, which ensured all manipulations were intact for the first round of camera trapping (Table 2). In a previous short-term study, the above methods were shown to reduce bird species richness at ponds by 40–50% when analyzed prior to the deployment of deterrents (July 21-22, 2013), three days during deployment (July 23-26, 2013), and two days after removal of deterrents (July 28-30, 2013) (Wood and Johnson unpublished data).

#### Attractant manipulations

To attract birds to randomly selected "attractant" sites, we added perching habitat, added nesting habitat, and deployed two mallard duck decoys (one male, one female) at each pond. Any natural perching habitat available in the vicinity of the pond, such as large branches, was haphazardly distributed closer to the water's edge. If no perching habitat was available in the vicinity of the pond, we brought branches in from the nearby forest. This process was completed by June 10<sup>th</sup> 2015, in time for the first round of camera trapping.

One wood duck nesting box and one generic bird nesting box (Backyard Boys Woodworking, Green Bay, WI) were installed at each site. We pounded one 2-m rebar fencepost into the ground at a point far enough from the center of the pond to ensure stability when water levels are at their highest. We then affixed another 1.6-m rebar fence-post to the stable 2 m post with cable ties, in order to ensure maximum stability of the boxes. Predator guards, which we constructed from 5-gallon bucket lids, were installed approximately 1 meter below the bird boxes to prevent nest predation (Huesmann 1975), and boxes were placed away from tree branches that might allow predators to drop down onto boxes. Two mallard decoys, one of each sex, were added to the pond. Each mallard was secured to a cinder block by a rope long enough to allow for natural looking movement of the decoy (Figure 11). The above manipulations were installed by July 7<sup>th</sup>, 2015.

#### Measuring bird activity

To assess bird activity at each pond and measure how it was affected by the manipulations, we used camera trapping to take time-lapse photos over extended periods of time, varying from 4–7 days per trapping session. The use of cameras offers many advantages over direct surveys of birds: it minimizes the effect of human presence on bird behavior and allows larger amounts of data to be collected with fewer person-hours of effort, relative to direct observation. We performed three deployments of cameras at each site during the summer of 2015 (Table 2). DLC Covert MP6 trail cameras with 8 GB memory cards were positioned at each pond for several days, for a total of approximately 15 hours each day. Each camera was programmed to take one time-stamped photo every 3 minutes from 15 minutes after sunrise to 15 minutes before sunset.

We deployed two cameras at each site – one with a narrow view of the pond and one with a broad view of the pond. Each camera was affixed to a 1-m rebar post using cable ties. The "narrow camera" was positioned to capture photos consisting of half-shore and half-water. Because this camera was close to the shoreline, it captured close-up photos that often allowed species-level identification of small birds, such as jays, phoebes, and quails, as well as small mammals, such as ground squirrels. The "broad camera" captured a wide-angle view of the site, allowing us to detect rare birds, such as raptors, as well as large mammals such as pigs, deer, and coyotes. Through the assessment of both cameras in conjunction, we were able to get a wide range of photos and angles capturing the maximum area of the pond and identifying both small and large bird species. Each species of bird was once quantified with either the broad or narrow method. This strategy allowed us to gain the most comprehensive view of bird activity at each site.

#### Photo analysis

Photos were uploaded to TimeLapse2 Image Analyzer (Saul Greenberg, University of Calgary, Calgary, Alberta), a program that enables efficient and accurate scoring, or review, of large quantities of photos. We excluded the first five minutes of photos (i.e., the first two photos) after the camera was deployed, in order to account for disturbance of bird behavior by the researcher deploying the camera. Any photos with obstructed views due to tall grass, sun glare, or darkness that impaired the scorer's ability to see birds were also excluded from the data set. Occasionally, cameras were completely dislodged from the rebar pole (often due to human or animal disturbances); in these cases, all photos after the time of disturbance were excluded, but the photos before the time of disturbance were included.

Sets of photos were organized into folders corresponding to the memory card used for their collection. Each folder was claimed and scored by one observer to avoid discrepancies in bird identification among pictures in one data set. Each photo included in the data set was analyzed individually using TimeLapse2<sup>®</sup>. Observers marked the presence of birds or mammals in each photo. We documented the presence of birds or mammals by clicking the individual in the photo, which would then automatically save the identification associated with that image to an internal

database. Different camera angles and photo clarity led to varying levels of specificity in bird identification. When possible, each bird was identified to species. If the image was unclear and confident identification of the bird was impossible, the bird was marked as "unknown". When the scoring of all photos in a folder was completed, TimeLapse automatically generated a .csv file of the photo data for analysis in other programs (Excel, R).

#### Snail data collection and parasite identification

Quantification of non-avian hosts was also conducted in all 24 ponds. Our team sampled each of the 24 ponds twice over the course of the summer of 2015. At each pond, we performed a site analysis that assessed vegetation abundance and pond size, water chemistry, invertebrates, amphibians, and snails. For the purposes of this project, the most relevant data were the snail data.

At each pond, our crew employed both dip net and seine sampling techniques to quantify the abundance of snails. Dip net, or "sweep", samples were utilized to collect a more localized and smaller-scale sample while seining was used for longer sweeps covering larger areas. The number of dip nets and seines performed at each pond was determined by pond size, with a minimum of ten dip nets and three seines performed in each pond. For the dip net procedure, a net was dropped into the water and swept through while brushing the bottom of the pond. The lengths of these sweeps were approximately 1 m. Dip net sampling is effective for quantifying microinvertebrates (Freeman et. al. 1984) and smaller snail species, such as *Gyraulus* spp. Seine sweeps were performed by two researchers and consisted of dragging a 1 x 1.5-m net for approximately 3–5m through the water at depth of approximately 1m. Seining is an effective method of collection and sampling of larger invertebrates and fishes within a pond (Freeman et.

al. 1984) and was useful for collecting large quantities of snails in one seine. At each pond, our crew aimed to collect 50 individuals of each snail species found at the site (*Lymnaea* spp., *Gyraulus* spp., *Helisoma trivolvis*, or *Physa* spp.). Collected snails were measured and dissected in the lab. After measurement, we cracked and dissected the snails in order to identify those infected hosting trematodes parasites, which are often found in the gonads of the snail. If trematodes were present, we identified, photographed, and estimated their abundance within the snail. This procedure was performed less than one week from the original date of collection of the snail.

#### Statistical Analysis

The focus of our analysis revolved around three central questions: Were our manipulations effective? What is the relationship between abundance and richness of host avian species? And is there a causal relationship between host richness and infection prevalence? To address whether our manipulations were effective in altering bird activity, we analyzed bird and mammal response to manipulations through the compilation of richness and abundance results from photo scoring. Bird and mammal data were compiled into an Microsoft Excel<sup>®</sup> file by the TimeLapse2<sup>®</sup> program. Results were organized by individual photo and labeled by date, time, treatment, and site code. Bird species were organized into five functional categories – passerine, wading and shore birds, raptors, waterfowl, and game (based on recommendations from Richgels et al. 2014). The separation of passerine and non-passerine species enabled us to focus on potential parasite hosts (i.e. non-passerines) when evaluating relationships between avian hosts and infection prevalence..

We analyzed the data using a Poisson generalized linear mixed model (lme4) with a log<sub>10</sub> offset (R Core Development team 2015). We used a generalized linear mixed model to account for our non-normal data set with a disproportionate number of zero values (i.e., photos with no birds present). A mixed model allows for both fixed and random effects, our fixed effects being the dates the photos were taken and our random effect being the study sites. A log<sub>10</sub> offset was applied to photo count. This enables Poisson model response as a rate (i.e. number of bird observed scaled by total number of photos) as opposed to a standard count (i.e. number of photos taken at each pond) which corrects for variation in total number of scored photos at each study site caused by exclusion of photos due to glare, darkness, or camera malfunction.

Infection prevalence and host richness were analyzed using two data sets, both snail dissection and parasite taxonomic data for each study site as well as photo analysis results for bird richness. Parasite data were collected in the summer of 2015 and compiled into a Microsoft Excel<sup>®</sup> spread sheet for analysis. We developed a linear regression model using JMP (JMP Statistical Discovery, SAS Institute 2016) for statistical analysis and a regression figure in Microsoft Excel<sup>®</sup>. We used a similar strategy to analyze bird richness as a response to bird abundance. Bird richness was assessed by counting the number of observed bird species. Birds that were unidentified or unknown were counted together as one species. When broken into passerine/non-passerine groups, unidentified passerines were counted as one species in the passerine group. We grouped photo scoring results by study site, corrected for number of photos taken at each study site by dividing total site photos by total photos per manipulation, assessed using a linear regression model in JMP<sup>®</sup>.

#### Data Set

In total, 45,532 photos from the second round of camera trapping (Table 2) were scored, all time and date stamped; of these, 7,931 were excluded due to glare, darkness, or disturbance. When separated into manipulation groups, this left 11,138 photos from attractant ponds, 10,603 photos from control ponds, and 15,860 photos from deterrent ponds for analysis. Within these photos, we identified 21 bird species and five mammal species. Of the 21 bird species, 14 were classified as passerine and 7 as non-passerine bird species (Table 3). The number of bird species per site ranged from zero to 11 on a single day whereas the number of individual birds per photo ranged from zero to three. The most common birds identified were Mallard ducks, Great Blue Heron, and California Quail (Figure 3) Mammal observations primarily involved ground squirrels and deer, which made up 72% of all mammal individuals identified (925 of 1,284 total mammals). Snail hosts were collected from 18 of 24 study sites over the course of two collection visits. Two sample sites were dry and four others did not have any snail hosts present during sampling. We collected and dissected a total of 1,938 individuals representing four different snail species; Helisoma trivolvis (1,172 dissected), Lymnaea columella (255 dissected), Physa spp. (367 dissected), and *Radix auricularia* (114 dissected). Eighty-one snails were infected with one of eight trematode parasite morphotypes.

#### **Bird Results**

Study sites assigned to the attractant manipulation supported a higher overall abundance (z = 2.659, p = 0.0078; Figure 6) and richness (z = 2.633, p = 0.0085; Figure 7) of birds than did control study sites. However, deterrent sites did not have significantly lower bird abundance (z = (z = 2.659, p = 0.0078; Figure 6))

1.425, p = 0.154) or richness (z = 1.756, p = 0.079) when compared to our control treatment study sites (Figures 6, 7), although the difference in richness was marginal and slightly higher than control sites. On average, all manipulation types had higher richness and abundance of passerine bird species than of non-passerine bird species. At attractant sites, of 3,312 individuals seen, 1808 were passerines (54.6%) and at deterrent sites of 3,172 total individuals, 2,121 were passerines (66.9%). Control sites differed in that of 2,703 individuals seen, 1,228 were passerine (45.4%). There was not a significant change in mammal abundance or richness among manipulation sites (Figures 8, 9).

Among all sampled sites, bird abundance was positively related to bird richness ( $R^2$  value = 0.863, P-value < 0.0001\*\*\*) (Figure 4). Study site SF30, an attractant site, had the highest levels of both abundance (0.65 birds/photo) and richness (0.32 species/photo) of any study site. SF27, a control site, had both the lowest bird abundance and richness of any site (0 and 0, respectively).

#### Parasite Results

We did not find definitive host richness and infection prevalence to be significantly correlated (R-squared value = 0.08962, P-value = 0.286) (Figure 4). Of our 24 study sites, 18 supported snail populations and of those 18, 13 had infected snails. We identified a total of eight different parasite morphotypes and the most commonly seen parasite was was an *Alaria* spp. (30 occurrences). *Helisoma trivolvis* was the most abundant snail (1,172 total individuals) and was present in 17 of the 18 sites where snails were found. *Helisoma trivolvis* was also the most highly infected snail species (63 infected individuals).

### Discussion

We observed varying effectiveness of our manipulations on controlling bird behavior at our 24 study sites. The increased perching/nesting habitat, mallard decoys and bird boxes that we employed at our attractant sites were effective in promoting bird activity in aquatic habitats (Figures 5, 6). Conversely, deterrent manipulations did not decrease bird richness or abundance relative to control treatments. The higher abundance of birds at attractant manipulations was driven largely by passerine bird species, birds that we do not suspect are potential parasite hosts. It is unclear whether non-passerine host species were unaffected by manipulations or if too few non-passerine individuals were observed at the study sites to provide sufficient statistical power for differentiating non-passerine host abundance and richness among treatments. We designed our environmental manipulations to affect bird populations only, and we were successful in that we did not observe significant differences in mammal richness or abundance at control versus deterrent/attractant sites (Figures 8, 9). Land managers should therefore not be concerned that these environmental manipulations will affect abundance and richness of native mammal species and/or cattle grazing behavior.

Birds can habituate to environmental disturbances (Simonsen et. al. 2016, Burger & Gochfeld 1991), which might explain why the deterrent manipulations were ineffective. The bulk of the manipulations were deployed in Fall 2014 and updated in Summer 2015, approximately one week prior to the first round of camera trapping. It is conceivable that this amount of time is sufficient for bird habituation, as previous studies showed habituation of some species after only a few days (Conover & Dolbeer 1989; Fukuda et al. 2008). Future studies are advised to assess both short- and long-term responses to bird deterrent manipulations. Furthermore, frequent

updates and/or changes to deterrent manipulations may increase their effectiveness over long periods of time (Simonsen et. al. 2016).

A strong, positive correlation existed between host abundance and host richness (Figure 5), suggesting that a freshwater ecosystem that supports a higher total number of bird individuals will also support a higher number of bird species. This suggests a lack of strong competition among potential trematode hosts within these communities. Competition between hosts, the "diluters" in the ecosystem, could cause either amplification or dilution depending upon intraspecies competition. Our results suggest that both competent and incompetent parasite hosts are contributing to community makeup which leads to variations in the total competence of the community (Johnson et al. 2015). One of the most highly cited mechanisms of dilution assumes that as the number of species (i.e., biodiversity) increases, the density of competent hosts in the community decreases, effectively "diluting" the competent host population and reducing transmission. But if there is a direct correlation between community-level abundance and richness, this suggests that the density of competent hosts may not necessarily decline as richness increases. This in turn will diminish the effects of dilution, because as we add species to an ecosystem we are not necessarily "diluting" the density of competent hosts. However, we have not yet identified the definitive host of many of these trematode parasites and therefore cannot draw a definitive conclusion regarding how infection prevalence of a particular trematode species will respond to this richness-abundance correlation. We can use this correlation information in conjunction with an understanding of community makeup to predict which diversity-disease relationship, dilution or amplification, will dominate in an ecosystem (Mihaljevic et al. 2014), but further research regarding competence of particular hosts is necessary to make these predictions. A logical next step to this study would be looking more

deeply into the community make up of potential hosts at ponds supporting infection to potentially illuminate key species for parasite transmission. A study done by Hall et al. (2009) found a negative correlation between host abundance and host richness when looking at *Daphnia* host species, suggesting strong competition between hosts. Beyond a deeper look into community make up, this discrepancy highlights the complexity of community ecology and the necessity for future research into predictors of disease outbreak.

Our study shows a clear increase in biodiversity as a result of increased overall abundance for bird hosts, that increased biodiversity does not translate to increased infection prevalence. Linear regression analysis did not present evidence to support either the dilution or amplification effects through our analysis of parasite richness as a response to host richness. While we recorded variation in biodiversity of both definitive hosts and parasites among study sites, there was not a significant correlation between host biodiversity and infection prevalence. This study contributes to a growing body of research focusing on the effects of definitive hosts on disease transmission. Hechinger & Lafferty (2005) found strong evidence supporting amplification when studying definitive avian hosts and infection prevalence, but their study was observational and was set in salt-water marsh ecosystems. These discrepancies between studies regarding definitive hosts and infection prevalence highlight the need for further investigation.

The factors affecting infection rates in a community extend beyond biodiversity of host species. While we controlled for environmental variation by randomization of treatments, many environmental factors may still influence host and parasite richness and abundance: these include water levels, wind speeds/directions, and temperature. California drought conditions in 2015 were described as a one-in-1,000 year event (Asner et al. 2015), and these conditions

undoubtedly have effects on freshwater ecosystems. Dry and extremely low-water ponds cannot support aquatic hosts or parasites, and therefore will result in low infection rates.

Correcting for baseline bird data from the previous year would be a logical next step for this project. Baseline data would be the observed abundance and richness of birds at each site before the manipulations were installed, collected during the same time of year as the observed bird abundance and richness after the manipulations were installed. These data would represent the bird populations that the ponds support in their natural state. We randomly assigned our study ponds to treatments, which was intended to control for bias and ensure that there were not systematic differences among the treatments in terms of the pond's natural abundance and diversity of birds. However, because each treatment had relatively low levels of replication (8 ponds per treatment), it is possible that randomization alone was not sufficient to eliminate systematic differences. Correcting for baseline data on natural levels of bird abundance and richness at each pond would allow us to detect the true, absolute, effect of the manipulation treatments on bird abundance and prevent us from overlooking these absolute effects due to natural variability among ponds.

#### Conclusion

The driving factors of infectious disease remain complex and poorly understood, but this study fits a piece into the puzzle. Ecosystems are complex and difficult to manipulate, and for this reason large-scale experimental studies are not common. Yet we showed it is possible to increase the abundance and richness of essential hosts, effectively controlling factors of interest to infectious disease researchers. While we did not find evidence for either dilution or amplification, we demonstrate the need for consideration of an entire ecosystem rather than just

one isolated factor when unraveling infectious disease dynamics. The fact that host abundance increases with host richness is an important conclusion that provokes questions regarding the mechanisms of dilution and amplification. This study contributes to a growing body of research in infectious disease and community ecology and helps to unveil the mysteries of our natural world.

#### Acknowledgements

I would like to thank Dr. Pieter Johnson for his mentorship, encouragment, and expertise that made this project possible. Thank you to Dr. Chelsea Wood for allowing me to collaborate on this project and providing tireless assistance and support from the beginning to the end. Thank you to my committee members Dr. Abby Hickcox and Dr. Rebecca Safran. Thank you to the entire Johnson lab, especially Dain Calhoun, and the Johnson field crew, especially Travis McDevitt, for their willingness to share their resources, ideas, knowledge, and time to make this project a reality. Thank you to the faculty at Blue Oaks Ranch Reserve, especially Mike Hamilton and Erik and Lucy Vikk for their help, encouragment and willingness to share beautiful Blue Oaks Ranch Reserve with young researchers. Thank you to Harlan Wittkopp for his assistance at San Felipe Ranch as well as the owners of San Felipe Ranch. Thank you to Karen Cotter and the managers at Joseph D. Grant Park for allowing me to conduct research on their property. Thank you to University of Colorado Undergraduate Research Opportunity Program (UROP) for the funding that enabled me to conduct independent research, and lastly thank you to the University of Colorado's Department of Ecology and Evolutionary Biology.

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Site Name	Park	Manipulation	Coordinates
Kammerer	Joseph D. Grant	Control	37.3626, -1221.7323
Heron	Joseph D. Grant	Control	37.3418, -121.7363
Manzanita	Joseph D. Grant	Control	37.3117, -121.6573
Pig	Joseph D. Grant	Control	37.3062, -121.6746
SF-31	San Felipe Ranch Control 37.300		37.3001, -121.7074
SF-27	San Felipe Ranch	Control	37.2894, -121.7029
SF-37	SF-37 San Felipe Ranch		37.2876, -121.6730
SF-101	SF-101 San Felipe Ranch		37.2799, -121.6640
Windmill	Joseph D. Grant	Attractant	37.3666, -121.7269
Ferrari	Joseph D. Grant	Attractant	37.3467, -121.6875
Glorious	Joseph D. Grant	Attractant	37.3203, -121.6729
No Talk	Joseph D. Grant	Attractant	37.3145, -121.6854
SF-34	San Felipe Ranch	Attractant	37.3036, -121.6957
SF-30	San Felipe Ranch	Attractant	37.2944, -121.7030
SF-19	San Felipe Ranch	Attractant	37.2921, -121.7062
SF-40	San Felipe Ranch	Attractant	37.2990, -121.6714
Washburn	Washburn Joseph D. Grant		37.3689, -121.7257
Yerba Buena	Joseph D. Grant	Deterrent	37.3361, -121.6898
Rattle Snake	Joseph D. Grant	Deterrent	37.3151, -121.6909
Sharptail Joseph D. Grant		Deterrent	37.3099, -121.6949
SF-26	San Felipe Ranch	Deterrent	37.2788, -121.6981
SF-25	San Felipe Ranch	Deterrent	37.2755, -121.7000
SF-41	San Felipe Ranch	Deterrent	37.3015, -121.6725
SF-85	San Felipe Ranch	Deterrent	37.2962, -121.6583

 $\label{eq:table1} \begin{array}{l} \textbf{Table 1} - \textbf{Manipulation assignments and coordinates of each study site, separated by park} \end{array}$ 

# **Table 2**Dates of camera deployments in each park

	Joseph D Grant Park	San Felipe Ranch
Round One	6/14/15 - 6/19/16	6/19/15 - 6/26/15
Round Two	6/29/15 - 7/2/15	7/12/15 - 7/17/15
<b>Round Three</b>	7/24/15 - 7/26/15	7/18/15 - 7/22/15

# Table 3

Bird and mammal species observed during round two of camera deployment split into functional groups

Passerine Latin Name	Common Name	Non-Passerine Latin name	Common Name	Mammal Latin Name	Common Name
Melanerpes formicivorus	Acorn Woodpecker	Buteo platypterus	Broad Winged Hawk	Canis latrans	Coyote
Corvus brachyrhynchos	American Crow	Ardea herodias	Great Blue Heron	Bos spp.	Cow
Turdus migratorius	American Robin	Ardea alba	Great Egret	Odocoileus hemionus	Deer
Sayornis nigricans	Black Phoebe	Anas platyrhynchos	Mallard	Otospermophilus beecheyi	California Ground Squirrel
Callipepla californica	California Quail	Buteo jamaicensis	Red-tailed Hawk	Sus. spp.	Pig
Melozone crissalis	California Towhee	Meleagris spp.	Turkey		
Charadrius vociferus	Killdeer				
Zenaida macroura	Mourning Dove				
Colaptes auratus	Northern Flicker				
Cyanocitta stelleri	Steller's Jay				
Sialia Mexicana	Western Bluebird				
Tyrannus verticalis	Western Kingbird				
Aphelocoma californica	Western Scrub Jay				
Pica nuttalli	Yellow-Billed Magpie				

**Figure 1** Trematode lifecycle employing definitive avian host



**Figure 2** Map of study sites in California's East Bay Area



Distribution of bird and mammal observations during round two of photo trapping, separated into passerine, non-passerine, and mammal functional groups



Infection prevalence in ponds as a response to non-passerine bird richness. Each data point represents one study site (i.e. pond).







Overall bird abundance of all functional groups, grouped by manipulation.





Overall mammal abundance observed grouped by manipulation type.









# **Figure 10** Example deterrent manipulations. (Left to right) Octopus Scare Eye Balloon, rebar post with mylar tape, raptor decoy



**Figure 11** Example attractant manipulations, (left to right) Bird nesting box with predator guard, duck box with predator guard, mallard decoy (top: male, bottom: female)