Multi-scale Transmission Ecology: How Individual Host Characteristics, Host Population Density, and Community Structure Influence Transmission in a Multi-host Snail Symbiont System

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ABSTRACT

We live in an era of global change, where emerging infectious diseases such as Ebola, Zika, bird flu, and white nose syndrome are affecting humans, wildlife, and domesticated species at an increasing rate. To understand and predict the dynamic spread of these infectious agents and other symbionts through host populations and communities, we need dynamic mathematical models that accurately portray host-symbiont transmission. But 'transmission' is an inherently difficult process to measure or study, because it is actually a series of interacting processes influenced by abiotic and biotic factors at multiple scales, and thus empirical tests of the transmission function within epidemiological models are rare. Therefore, in this dissertation, I explore factors at the individual, population, and community-levels that influence host contact rates or symbiont transmission success in a common snail-symbiont system, providing a detailed description of the multi-faceted nature of symbiont transmission. From a review of the ecological literature, I found that most models assume that transmission is a linear function of host population density, whereas most empirical studies describe transmission as a nonlinear function of density. I then quantified the net nonlinear transmission-density relationship in a system where ectosymbiotic oligochaetes are directly transmitted among snail hosts, and I explored the ecological mechanisms underlying the nonlinear transmission-density relationship observed in the field via intraspecific transmission success and contact rate experiments in the laboratory. I found that the field results could

be explained by heterogeneity in transmission success among snails with different characteristics and nonlinear contact-density relationships caused by non-instantaneous handling times. After I 'unpacked' population-level transmission dynamics into those individual-level mechanistic processes, I used this same approach to examine higher-level ecological organization by describing the mechanistic underpinnings of interspecific or community-level transmission in the same snail-symbiont system. I found that low interspecific transmission rates in the field were the product of opposing interactions between high population densities, high prevalences of infection, and very low interspecific transmission success caused by strong symbiont preferences for their current host species. Unpacking transmission in this way resulted in one of the most detailed empirical studies of transmission dynamics in a wildlife system, and yielded many surprising new insights in symbiont ecology that would not have been discovered with a purely phenomenological or holistic view of transmission. Though simple, linear, and holistic epidemiological models will always be important tools in disease ecology, 'unpacking' transmission rates and adding heterogeneity and nonlinearity to models, as I have done here, will become increasingly important as we work to maximize model prediction accuracy in this era of increased disease emergence.

GENERAL AUDIENCE ABSTRACT

Parasites and pathogens can have important implications for wildlife conservation, the production of domesticated animals and crops, and human health, and thus ecologists and epidemiologists need effective tools for understanding, predicting, and managing the spread of these important pathogens. Mathematical models that represent the transmission of pathogens within single wildlife host species (e.g., Ebola transmission within bat populations) and between different host species (e.g., Ebola transmission between bats and humans) are one such tool, and these same models can be used to understand the spread of beneficial symbionts that actually help the host by being present. But despite being critical tools, these mathematical models are not yet perfect. In fact, in this dissertation work, I demonstrate that the most commonly used models are not well-supported by data from real host-parasite systems, and that the fundamental assumptions underlying these models are rarely tested, because measuring transmission among individuals is often difficult. Therefore, I developed experimental methods to test some of these fundamental assumptions in a system where tiny annelid worms live on aquatic snails, and are only transmitted from one snail to the next during direct contacts between snails. In particular, I first used field studies in a Virginia pond to describe how the rate of worm transmission within and between snail species depends on snail density. I then used laboratory experiments to understand how the rate of contacts between snails and worm preferences for particular snail characteristics (i.e., size, species) influence worm transmission rates. Taken together, this work represents one of the most detailed studies of transmission dynamics in a wildlife system, and yielded many important new insights regarding how to make epidemiological models more biologically realistic.

Though the simplest epidemiological models that we have relied on for decades will continue to be useful, the more complicated, biologically-realistic models explored here will become increasingly important as we work towards improving our abilities to precisely and accurately predict and manage parasite transmission.

DEDICATION

To snails, underground trails, and puppy-dog tails.

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ATTRIBUTION

Chapter 1 was co-authored with my advisor, Lisa Belden, committee member Jeremy Wojdak, and Arietta Fleming-Davies. All co-authors helped analyze subsets of the papers to ensure that multiple experts would reach the same conclusions. JW and AF helped to design the model fitting simulations, run the simulations, and analyze the output. All co-authors contributed substantially to manuscript revisions.

Chapter 2 was co-authored with my advisor, Lisa Belden, committee member Jeremy Wojdak, and an undergraduate research assistant, Lindsey Boyle. All co-authors helped to conceive and design the experiments and contributed to editing the manuscript. LJB performed the experiments with my assistance.

Chapter 3 was co-authored with my advisor, Lisa Belden, committee member Jeremy Wojdak, and an undergraduate research assistant, Cari McGregor. All co-authors helped to conceive and design the field study and experiments. CM helped with the laboratory experiments, JW helped with data analysis, and LB and JW contributed to editing the manuscript.

Chapter 4 was co-authored with my advisor, Lisa Belden, committee member Jeremy Wojdak, and an undergraduate research assistant, Cari McGregor. All co-authors helped to conceive and design the field study and experiments. CM helped with the laboratory experiments, JW helped with data analysis, and LB contributed to editing the manuscript.

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INTRODUCTION

From both theoretical and applied perspectives, predicting the dynamics of infectious disease epidemics in host populations and communities remains a priority and a challenge (De Castro and Bolker 2005, Johnson et al. 2015). Accurate predictions are a priority because parasitic viruses, bacteria, protozoa, worms, and arthropods (hereafter "parasites") can influence the distribution and abundance of their hosts, including driving host species to extinction (e.g., van Riper et al. 1986, LaDeau et al. 2007, McCallum et al. 2009, Kilpatrick et al. 2010, McCallum 2012, Frick et al. 2015). However, making accurate predictions is a challenge because host and parasite communities are complex and dynamic ecological systems, with many interacting and variable components (Keesing et al. 2006, Dobson et al. 2008, Rudge et al. 2013, Johnson et al. 2015). Fortunately, that complexity can be distilled by mathematical models that provide ecologists, epidemiologists, and policy makers with a tool for turning otherwise intractable systems into tractable entities for study (Alexander et al. 2012, Heesterbeek et al. 2015).

In particular, variants of the Susceptible-Infectious-Recovered (SIR) epidemiological models have been important tools for understanding and managing the spread of parasites among humans, wildlife, and domesticated species for several decades (*sensu* Hamer 1906, Kermack and McKendrick 1927, Anderson and May 1979, May and Anderson 1979). Since their original conception, these models have been expanded to include multiple host and/or multiple parasite species (e.g., Dobson 2004), evolution in the host and/or parasite populations (e.g., Galvani 2003), and many other system-specific details. However, one component of the earliest epidemiological models has remained largely unchanged: the central transmission function that describes how susceptible hosts interact with infected hosts and/or how susceptible hosts interact

with infectious agents. But were the earliest, simplest proposed transmission functions the best choices for the ensuing decades of ecological studies, or have we missed opportunities to improve transmission theory?

Evaluating transmission functions is complicated because "transmission" is a difficult ecological phenomenon to define, measure, and model (e.g., Lello and Fenton 2017). Though we often imply that transmission is a single process represented by a single model parameter (the transmission rate, β), transmission is actually a series of discrete processes acting at multiple ecological scales (e.g., Lello and Fenton 2017, McCallum et al. 2017; Figure Intro.1). For instance, in order for an uninfected, susceptible host to become infected, that host must first encounter an infectious host and/or an infectious stage of the parasite (=contact), and then the parasite must successfully navigate to the appropriate host tissues and survive potential resistance from the host's immune system (=transmission success). Each of those processes can be influenced by many abiotic and biotic factors, including host movement behavior, host physiology, within-host dynamics, and environmental conditions (e.g., Donnelly et al. 2007, Rosenthal 2009, Plowright et al. 2016). Given this complexity, several authors have recently suggested that epidemiological models could be improved if disease ecologists were to "unpack" the transmission rate into its distinct 'contact rate' and 'success rate' components (e.g., Civetello and Rohr 2014, McCallum et al. 2017). But on the other hand, reductionist approaches in ecology can miss important emergent or synergistic effects that more holistic approaches would include (e.g., Lidicker 1988). Ideally, epidemiological models will balance holistic and mechanistic approaches, such that they provide accurate descriptions or predictions of higher-order transmission dynamics using parameters that have straightforward biological interpretations.

The overarching goal of my dissertation work was to holistically describe transmission dynamics, and then to identify and quantify how contact rates and transmission success rates work independently and collectively to influence net transmission dynamics at multiple scales. I began with an extensive review of the ecological literature (Chapter 1), where I quantified which transmission functions are used by ecologists, which transmissions are best supported by ecological data, and the consequences of the mismatch between modeling practices and real transmission ecology. My review revealed a paucity of empirical tests of transmission theory, so I developed methodologies to use a tractable snail-symbiont system as a model for directly-transmitted parasites or other symbionts (Chapter 2). I then quantified net intraspecific (Chapter 3) and interspecific (Chapter 4) symbiont transmission in the field using a flexible, phenomenological function. Finally, I used a series of laboratory contact rate and transmission success experiments to "unpack" those broad intraspecific (Chapter 3) and interspecific transmission (Chapter 4) patterns into their underlying mechanistic explanations. Taken together, these studies disassembled symbiont transmission into its component parts, and demonstrated how ecological processes collectively influence higher-order symbiont transmission dynamics.

Study system:

Chaetogaster limnaei (hereafter *Chaetogaster*) is an ectosymbiotic oligochaete worm that lives on the headfoot of aquatic snails. *Chaetogaster* has been documented on upwards of 15 snail genera and there are records of the oligochaete from all continents except Antarctica (Smythe et al. 2015). In Virginia in particular, *Chaetogaster* is found on both prosobranch and pulmonate snails in ponds and streams (S. Hopkins, unpublished data). During the spring and summer, the oligochaetes asexually reproduce (Vaghin 1946) and, as far as we know, are only found on their snail hosts and predominantly disperse to new hosts during direct snail contacts

(Chapter 2). During the fall and winter, some *Chaetogaster* might sexually reproduce and produce cocoons, but it is unclear how often sexual reproduction occurs and whether the cocoons spend the winter on snails or in pond sediments (Vaghin 1946). Therefore, my dissertation work focused specifically on *Chaetogaster* transmission during the late spring and summer.

The symbiont continuum, from mutualism to parasitism:

Symbiotic relationships vary on a continuum from beneficial (=mutualism, net increase in host fitness) to detrimental (=parasitism, net decrease in host fitness), and a given symbiont's location on this continuum is often context-dependent (e.g., Bronstein 1994). It is currently unclear whether *Chaetogaster* is predominantly a mutualist or parasite of snails, or the extent to which the relationship is context-dependent (Rodgers et al. 2005, Stoll et al. 2013, Hopkins et al. 2017). But the transmission functions in epidemiological models work equally well for mutualistic or parasitic symbionts, so I consider the *Chaetogaster*-snail system a good testing ground for transmission theory, especially because beneficial symbionts have mostly been ignored as ecologists have worked towards developing a general predictive framework for symbiont transmission.

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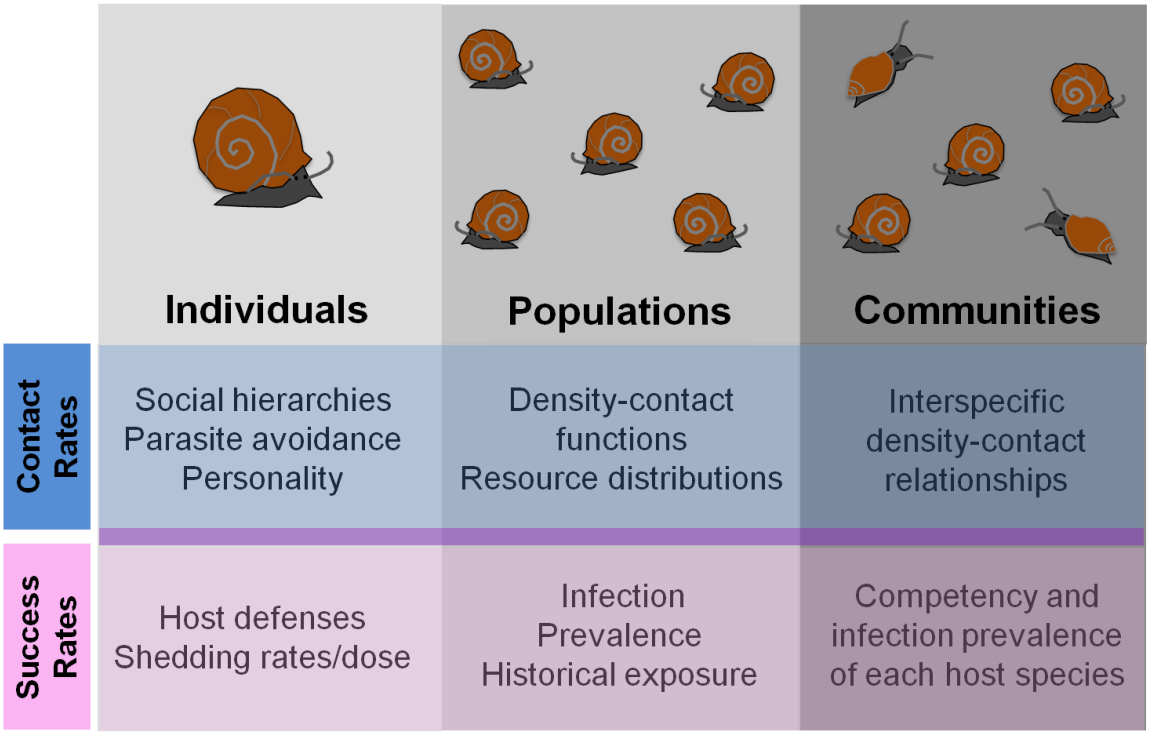
Figure Intro.1. Transmission is the product of ecological processes involving transmission-relevant contacts (contact rates) and parasite transmission success given a contact (success rates). These interacting processes are influenced by factors at multiple hierarchical scales, examples of which are given in the cells of this table.



	Individuals	Populations	Communities
Contact Rates	Social hierarchies Parasite avoidance Personality	Density-contact functions Resource distributions	Interspecific density-contact relationships
Success Rates	Host defenses Shedding rates/dose	Infection Prevalence Historical exposure	Competency and infection prevalence of each host species

CHAPTER 1: NONLINEAR TRANSMISSION FUNCTIONS OUTPERFORM CANONICAL LINEAR FUNCTIONS IN HOST-PARASITE MODELS.

Co-authors: Arietta E. Fleming-Davies, Lisa K. Belden, and Jeremy M. Wojdak

Abstract:

In all dynamic species interaction models, there are assumptions regarding how individual contact rates scale with population densities. In epidemiological models, contact rates are incorporated into a 'transmission function' that affects several aspects of model dynamics and stability, including population invasion thresholds and whether the host and parasite can coexist. Though choosing an appropriate transmission function is a critical step in building models for understanding and predicting disease dynamics, existing guidelines for these decisions are unclear. In particular, there is limited guidance as to when a practitioner should use a particular linear, canonical transmission function (density or frequency dependent transmission) or one of the many other proposed transmission functions. To determine which transmission functions are most commonly used by ecologists, and to identify the ecological contexts under which those functions are used, we performed a quantitative review of the recent ecological literature. We then performed a vote-counting meta-analysis of empirical studies to determine whether existing model practices are supported by observed relationships between host contact rates and host density and between parasite transmission rates and host density. Finally, we used model simulations to assess how mismatches between epidemiological theory and biological data influence model fits and predictions. We found that the majority of epidemiological models in the ecological literature assume that host contact rates and parasite transmission rates are linear functions of host density, even though these relationships are typically nonlinear in real host-parasite systems. This mismatch between theory and realty biases

estimates of important epidemiological parameters, especially when the basic reproductive rate of the parasite is low to moderate. Therefore, we recommend that as ecologists continue to improve epidemiological models, we should renew our focus on the backbone of all epidemiological theory: the transmission function.

Introduction:

Before a lion can eat a giraffe, a bee can sip nectar from a flower, or a parasite can worm its way inside of a host, the two individuals must contact. Ecologists have agreed that such contacts are vital ecological phenomena, and thus every dynamic species interaction model contains a central function that describes the contact rates among organisms (e.g., Lafferty et al. 2015). But which contact function(s) should be used? Mechanistic models typically start with the underlying assumption that there is a linear relationship between contacts and density, a so-called "mass-action" assumption or a Type I functional response (e.g., Holling 1965). However, nonlinear functions that saturate with density have often been proposed as more biologically realistic alternatives to simple linear functions (e.g., Type II functional response). The choice of contact function is critical because it influences the dynamics and stability of consumer-resource interactions, and thus our ability to understand and predict outcomes in natural systems (e.g., Holling 1965; Fenton et al. 2002). In this paper, we explore the congruence between the contact functions used in models (ecological theory) and the contact functions that occur in real systems (data) for an important class of ecological models: those that describe the dynamics of host-associated viruses, bacteria, protozoa, worms, and arthropods (hereafter “parasites”).

In particular, variants of the Susceptible-Infectious-Recovered (SIR) epidemiological models are important tools for understanding and managing the spread of parasites among humans, wildlife, and domesticated species (*sensu* Hamer 1906; Kermack and McKendrick

1927; Anderson and May 1979; May and Anderson 1979). These epidemiological models always contain at least one function that describes how susceptible hosts interact with infected hosts and/or how susceptible hosts interact with infectious agents in the environment. This function includes terms representing both contact rates and transmission success rates (see below), and thus we will refer to it as the "transmission function." The transmission function plays a large role in determining the dynamics and stability of the host-parasite model (e.g., Getz and Pickering 1983; Hochberg 1991; Fenton et al. 2002), including dictating the host densities at which a given parasite can invade a host population or community (Kermack and McKendrick 1927) and whether the host and parasite can coexist (Getz and Pickering 1983). However, despite its central importance, the transmission function has typically remained a simple linear function, even as ecologists have worked for decades to increase epidemiological model realism by adding novel components to the basic SIR modeling framework (e.g., multiple host and/or multiple parasite species *sensu* Dobson 2004, evolution in the host and/or parasite populations *sensu* Galvani 2003).

Our epidemiological theory needs to accurately reflect empirical data because infectious diseases are emerging at an increasing rate, parasites can dramatically influence the distributions and abundances of their hosts, and we rely heavily on epidemiological models to predict the epidemic dynamics of important parasites (van Riper et al. 1986; LaDeau et al. 2007; Jones *et al.* 2008; McCallum et al. 2009; Kilpatrick *et al.* 2010; McCallum 2012; Thogmartin et al. 2013; Frick et al. 2015). We therefore provide the answers to three pressing ecological questions: what transmission theory are we using, what transmission theory should we be using, and what are the consequences of the discrepancies between theory and data? Specifically, we first quantify the transmission functions that are most commonly used in ecological epidemiological models. We

then show that the commonly-used, linear functions do not match the qualitatively nonlinear relationships between host density and contact rates and/or host density and transmission rates that we found in the empirical literature. To illustrate how problematic this mismatch between theory and data is, we use model simulations to demonstrate the extent to which choosing an inappropriate, linear function compromises biological understanding and predictions. Finally, we suggest several important future directions of study that will ensure that our theory better reflects real host-parasite ecology.

Methods:

Overview of transmission functions

In the density-dependent transmission function (DD), transmission-relevant contact rates are a linear, increasing function of host density (N)(Figure 1.1). Assuming that no contacts occur when host density is zero, this line passes through the intercept and the contact rate function (g_{DD}) can be described with just a slope: $g_{DD}=0+c_{DD}N$. In contrast, the frequency-dependent transmission function (FD) assumes that transmission-relevant contact rates are not influenced by host density (Figure 1.1). In that case, the contact rate function (g_{FD}) can be described with just an intercept ($g_{FD}= n + 0*N$), but for our purposes we will consider it a coefficient: $g_{FD}= 0 + c_{FD}*N^0$ (see below). The FD function is typically recommended for host-parasite systems with any one of the following three characteristics: (1) the parasites are sexually-transmitted or vector-transmitted (Thrall et al. 1993; Antonovics et al. 1995; Anderson and May 1991; Keeling and Rohani 2008; but see Wonham et al. 2006); (2) the parasites are directly-transmitted parasites of humans, whether transmission is venereal or non-venereal (Bjørnstad et al. 2002; Mills et al. 2004; Keeling and Rohani 2008); or (3) host density remains constant as population size changes (e.g., fixed territory sizes, fixed group or family sizes), such that the rate of local

contacts remains constant with increasing population size (e.g., Begon et al. 2002; McCallum et al. 2002). The DD transmission function has historically been used for all other systems (i.e., non-human hosts, direct non-venereal transmission, and homogeneous contact structures).

However, more than two decades ago, researchers began to propose nonlinear transmission functions that often fall somewhere between the DD and FD transmission functions (e.g., Hochberg 1991; Heesterbeek and Metz 1993; Antonovics et al. 1995; Dwyer et al. 1997). The broader justification for these nonlinear functions is that functions that incorporate nonlinear relationships might be more biologically realistic than assuming that contact rates increase infinitely with host density (i.e., DD transmission function) or assuming that contact rates never decline, even when population density is vanishingly small (i.e., FD transmission function). We use one particular nonlinear contact-density function in this paper (see below; Figure 1.1), which allows for both saturating and concave up relationships between transmission-relevant contact rates and host density: $g_{NL} = 0 + c_{NL}N^K$, where K is the dimensionless density dependence parameter. When K=0, the FD function is recovered, and when K=1, the DD function is recovered (Figure 1.1).

A typical SIR epidemiological model assumes that a population of hosts can be divided into discrete subpopulations where S is the number of susceptible hosts, I is the number of infected and infectious hosts, and R is the number of hosts which are resistant to infection. To simplify the derivation, we assume that the area (A) occupied by hosts is constant, so that host abundances and densities are equivalent, and we assume that the total host population density (N) is constant through time (i.e., no host demography). If we assume that the host population mixes homogeneously and continuously, the change in the number of susceptible hosts over time can be described by the following differential equation:

$$\frac{dS}{dt} = -gv\frac{I}{N}S$$

where the probability that a susceptible individual becomes infected is a function of the probability of a host-host contact (given by the contact rate function, g), the probability that a given contact between susceptible and infected individuals leads to successful transmission of the parasite (v), and the probability that any given contact made by a susceptible individual is a contact with an infected individual (I/N). The parameters g*v are often combined into one term, called the transmission rate (β), and g*v*(I/N) is the force of infection (FOI).

After substituting the appropriate contact rate function – g_{DD}, g_{FD}, or g_{NL} – into the above equation and simplifying, we obtain the familiar canonical epidemiological models and a flexible nonlinear model that can simplify to the two canonical models.

$$DD: \frac{dS}{dt} = -C_{DD}vSI$$

$$FD: \frac{dS}{dt} = -C_{FD}v\frac{I}{N}S$$

$$NL: \frac{dS}{dt} = -C_{NL}vN^{K}\frac{I}{N}S$$

We refer interested readers to the more detailed derivations in Begon et al. (2002) and Smith et al. (2009), which include area as a model parameter and account for differences in the units of the contact rate coefficients. We also emphasize that there are many alternative nonlinear functions (e.g., McCallum et al. 2001), including a commonly-used, phenomenological "power function" that allows for nonlinearities in the effects of both susceptible hosts and environmental stages (or infectious hosts) on transmission rates (Hochberg 1991; Fenton et al. 2002).

Review of epidemiological theory used in the recent ecological literature

To determine which functions are the most commonly used for any given transmission mode (i.e., sexually-transmitted, vector-transmitted, environmentally-transmitted, and directly-

transmitted non-venereal parasites), we quantitatively reviewed the recent ecological literature published between 2001 and 2015. We first used Web of Science to obtain a sample of the transmission functions used in epidemiological models by performing relevant keyword searches for papers in seven representative journals: American Naturalist, Ecology, Ecology Letters, Journal of Animal Ecology, Journal of Applied Ecology, Proceedings of the National Academy of Sciences, and Proceedings of the Royal Society B. Using this method, we collected 222 papers containing SIR-type models describing the horizontal transmission of an infectious agent among animal hosts, including humans. We then determined whether each study used a single transmission function - the DD transmission function, the FD transmission function, or a single nonlinear transmission function - or whether the paper qualitatively or quantitatively evaluated multiple transmission functions (e.g., Rachowicz and Briggs 2007; Civitello and Rohr 2014). Papers that evaluated multiple transmission functions may have included a nonlinear option, but many only compared the two canonical functions.

Review of comparative evaluations of multiple transmission functions

To determine whether the functions that are most commonly used for any given mode of transmission accurately reflect the biological relationships in real host-parasite systems, we then quantified which transmission functions or contact functions were best supported by data from the existing literature. In particular, we sought out papers that used experimental or observational data to decide which function best described contact rates or transmission rates in a particular host-parasite system. We considered all papers that evaluated multiple transmission functions in our review of the recent literature (see above), but limited this analysis to only those papers that quantitatively evaluated multiple functions by fitting models to data, which resulted in a dataset of 11 papers. We also performed literature searches to find papers that were published before

2001 or that were published in journals other than the ones considered in our initial literature review. This added 24 papers to our dataset, for a total of 35 papers. For each paper, we determined which transmission functions were evaluated and which function the authors determined to be the most parsimonious description (best fit balanced against number of parameters) of the given host-parasite system. We categorized the papers into groups that tested (1) the DD versus FD transmission function (12 papers) or (2) any nonlinear function and/or power function versus the DD and/or FD transmission functions (23 papers).

Model simulations: consequences for mismatches between transmission theory and data

After finding that most epidemiological models in the ecological literature assume that contact rates and transmission rates are linear functions of density, despite the fact that these relationships are typically nonlinear in real host-parasite systems, we were faced with the most famous question in mathematical modeling (*sensu* Box and Draper (1987), "all models are wrong; the practical question is how wrong do they have to be to not be useful?" It could be that the assumed linear relationships provide close approximations to nonlinear dynamics, such that simplifying from a nonlinear option to a canonical option does not appreciably change model fits or predictions. To our knowledge, previous work has not evaluated the accuracy of the canonical DD and FD transmission functions when applied to systems with truly nonlinear transmission dynamics, or the accuracy of a DD model when applied to truly FD data, and vice versa.

To evaluate the importance of choosing transmission assumptions that match the underlying transmission ecology, we simulated sample data from parasite epidemics using the NL transmission function, where the density dependence parameter (K) ranged from 0 (FD model) to 1 (DD model) by intervals of 0.1 (Figure 1.1). Epidemic dynamics are also controlled by additional model parameters besides K, such as the transmission rate (β) or force of infection

(FOI) and the rate that infected individuals recover from infection (γ). Therefore, we conducted our simulations under nine combinations of FOI and γ, where the full parameter space covered a wide range of possible epidemic dynamics. For each value of K, FOI, and γ, we (1) simulated three epidemics with different starting host population densities (N=500, 1000, and 1500 animals/unit space); (2) generated random sample data from 20 time points during each epidemic by sampling with a binomial error distribution around the true fraction infected; (3) fit the DD, FD, and NL models to the complete dataset of samples from the three epidemics using maximum likelihood estimation; and (4) quantified model fits and the biases in estimated model parameters for each model. For each of the nine combinations of FOI and γ and each of the 11 values of K, we repeated the simulation and fitting process 100 times, for a total of 9900 simulated datasets. We were particularly interested in the models' abilities to accurately estimate the known basic reproductive rate of the parasite, R_0, which is a conglomerate parameter where $R_0 = \beta N^K \gamma^{-1}$.

Results:

Review of epidemiological theory used in the recent ecological literature

Most ecological papers used the DD transmission function as the sole transmission function in their epidemiological models (55%; 123/222 papers). While the DD function was used most commonly for directly-transmitted, non-venereal parasites, it was also used in some models for parasites with sexual transmission, environmental transmission, and vector transmission (Figure 1.2). Because only 22% (27/123) of papers that used the DD transmission function explicitly justified the use of that particular function for the given host-parasite system or research question – in contrast with 60% (59/99) of papers using any other single function or combination of functions – we conclude that ecologists still view the DD function as the default function for epidemiological models.

Surprisingly, of the three characteristics of host-parasite systems for which the FD function has been recommended – vector or sexual transmission, human hosts, and constant density with changing population size – the presence of human hosts in the system was the most common reason that studies used the FD function. However, there was no consensus in the ecological literature regarding which function to use for non-venereal, directly-transmitted human parasites: only 47% (22/47) of those models used the FD transmission function, and the rest used the DD transmission function or tested multiple functions.

Equally surprisingly, only 33% (4/12) of models for vector-transmitted parasites of non-human hosts used the FD function (Figure 1.2), despite the well-known guideline to use the FD transmission function for sexually-transmitted and vector-transmitted parasites of non-human hosts (*sensu* Thrall et al. 1993; Antonovics et al. 1995; Anderson and May 1991; Keeling and Rohani 2008). Furthermore, only 2 out of 51 papers (4%) that used the FD function explicitly stated that they chose the FD function because host density remained constant as population size changed in their system of interest (e.g., fixed territory sizes, fixed group or family sizes). This suggests that using the FD function for constant host densities is either rarely relevant or poorly known. Overall, there was little consensus regarding when to use the FD function in epidemiological models.

Only a small subset of studies used a single transmission function that was not the DD or FD transmission function (6%; 13/222 papers). Furthermore, only 16% (35/222) of studies evaluated multiple transmission functions (Figure 1.1), despite McCallum et al.'s (2001) recommendation to "evaluate several alternative models of transmission, if possible." Authors appeared to use an alternative function if the function was previously demonstrated to work well for the given system, or because the authors were not sure which function to use and opted for a

more flexible function. For instance, some researchers used the negative binomial function for aggregation of macroparasites among hosts (e.g., Townsend et al. 2009), a nonlinear function that accounted for heterogeneity in parasite attack rates among hosts (e.g., Elderd et al. 2008), or the flexible power function to allow for nonlinearity in the relationships between transmission rates and host and parasite densities (e.g., Duffy *et al.* 2009).

Review of comparative evaluations of multiple transmission functions

When considering all studies that compared the DD and/or FD function to a nonlinear and/or power function, the majority (19/23) found that the nonlinear function was the most parsimonious description of transmission (Figure 1.2). Additionally, two of the studies that tested whether the DD, FD, or a NL transmission function provided the best fit to data noted that they could not determine whether the NL function outperformed the DD or FD functions given their datasets (Rachowicz and Briggs 2007; Cross *et al.* 2010), meaning that a NL function was either equivalent to, or better than, the canonical functions in 91% (21/23) of studies.

Most papers that compared multiple transmission functions considered directly-transmitted or environmentally-transmitted parasites, with few to no tests for sexually-transmitted or vector-transmitted parasites (Figure 1.2). To our knowledge, there have been no tests of multiple transmission functions for sexually-transmitted parasites of human or non-human animals since the work of Ryder et al. (2005) on two-spot lady beetles and Ji et al. (2005) on possums. In those systems, and in several other systems reviewed by Ryder et al. (2005), the probability of sexual contacts and/or parasite transmission increased linearly (DD) or nonlinearly with host density. Though it is difficult to generalize from so few studies, these trends are contrary to the paradigm that frequency-dependent models should be used for sexually-

transmitted parasites (Thrall et al. 1993; Antonovics et al. 1995; Anderson and May 1991; Keeling and Rohani 2008).

Model simulations: consequences for mismatches between transmission theory and data

Models based on the DD transmission function consistently produced substantially biased estimates of R_0 when the true underlying contact-density relationship was not DD ($K \neq 0$; Figures 1.3-4), even when the true underlying contact-density relationship was only slightly nonlinear (e.g., K=0.9). The FD function also poorly estimated the true R_0 over a large region of parameter space, but particularly when $K > 0.7$. For instance, in simulations where K=1 (truly DD data), FOI=0.0005, γ=0.05, and N= 500 individuals, the FD function predicted an epidemic peak of 70% prevalence at Day 7, whereas the true epidemic peaked at 50% prevalence on Day 42 (bottom left panel in Figure 1.3). Together, these biased estimates suggest that (1) choosing the wrong canonical function (e.g., using DD when the underlying relationship is truly FD) or (2) choosing a linear canonical function when the true underlying relationship is nonlinear can compromise ecologists' abilities to understand and predict disease dynamics.

The FD function tended to be the better of the two canonical functions for much of the parameter space; in particular, the FD function produced estimates of R_0 that were just as accurate as those from the NL function when the true R_0 was large (Figure 1.4). This occurred because at large R_0s, the prevalence of infection peaked rapidly regardless of density, and thus the importance of density was removed. However, it is notable that the R_0 values in those regions of parameter space were so high that they would only be relevant for particularly fast-spreading diseases (e.g., measles). It also important to note that the estimation biases for FOI and γ often differed in magnitude and direction from the estimation biases for R_0. In fact, propagation of

error sometimes produced accurate R_0 estimates even though FOI and γ estimates were biased (see curvilinear bias for FD function when FOI=0.0001 and FOI=0.05 in Figure 1.4).

Discussion:

The majority of epidemiological models published since 2001 assume that homogeneous organisms move randomly through their environments like gas molecules, instantaneously interacting with other organisms and creating linear relationships between transmission and host density. In contrast, 19/23 empirical studies in real host-parasite systems concluded that transmission functions are nonlinear, suggesting that the majority of epidemiological models do not reflect the biological reality of these systems. Despite being biologically inaccurate, a linear transmission function assumption might be a good first approximation under some ecological and epidemiological conditions, such as when R_0 is large (Figure 1.4). However, under most conditions, choosing an appropriate transmission function is not a trivial methodological detail. We have long known that transmission functions determine the dynamics and stability of host-parasite systems (e.g., Kermack and McKendrick 1927; Getz and Pickering 1983; Hochberg 1991; Fenton et al. 2002). For instance, simple linear models cannot simulate realistic dynamics for tuberculosis in possums no matter which parameter estimates are used, whereas a nonlinear model based on heterogeneous mixing in the possum population produces reasonable dynamics (Barlow 2000). Our model simulations further show that even in systems where the long-term dynamics are as simple as biologically possible, ecologists cannot rely on model predictions if they (1) choose the wrong canonical function (e.g., using DD when the underlying relationship is truly FD) or (2) choose a linear, canonical function when the true underlying relationship is nonlinear (Figure 1.4). Given that most existing epidemiological models use a single canonical function, while real transmission functions are typically nonlinear, this mismatch between theory

and data might be substantially compromising our current abilities to understand and predict transmission dynamics.

Throughout this paper, we have primarily discussed nonlinear transmission functions that arise due to nonlinearity in contact-density relationships. However, other mechanisms can give rise to nonlinear transmission functions, and these other types of transmission functions were prevalent in our literature review. For instance, the nonlinear transmission function that represents aggregation of macroparasites among hosts can arise from a linear contact-density relationship and variation in host exposure or susceptibility to parasites (Anderson and May 1978). Unfortunately, only one study in our dataset considered how both contact rates and transmission rates changed with host density (Ryder et al. 2005), so we cannot say whether transmission heterogeneity or nonlinear contact-density relationships are greater drivers of nonlinear transmission-density relationships. Both mechanisms probably occur in many systems, and thus future studies that quantify both contact rate functions and overall transmission rate functions remain a priority.

Based on our results, we suggest some simple new guidelines for ensuring that future theoretical models align more closely with real host-parasite ecology. First, we recommend that ecologists experimentally test contact-density and transmission-density relationships in their own host-parasite systems whenever possible. If experiments are not possible, ecologists should evaluate multiple transmission functions when fitting epidemiological models to their observational data. At the very least, ecologists should consider both the DD and FD transmission functions, but ideally they should also try mechanistically-relevant nonlinear transmission functions. The flexible nonlinear function that we used here is easy to use, but it may not be relevant to all systems. Finally, we recommend that ecologists always report (1)

which transmission functions they tested, (2) which transmission function they used and why (see examples in Caley and Hone 2005; Webb et al. 2006; Smith et al. 2009), and (3) how host density and/or area were incorporated in their models (see de Jong et al. 1995; McCallum et al. 2001; Begon et al. 2002). Ideally, this information will be provided both mathematically and verbally, because mathematical equations give no indication of the reasoning behind using the given transmission function. Peer reviewers can help the field to advance by insisting that these details are included in manuscripts.

We also suggest several steps that authors can take to make their results more valuable for third parties and/or future meta-analyses. Most importantly, authors should make their data widely available by archiving them in data repositories (e.g., Heesterbeek et al. 2015). For instance, we found several potentially useful empirical tests of multiple transmission functions in the literature that reported only qualitative results. We had hoped to include those studies in our analyses by re-analyzing the data for quantitative results, but upon contacting the authors, we found that the data for the studies had been lost in the past decade. In addition to prioritizing data management and open access archiving, reporting modeling results in a standardized way should be a priority. In particular, we suggest that authors not only decide which transmission function produces the best fits to data with the fewest parameters (e.g., by comparing model AICs), but that authors also report the improvement in model fits provided by the winning model (e.g., comparing model R^2s or reporting effect sizes). Additionally, authors should report their parameter estimates, model selection criteria, and model fit statistics for all candidate models, not just the winning model.

Our literature review focused specifically on the hundreds of papers in the recent ecological literature that included dynamic host-parasite models, but contact functions are used

far more broadly in ecology (e.g., models for predation, mutualism, and competition). Furthermore, the uncertainty surrounding these functions is not limited to host-parasite models; even in the predator-prey literature, there is ongoing debate regarding these functions (i.e., ratio-dependent predation; Abrams and Ginzburg 2000; Barraquand 2014; Abrams 2015). Though many alternative contact functions have been proposed for dynamic species interaction models (e.g., Holling 1965; McCallum et al. 2001), empirical tests of these functions in diverse systems are relatively rare, reflecting a broader trend in the ecological literature where papers developing new theories outweigh those testing existing theories or assumptions (Scheiner 2013).

Until we accumulate enough empirical evidence to strongly reject particular contact functions or to develop better frameworks for understanding when a given assumption will best describe real ecological systems, uncertainty and debate regarding these theories will continue. Therefore, as we continue to add new components to our basic modeling frameworks, we should not forget to question and test the assumptions that form the backbone of all dynamic species interaction models. As McCallum et al. (2001) said more than a decade ago, "We also are still a long way from being able to use transmission parameters estimated for a particular host–pathogen pair in one environment for the same pair in a different environment, particularly if densities are also different." However, we are confident that confronting the existing transmission functions with more and better data and changing existing modeling practices will lead to the development and use of models that excel at both describing and predicting the behavior of dynamic ecological systems.

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Figure 1.1. The nonlinear (NL) transmission function used here ($g_{NL} = 0 + c_{NL}N^K$) is a flexible function that simplifies to the density-dependent (DD) function when K=1 and to the frequency-dependent function (FD) when K=0. To create this particular figure, we set c_{NL}=0.1 and varied K from 0 to 1. We do not show lines for K=0.1, K=0.2, and K=0.3 to avoid cluttering the figure.

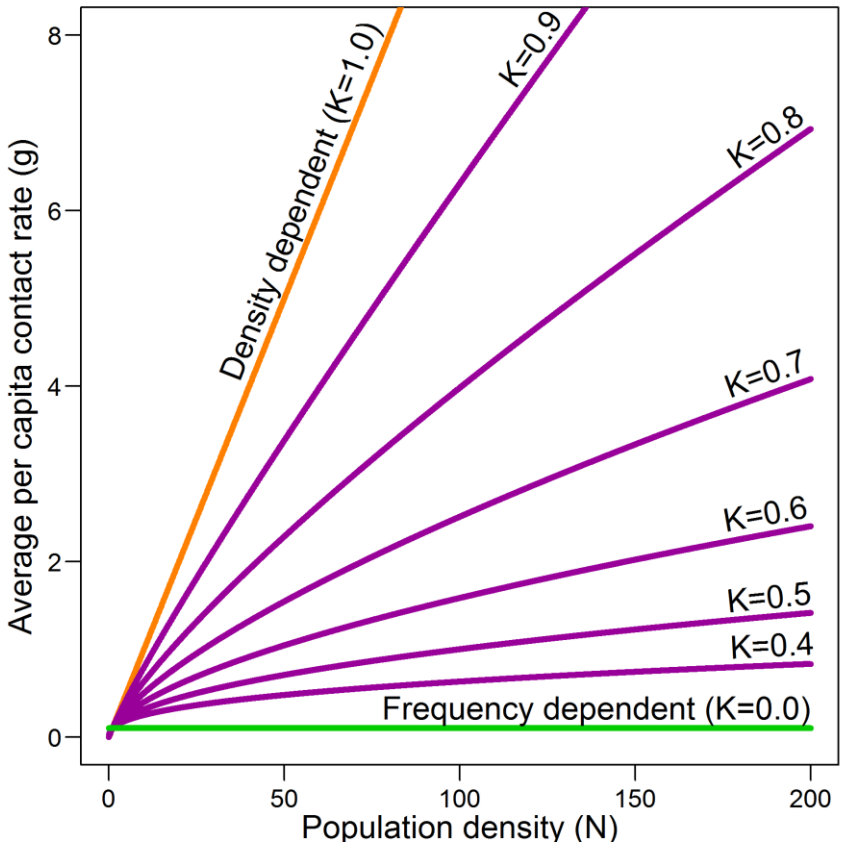


Figure 1.2. In 222 papers using SIR models in the recent ecological literature, the majority used a single, linear, canonical transmission function ("theory"). In contrast, 19/23 papers that tested whether linear or nonlinear transmission functions were more parsimonious descriptions of transmission dynamics in real host-parasite systems found best support for nonlinear functions ("data"). The orange, green, and dark purple bars represent the proportion of papers that used ("theory") or supported ("data") the DD, FD, and NL functions, respectively. The light purple bars indicate the proportion of the 222 surveyed papers that considered multiple transmission functions, either by comparing the two canonical functions or by comparing at least one canonical function to a nonlinear/power function. The numbers at the top of each bar indicate the number of papers used to generate the proportional data.

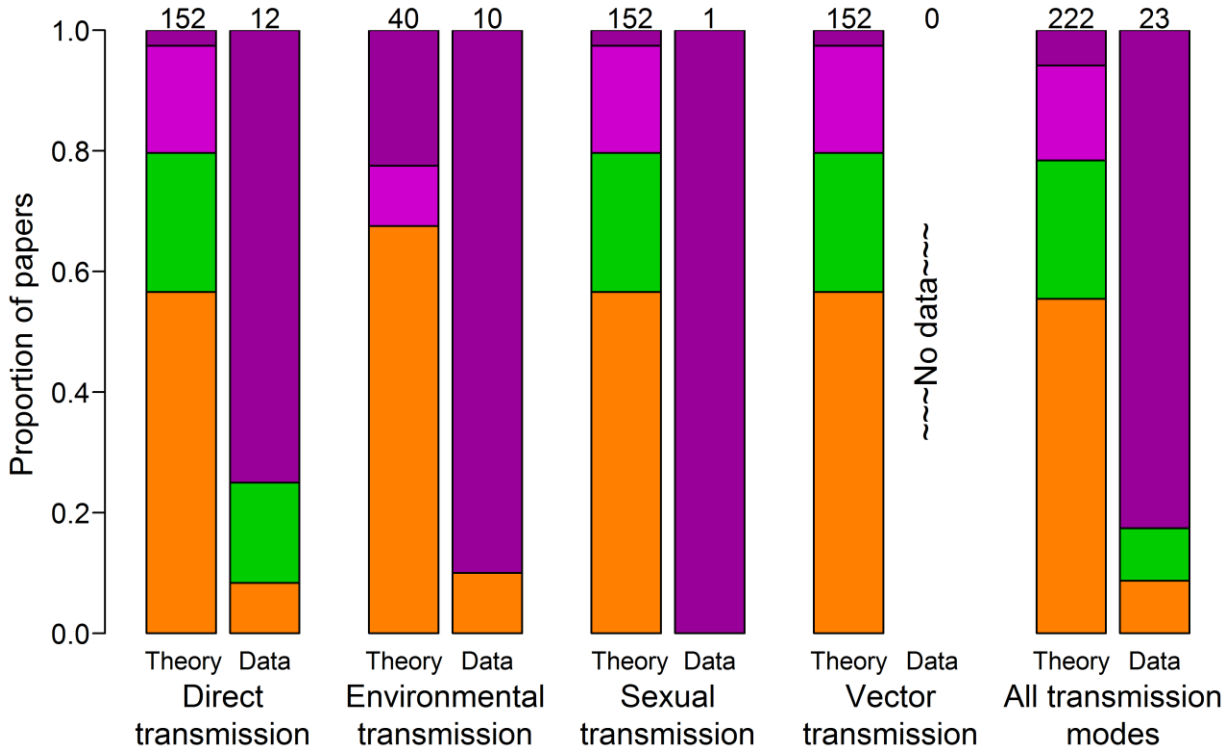


Figure 1.3. The flexible, NL transmission function always fit the time series data generated by the three simulated epidemics well. The FD and DD transmission functions fit the simulated data well when the true underlying transmission function was FD (K=0) or DD (K=1), respectively, but the canonical functions typically performed poorly otherwise (also see Figure 1.4). The points are samples taken from three epidemics with different host densities, where panels in the same row show the same simulated data and different columns show the best fits achieved by the FD (green), NL (purple), and DD (orange) models. Similar figures could be created for all combinations of FOI and γ, but in this case FOI=0.0005 and γ=0.05.

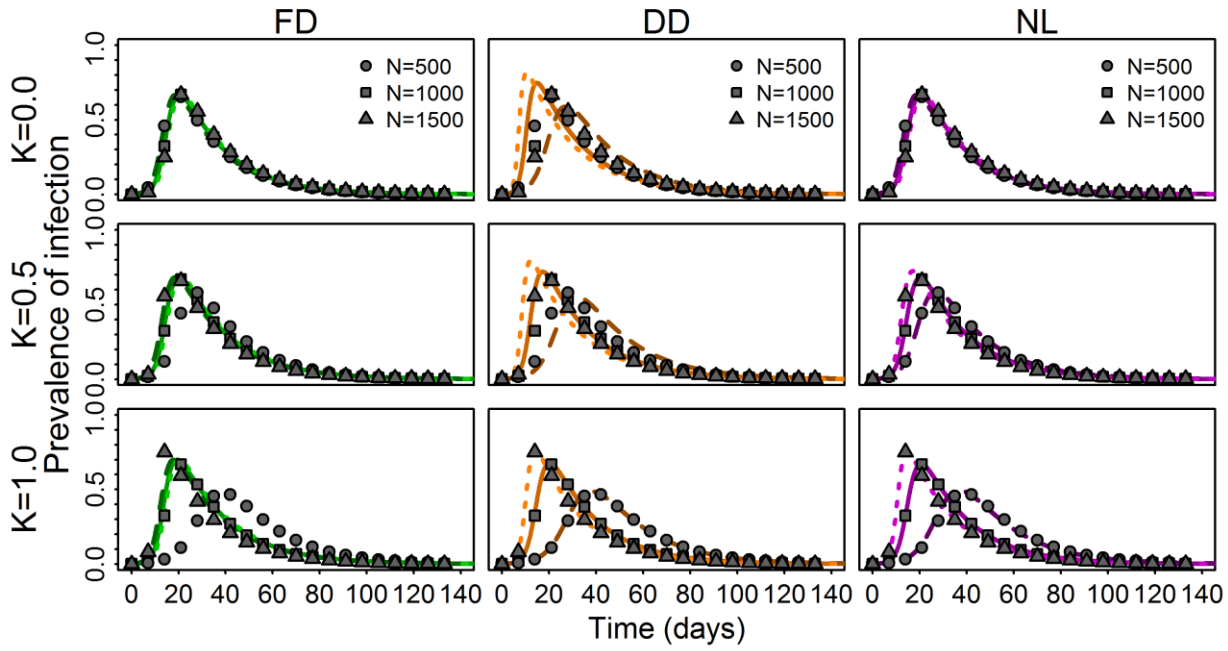
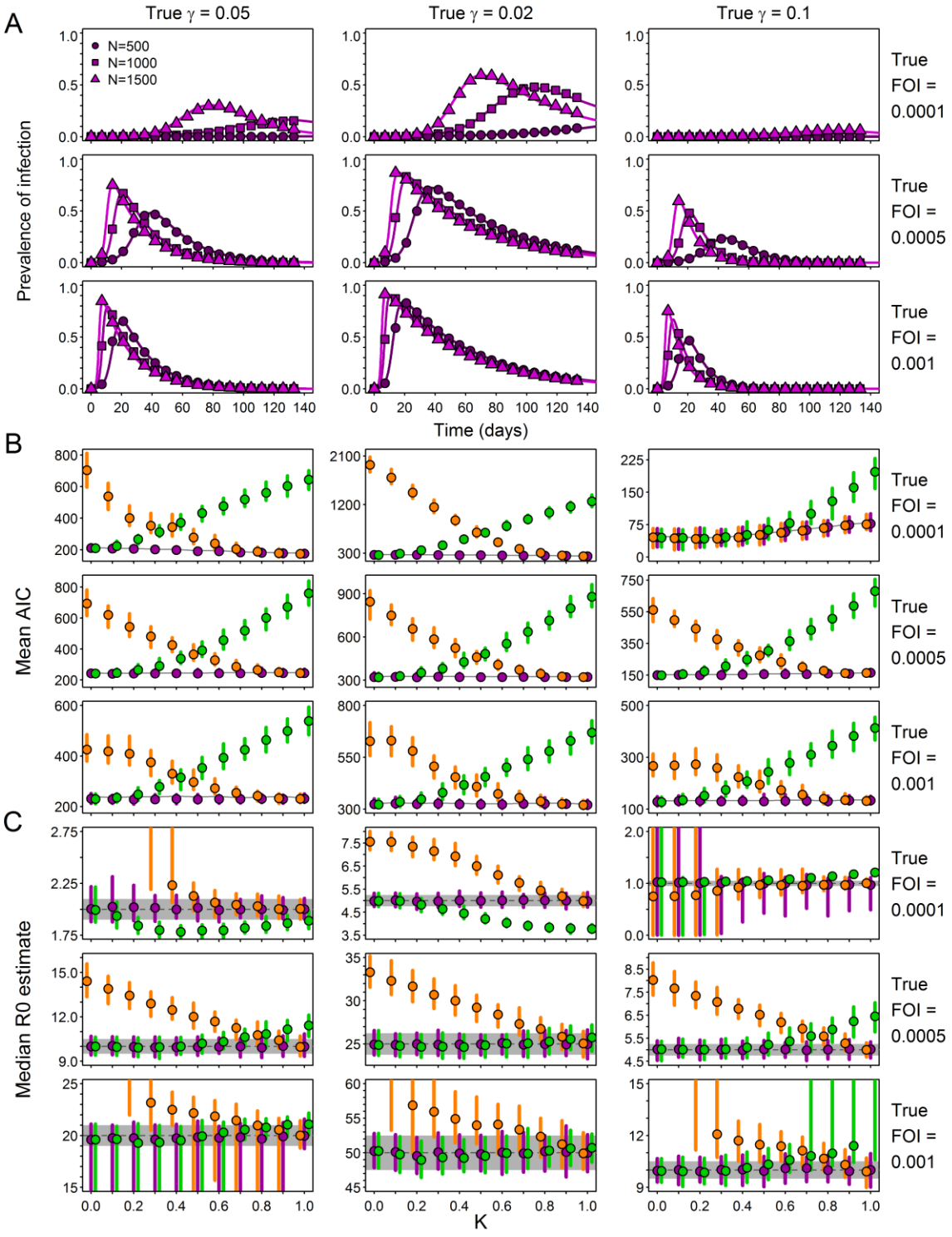


Figure 1.4. When considering a wide range of parameter space (A; top nine panels), the NL model could always achieve the best possible fit to simulated data (B; middle nine panels) and could also accurately recover the R_0 used to generate the simulated data (C; bottom nine panels). Conversely, the best fits obtained by the FD and DD models were typically poor whenever the underlying data were not FD (K=0) or DD (K=1), respectively, and the canonical transmission functions often provided biased estimates of the true R_0. In the top nine panels (A), examples of three simulated epidemics for host populations with different densities (N) are indicated by the three point types, with the best fit lines produced by the NL model overlain. In all other panels, the purple, orange, and green points represent the mean AICs (B panels) and median R_0 estimates (C panels) produced by fitting the NL, DD, and FD models, respectively, to 100 simulated datasets of each combination of FOI, γ, and K. Vertical lines show the 95% percentile for AICs and R_0 estimates for the 100 simulated datasets. The gray lines in the middle nine panels (B) show the best possible AICs, as calculated from the average true negative log likelihood for each dataset. The gray polygons in the bottom nine panels (C) show a range 5% above and 5% below the true R_0 values to provide an idea of the relative scale of the R_0 estimates under different parameter combinations.


A
True γ = 0.05
True γ = 0.02
True γ = 0.1
N=500
N=1000
N=1500
Prevalence of infection
True FOI = 0.0001
True FOI = 0.0005
True FOI = 0.001
Time (days)
B
Mean AIC
C
Median R0 estimate
K

CHAPTER 2: DISPERSAL OF A DEFENSIVE SYMBIONT DEPENDS ON CONTACT BETWEEN HOSTS, HOST HEALTH, AND HOST SIZE

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Abstract:

Symbiont dispersal is necessary for the maintenance of defense mutualisms in space and time, and the distribution of symbionts among hosts should be intricately tied to symbiont dispersal behaviors. However, we know surprisingly little about how most defensive symbionts find and choose advantageous hosts or what cues trigger symbionts to disperse from their current hosts. In a series of six experiments, we explored the dispersal ecology of an oligochaete worm (*Chaetogaster limnaei*) that protects snail hosts from infection by larval trematode parasites. Specifically, we determined the factors that affected net symbiont dispersal from a current "donor" host to a new "receiver" host. Symbionts rarely dispersed unless hosts directly contacted one another. However, symbionts would overcome their reluctance to disperse across the open environment if the donor host died. When hosts could directly contact, net symbiont dispersal varied with both host size and trematode infection status, whereas symbiont density did not influence the probability of symbiont dispersal. Together, these experiments show that symbiont dispersal is not a constant, random process, as is often assumed in symbiont dispersal models, but rather the probability of dispersal varies with ecological conditions and among

individual hosts. The observed heterogeneity in dispersal rates among hosts may help explain symbiont aggregation among snail hosts in nature.

Introduction:

There is an important category of mutualisms that involves at least three interacting species, where symbionts protect their hosts from natural enemies (Janzen 1985, White and Torres 2009). Examples of these defensive symbionts include ants that protect their plant hosts from herbivores (Janzen 1966), bacteria that protect a variety of taxa against fungal pathogens (Gil-Turnes et al. 1989; Woodhams et al. 2007), mites that protect hymenopteran larvae from parasitoid wasps (Okabe and Makino 2008), and 'guard' crustaceans that protect corals from starfish predators (Glynn 1976). These defensive symbionts can play important roles at all levels of ecological organization, from individual host fitness to community-level dynamics to ecosystem structure and function (Glynn 1983; Glynn 1987; Osman and Haugsness 1981; Heil et al. 2001; Hay et al. 2004; Oliver et al. 2014). It is therefore imperative that we understand how defense mutualisms are maintained across time and space.

Maintenance of defense mutualisms depends on the ability of symbionts to disperse among hosts. This dispersal may happen vertically, from host parent to offspring, or horizontally among conspecific hosts (reviewed in Bright and Bulgheresi 2010). Vertical dispersal of 'heritable' defensive symbionts has been considered most frequently, especially with regards to defensive bacteria (e.g., Walke et al. 2011; Oliver et al. 2014). However, many defensive symbionts use exclusively horizontal dispersal or a combination of horizontal and vertical dispersal (Bright and Bulgheresi 2010; Henry et al. 2013). For instance, in ant-plant mutualisms, winged queen ants disperse by flying from their natal host plant to a new host plant (Edwards et al. 2006). In systems where symbionts and hosts need to find each other anew each generation in

this way, the symbionts presumably experience strong selection for mechanisms that facilitate finding hosts and assessing host suitability. For instance, ants living symbiotically with plants can locate potential hosts via plant volatiles (Edwards et al. 2006). However, in most systems, we know surprisingly little about how defensive symbionts find and choose advantageous hosts or what cues trigger symbionts to disperse from their current hosts in the first place.

While our understanding of the horizontal dispersal of defensive symbionts among hosts is limited to a few systems, there is a rich literature regarding the dispersal of free-living species among habitat patches (e.g., Bowler and Benton 2005; Bonte et al. 2012), and these ideas may be usefully employed to understand symbiont dispersal. For free-living species, the benefits of emigration include avoiding inbreeding (Daniels and Walters 2000), low resource conditions (Fred and Brommer 2009), or competition (Léna et al. 1998; Sutherland et al. 2002; Cote and Clobert 2010; Mehrparvar et al. 2013; Waser et al. 2013). Successful dispersal strategies for free-living species are a tradeoff between the future fitness benefits that accrue because of an improvement in local conditions and the costs or risks of dispersal itself (Bonte et al. 2012). Similarly, we expect defensive symbionts to emigrate from hosts where symbiont fitness is low and preferentially immigrate to hosts where symbiont fitness is high, but only when the costs of dispersing are relatively low in comparison to fitness gains. For instance, guard crabs will leave their host coral colony when there is a coral bleaching event (Stella et al. 2011), when the crabs do not have suitable mates on the same host colony (Castro 1978), and when the host colony is too small (Castro 1978). However, guard crabs are more likely to remain on hosts that provide suboptimal crab fitness if crab predation risk in the environment is high and dispersal is therefore particularly risky (Castro 1978).

Importantly, just as the distributions of free-living species are affected by dispersal, the distributions of symbionts among hosts depend on dispersal/transmission processes. Specifically, aggregation of symbionts among hosts can arise if there is variation in symbiont immigration rates among hosts or if symbionts reproduce directly on their hosts (Anderson and Gordon 1982). However, these aggregating processes can be overwhelmed by negative density-dependent death or emigration processes (Anderson and Gordon 1982), which act to homogenize symbiont distributions. For example, guard crabs preferentially disperse from suboptimal corals to more advantageous hosts (i.e., aggregating process; Castro 1978), but they are also territorial and emigrate to avoid intraspecific competition (i.e., density-dependent process; Castro 1978; Steir et al. 2012), and the net result is just one pair of adult crabs on most corals (Castro 1978; Steir et al. 2012). Because symbiont density can determine the net outcome of host-symbiont interactions (i.e., mutualism or parasitism; Holland et al. 2002; Brown et al. 2012), consideration of the processes leading to aggregated symbiont distributions is an important step in understanding host-symbiont interactions.

In this study, we considered the dispersal ecology of an oligochaete worm, *Chaetogaster limnaei limnaei* K. von Baer (hereafter *Chaetogaster*), which lives symbiotically on aquatic snails. *Chaetogaster* is an important defensive symbiont because the oligochaetes may reduce aquatic trematode parasite transmission to snail hosts (e.g., Sankurathri and Holmes 1976; Fernandez et al. 1991; Rodgers et al. 2005; McKoy et al. 2011; Hopkins et al. 2013), including trematodes that are important to human and livestock health (e.g., schistosomiasis, Rogers et al. 2005; fascioliasis, Khalil 1961). In our first three experiments, we determined whether *Chaetogaster* would disperse if the snail hosts could not directly contact one another. Because symbionts should avoid dispersing when the risks associated with dispersal are high, we

predicted that there would be more *Chaetogaster* dispersal when host snails could directly contact than when *Chaetogaster* had to cross an open environment to find new hosts (Experiments 1-2). However, we predicted that *Chaetogaster* would be more likely to disperse across the open environment if the current host died, a circumstance maximizing the benefits of dispersal (Experiment 3). In our next three experiments, we considered which host and symbiont conditions favor symbiont dispersal. We predicted that emigration would be greatest from snails with the highest *Chaetogaster* densities (Experiment 4), because intraspecific and/or kin competition for resources might negatively impact *Chaetogaster* fitness when densities are high. We also predicted that *Chaetogaster* would preferentially disperse to snails that were first intermediate hosts for trematodes (Experiment 5) and to larger snails (Experiment 6), because those snails should provide the most per capita resources for *Chaetogaster* (Shigina 1970; Fernandez et al. 1991). Contrary to our predictions, symbiont density did not influence symbiont dispersal, symbionts preferentially dispersed from large to small snails, and symbionts were less likely to disperse to snails infected by trematodes than to uninfected snails.

Methods:

Study System:

C. l. limnaei is an obligate symbiont that can be found globally, and the oligochaetes use a wide array of snail species as hosts (e.g., Gruffydd 1965; Buse 1968; Ibrahim 2007). The life cycle of a second *C. limnaei* subspecies, *C. l. vaghini*, includes asexual reproduction of sexually immature individuals throughout most of the year, sexual maturation and reproduction in the fall and winter, and the production of cocoons shortly after sexual maturation (e.g., Vaghin 1946; Buse 1968). Because *C. l. vaghini* exclusively occupy snail kidneys, *C. l. vaghini* cocoons have mostly been found in the snail kidney, but some cocoons may be expelled from the kidney into

the environment (Buse 1968). *C. l. limnaei*, the subspecies used in this study, lives on the snail headfoot and in the mantle cavity and also reproduces asexually for most of the year (e.g., Vaghin 1946; Gruffydd 1965). Sexually mature individuals of *C. l. limnaei* are rare but do occur in the fall (Vaghin 1946). However, despite detailed searches, *C. l. limnaei* cocoons have not been found (Vaghin 1946; Gruffydd 1965). It is possible that some *C. l. limnaei* dispersal occurs via expulsion of cocoons, subsequent hatching of immature *C. l. limnaei* in the environment, and then encounters with new hosts. However, transmission/dispersal to new hosts often occurs during periods without sexually mature individuals (e.g., Gruffydd 1965). Additionally, the number of sexually mature *C. l. limnaei* (and thus cocoons) produced by a *C. l. limnaei* population is very small, so it is unlikely that cocoons are the predominant form of dispersal (Gruffydd 1965). Alternatively, dispersal can occur via direct contact between two snail hosts or via the oligochaetes leaving one host and crossing the open environment to a new host (Gruffydd 1965). *C. l. limnaei* are capable of detecting host mucus trails (Shaw 1991; Buse 1972), host chemical cues (Buse 1972), and host egg masses (Buse 1972), indicating that these oligochaetes have some ability to actively find new hosts if they leave their current host.

Snails are first intermediate hosts for many aquatic trematode species. When infected as first intermediate hosts, snails may release tens to thousands of free-living larval trematode stages called cercariae into the aquatic environment each day, depending on the trematode species. *Chaetogaster* consume these cercariae. Therefore, first intermediate host snails are high resource environments for *Chaetogaster*, where *Chaetogaster* asexual reproduction rates are often higher on snails infected as first intermediate trematode hosts than on uninfected snails (e.g., Fernandez et al. 1991).

General Methods:

We performed five experiments in which experimental units consisted of two snails (*Helisoma trivolvis*) paired by size and kept in 150 mL plastic cups filled with 30 mL of spring water, and a sixth (Exp. 5) where the snail pairs were in l000 mL plastic cups with 900 mL of spring water. Snails were raised from eggs in the laboratory or in cattle tanks at Virginia Tech's Kentland Farm, Virginia, USA so that snails had no prior trematode infection or *Chaetogaster* infestation. In all of the experiments, we added *Chaetogaster limnaei limnaei* to one of the snails in each pair (hereafter referred to as the "donor" snail) and the second snail received no experimental addition of *Chaetogaster* (hereafter "receiver" snail). Size-matched snail pairs were always randomly assigned to treatment groups, and individual snails within each pair were randomly assigned to be the donor or receiver snail.

The *Chaetogaster* were acquired from field-collected *H. trivolvis* snails or from *H. trivolvis* maintained in a separate cattle tank. To collect the *Chaetogaster*, we first dislodged most of the oligochaetes from each snail by squirting spring water into the snail's shell, and then dissected the snail to collect remaining *Chaetogaster*. The *Chaetogaster* from the individual snails were pooled prior to being distributed to donor snails. Fifteen *Chaetogaster* were added to each donor snail by placing the snail in an individual cup with the *Chaetogaster* overnight. While pooling *Chaetogaster* for the experiments, snail hemolymph from the dissected snails was inevitably added along with *Chaetogaster* to the individual donor snail cups, so receiver snails were also exposed to one pipetteful (~1.2 mL) of water with snail hemolymph as a control.

The morning after *Chaetogaster* additions, donor and receiver snails were both moved into experimental cups and were left together for 24 hours. After the allotted time, the donor and receiver snails were separated and dissected, and we comprehensively counted all *Chaetogaster* on each snail.

Experiment 1: Dispersal without host contact on artificial substrate

The goal of Experiment 1 was to determine whether *Chaetogaster* would disperse to a new host without direct host contact. In one treatment group, individual snails were tethered to opposite sides of the cup so that they could move freely, but not get closer than 20 mm to the other snail. This distance would take *Chaetogaster* only seconds to traverse, but it is far enough that no part of the tethered snails (e.g., antennae) could contact across the distance. In the second treatment group, snails were tethered such that the snails could easily contact each other. There were 10 replicate snail pairs in each treatment group.

To tether the snails, a length of white sewing thread was affixed to the back of the shell using Krazy Glue. After the glue dried, snails were dipped briefly in spring water to remove any glue residues. Snails were left for one hour to acclimate to the tethers in individual cups of spring water before adding *Chaetogaster* to the donor snails and sham-exposing the receiver snails to hemolymph. On the morning of the experiment, snails were paired in the appropriate cups, and the free end of each tether was threaded through a hole in the side of the cup and secured with tape.

Experiment 2: Dispersal without host contact on leaf substrate

When removed from a host snail, the oligochaetes appear to have no difficulty traversing the plastic surface of the cups used in our experiments (S.R.H., pers. obs.; Buse 1972), but they may not have been willing to leave a snail host to disperse across such an unnatural substrate. Therefore we repeated Experiment 1 while providing the *Chaetogaster* with natural substrate. For natural substrate, dried white oak leaves were cut in circles that fit the bottom of the 150 mL cups. A drop of Krazy Glue was used to anchor each leaf circle to the bottom of each cup. After the glue had dried, 30 mL of spring water was added to each cup, and the leaf circles were

soaked for four days to allow tannins to leach out of the leaves and to remove any glue residues. On the morning of the experiment, the water in each cup was changed to fresh spring water prior to tethering the snails in the cups. There were initially 10 replicate snail pairs in each treatment group. However, one snail died in the no-contact group during the experiment, and we removed that replicate from our analysis.

Experiment 3: Dispersal following host mortality

We then determined whether *Chaetogaster* would move to a new host without direct host contact if the current host died. In this experiment, all pairs of snails were tethered so that there was a 20 mm gap between the donor host and receiver snail. In one treatment group, both the donor and receiver snails were alive. In the second treatment group, the donor snail was euthanized at the start of the experiment by making a single puncture through the shell and heart of the snail using a dissecting needle, without disturbing the head and mantle region where the *Chaetogaster* reside. There were initially 10 replicate snail pairs in each treatment group, but a tether broke in one replicate in the control treatment group, and that replicate was removed from our analysis. At the end of the experiment, we examined all cups for dead and living *Chaetogaster* in addition to the snail dissections.

Experiment 4: Density-dependent dispersal

In all previous experiments, each donor snail had 15 *Chaetogaster* and the experiments lasted 24 hours. To evaluate whether *Chaetogaster* dispersal was density-dependent and to explore the effect of contact time on dispersal, we performed a 3x4 factorial experiment where donor snails with 5, 15 or 30 *Chaetogaster* per snail were paired with receiver snails for 1, 4, 8, or 24 hours. We chose the initial *Chaetogaster* densities based on the natural range that we observe in the field (range = 0-43 *Chaetogaster*/snail; median = 0 *Chaetogaster*/snail; mean

intensity = 3.96 *Chaetogaster*/snail; unpublished data; Hopkins et al. 2013). In this experiment, there were no tethers; snails were allowed to directly contact in all replicates. To identify donor and receiver snails, we marked the back of each donor and receiver snail's shell with a small dot of Sally Hansen's Insta-Dry nail polish prior to *Chaetogaster* additions.

Each of the 12 treatment groups had six replicate snail pairs, for a total of 72 experimental units. It was not logistically feasible to perform all replicates on a single day, so we performed two trials with three replicates of each treatment per trial. Neither dispersal nor percent *Chaetogaster* survival varied between the trials, so data were combined for analyses.

Experiment 5: Dispersal with trematode-infected hosts

In all previous experiments, the donor and receiver snails were parasite-free. In Experiment 5, we examined whether *Chaetogaster* dispersal was affected by host infection by trematode parasites (i.e., infected snails were releasing free-swimming trematode cercariae), where cercariae provide *Chaetogaster* with abundant food resources (e.g., Fernandez et al. 1991). There were three treatment groups in this experiment, each with 10 replicates: (1) the donor snail was uninfected and the receiver snail was infected as a first intermediate host for trematodes, (2) the donor snail was infected and the receiver snail was uninfected, or (3) both snails were uninfected.

Unlike the previous experiments, experimental snails were not lab-reared for Experiment 5 because we needed to use trematode-infected snails. To find first intermediate trematode-infected snails, *H. trivolvis* ($n \approx 300$) were collected from a pond in Montgomery County, Virginia, USA. Snails were housed in individual 50 mL centrifuge tubes in the lab for two hours, and then the spring water from each tube was examined under a dissecting microscope for trematode cercariae. Cercariae were morphologically identified under 40x darkfield

magnification (using Schell 1985). Twenty snails shedding echinostome cercariae were found. We size-matched the 20 snails that were actively shedding cercariae with 20 field-collected snails that were not shedding cercariae. We randomly assigned these pairs to have either donor or receiver first intermediate hosts. From the remaining non-shedding field-collected snails, 20 additional snails were randomly selected and size-matched in pairs to act as the control group, with one individual randomly selected in each pair to be the donor snail. All snails were again marked with nail polish in all treatment groups so that we could differentiate between the donor and receiver snails.

Because the snails used in this experiment were taken from a pond with an existing *Chaetogaster* population, snails had *Chaetogaster* infestations when we brought them into the laboratory. To remove the existing *Chaetogaster*, four pipettefuls (~4.8mL) of spring water were squirted into the mantle cavity of each experimental snail. This method leaves few, if any, *Chaetogaster* per snail, and snails in all treatment groups should have started with the same low *Chaetogaster* infestations.

Snails were paired in larger cups in this experiment (1000 mL with 900 mL of spring water) to reduce the density of trematode cercariae and thus snail mortality. During dissection at the end of the experiment, two non-shedding snails were found to have early trematode infections as first intermediate hosts; one was in a control replicate and one was in the treatment group where the donor snail was infected with trematodes. These replicates were removed from the analysis.

Experiment 6: Dispersal based on host size

In Experiment 6, we investigated whether snail host size influenced *Chaetogaster* dispersal among hosts. Experimental snails were first split into two groups: large snails ranging

from 9.7-15.3 mm in diameter and small snails ranging from 3.0-5.6 mm in diameter, corresponding to reproductively mature adults and not yet reproducing juveniles (Negovetich and Esch 2008). The following size combinations were used as treatment groups: a small donor snail paired with a large receiver snail (n=12 pairs), a large donor snail paired with a small receiver snail (n=12 pairs), a large donor snail paired with a large receiver snail (n=5 pairs), and a small donor snail paired with a small receiver snail (n=5 pairs). The last two treatment groups were control groups comparable to previous experiments where snails had been size-matched. We use an asterisk to refer to the donor snail hereafter (i.e., small*-large means the small snail is the donor snail).

Ten large snails and ten small snails were randomly selected to be the sized-matched control groups (large*-large and small*-small), and a snail in each pair was randomly assigned to be the donor host snail. Control snails were marked with nail polish so that they could be differentiated. In the treatment groups where snail sizes differed, snails were paired so that the smallest small snail and the smallest large snail were one replicate, etc. Then pairs were assigned randomly to treatment groups (small*-large or small-large*).

Statistical Analysis:

The response variable in all experiments was the proportion of *Chaetogaster* on the receiver snail at the end of the experiment, which measures net *Chaetogaster* dispersal (e.g., movement in both directions). We tested for differences in net *Chaetogaster* dispersal among treatment groups with generalized linear models (GLMs) with log links. To account for overdispersion in net dispersal, we used negative binomial error distributions and the natural log of the total number of *Chaetogaster* that survived on both snails as an offset (*sensu* Zuur et al. 2009). Analysis of residual plots indicated that these models provided equal or better fits to our

data than binomial error distributions. We initially included the average diameter of each pair of size-matched snails as a covariate in the GLMs for Experiments 1-5, and then dropped that covariate from each model if it was not a significant predictor of net dispersal.

For Experiment 4, where we examined dispersal at four different time points, we explored dispersal over time using two approaches. One approach was the NB GLM statistical model describe above, where time was natural log transformed because the relationship between time and net dispersal was non-linear. We added a second approach to determine if the observed non-linear pattern of net dispersal could be explained by the simplest mechanism we could envision: *Chaetogaster* moving in both directions (from donor to receiver, and back) at constant and equal per capita rates. We used the average proportion of *Chaetogaster* on the receiver snail after one hour of dispersal as the estimate for the per capita movement rate in a simple two-patch differential equation model of dispersal without demographics (Eqns. 1 and 2), and we plotted the predicted proportion of *Chaetogaster* on the receiver snail through time with our data from Experiment 4. Below, D is the number of *Chaetogaster* on the donor snail at a given time, R is the number of *Chaetogaster* on the receiver snail at a given time, and e and i are the constant and equal (e=i) emigration and immigration rates. The receiver snails always started with zero *Chaetogaster* ($R(0)$=0), and the donor snails started with 97% of the initial applied density, to account for the low levels of *Chaetogaster* mortality during experimental additions.

Eqn. 1: $$\frac{dD}{dt} = -eD + iR$$

Eqn. 2: $$\frac{dR}{dt} = -eR + iD$$

Finally, because some *Chaetogaster* mortality always occurred during *Chaetogaster* additions, and because *Chaetogaster* are known to rapidly asexually reproduce (e.g., Hopkins et al. 2013), we also used GLMs with Poisson error distributions to determine whether the number

of *Chaetogaster* remaining in each replicate at the end of the each experiment varied by treatment group. Visual inspections of all model predictions and Pearson residuals plots confirmed that our models were appropriate. All analyses were performed in R v2.15.3 using functions *glm* and *glm.nb* from package MASS (R Development Core Team 2014). Parameter estimates for all models are included in Table 2.1.

Results:

Experiment 1: dispersal without host contact on artificial substrate

When snails could not contact each other, only one *Chaetogaster* was acquired by a single receiver snail during the experiment. In contrast, when snails could contact, receiver snails acquired 40% of the total *Chaetogaster* by the end of the experiment, on average (contact vs. no contact; NB GLM; $P<0.001$; Table 2.1; Fig. 2.1a). The total number of *Chaetogaster* per replicate at the end of the experiment (13-22; mean = 16.9) did not vary among treatment groups ($P=0.42$).

Experiment 2: dispersal without host contact on leaf substrate

The presence of natural substrate had no effect on dispersal. As before, in the no contact treatment, only one *Chaetogaster* dispersed onto a single receiver snail, and in the contact treatment, all receiver snails acquired *Chaetogaster* during the experiment (Fig. 2.1b). Approximately 39% of the total *Chaetogaster* dispersed to the receiver snails in the contact treatment during the 24-hour experiment (contact vs. no contact; NB GLM; $P<0.001$; Table 2.1). The total number of *Chaetogaster* per replicate at the end of the experiment (10-17; mean = 13.3) did not vary among treatment groups ($P=0.44$).

Experiment 3: dispersal following host mortality, without host contact

When the donor snail remained alive, only one receiver snail acquired *Chaetogaster*, just as in Experiments 1 and 2. However, when the donor snail was euthanized, all receiver snails acquired *Chaetogaster*, even though the snails could not contact (Fig. 2.1c). On average, receiver snails in that treatment group had 46% of the total *Chaetogaster* at the end of the experiment (donor alive vs. euthanasia treatment; NB GLM; $P<0.001$; Table 2.1).

There was also a non-significant trend towards reduced *Chaetogaster* survival in the euthanasia treatment group, where there were 22% fewer remaining *Chaetogaster* on snails in that treatment group than the control, on average (NB GLM, $P=0.063$). Of the 49 *Chaetogaster* lost from snails in the euthanasia treatment, four were found alive in the cups but unattached to the snails, and five were found dead in the cups. The rest presumably died and their bodies were lost. Assuming mortality of those individuals, four of the replicates in the euthanasia treatment group had 40% or greater *Chaetogaster* mortality. No living or dead *Chaetogaster* were found off the snails in the control treatment, and the total end *Chaetogaster* in that treatment group ranged from 11-15 with a mean of 13.

Experiment 4: density-dependent dispersal

In Experiment 4, where paired snails could contact freely for up to 24 hours, the proportion of *Chaetogaster* that dispersed to the receiver snail increased with the time available for snail contacts (ln(time); NB GLM; $P<0.001$; Table 2.1). Specifically, the number of *Chaetogaster* on the receiver snail tended to saturate after several hours so the donor and the receiver snails had ~50% of the *Chaetogaster*, on average (Fig. 2.2). Contrary to our initial hypothesis, the proportion of *Chaetogaster* that moved to the receiver snail in a given period was not density-dependent, but rather our results suggest a single, constant per-capita rate of movement in both directions (density treatment; NB GLM; $P=0.14$; Table 2.1). For example,

16% of *Chaetogaster* dispersed within the first hour, averaged across all density treatment groups. We used this estimate as the emigration and immigration rates in a simple two-patch differential equation model of dispersal (simulating *Chaetogaster* moving back and forth from snail to snail at a constant rate) to plot the predicted proportion of *Chaetogaster* on the receiver snail for each time point (Fig. 2.2). Finally, the proportion of *Chaetogaster* on the receiver snail was not affected by the interaction between time and density (interaction term; NB GLM; P=0.092; Table 2.1).

Experiment 5: dispersal with trematode-infected hosts

In Experiment 5, we varied host trematode infection, and unlike our previous experiments, *Chaetogaster* abundance did not remain constant across treatment groups (Fig. 2.3a). Specifically, there were ~10 more total *Chaetogaster*, on average, in the treatment group where the donor snail was infected as a first intermediate host than in the other two treatment groups (NB GLM; P=0.0027). That represents a 40% increase in the *Chaetogaster* population in just 24 hours.

As in the previous experiments where snails could contact, when two uninfected snails were paired together, *Chaetogaster* tended to disperse towards an equal distribution among snails. Receiver snails ended up with ~49% of the *Chaetogaster* in that treatment group (Fig. 2.3b). There was much less dispersal in the treatment group where the donor snail was uninfected and the receiver snail was infected compared to the control group (~24%; control vs. infected receiver; NB GLM; P=0.006; Table 2.1; Fig. 2.3b). There was an intermediate level of dispersal in the treatment group where the donor snail was infected and the receiver snail was uninfected (~37%), where net dispersal in that group did not differ from either of the other

treatment groups (25% less than control; NB GLM; P=0.21; Table 2.1; 51% more than receiver infected; NB GLM; P=0.097; Fig. 2.3b).

Experiment 6: dispersal based on host size

In Experiment 6, there was more dispersal from large to small snails than in any other treatment group (Fig. 2.4). Net dispersal was 49% from larger donor snails to smaller receiver snails. In contrast, there was only 33% net dispersal from smaller donor snails to larger receiver snails (large*small vs. small*large; NB GLM; P=0.044), 24% net dispersal between two large snails (large*small vs. large*large; NB GLM; P=0.022), and 32% net dispersal between two small snails (large*small vs. small*small; NB GLM; P=0.087; Table 2.1; Fig. 2.4). The total number of *Chaetogaster* per replicate at the end of the experiment (7-16; mean = 11.2) did not vary among treatment groups (P=0.63).

Discussion:

Theory regarding the dispersal of free-living species leads us to expect that defensive symbionts will balance the potential fitness benefits of moving to a better host with the risks of dispersing (Bowler and Benton 2005; Bonte et al. 2012). By considering the net movement of *Chaetogaster* between donor and receiver snails under different ecological conditions, we present a detailed account of *Chaetogaster* dispersal ecology that fits into this fitness-based dispersal framework. In our experiments, symbionts readily dispersed to new hosts when the risks of dispersing were low (i.e., hosts could contact; Experiments 1,2,4,5,6), whereas net symbiont dispersal was almost zero when the risks of dispersing were higher (i.e., hosts could not contact; Experiments 1-3). However, as predicted, symbionts would overcome their reluctance to disperse across an open distance if the donor host died (Experiment 3). Finally, when hosts could contact, symbiont dispersal rates varied with host size (Experiment 6) and host

infection status (Experiment 5), but not in the ways that we predicted. Together, these experiments show that symbiont dispersal is not a constant, random process, as is often assumed in symbiont dispersal models (e.g., Lloyd-Smith et al. 2005), but rather the probability of dispersal varies with ecological conditions and among individual hosts.

Dispersal of symbionts during host contacts is similar to the dispersal of free-living species among connected habitat patches, and dispersal of symbionts through the open environment between hosts is similar to dispersal of free-living species through the non-habitat matrix between unconnected patches. For free-living species, dispersing through the non-habitat matrix may be strongly avoided (Cooper and Walters 2002; Desrochers and Hannon 1997), because matrix dispersal can be risky (de Kock and Robinson 1966; Lubin et al. 2003). For *Chaetogaster*, dispersing across an open distance between snail hosts may be risky because the oligochaetes are exposed to predators and currents (Gruffydd 1965), the oligochaetes experience reduced foraging efficiency when off the snail host (Gruffydd 1965), and there is no guarantee that another host is nearby. Concordantly, in our experiments, *Chaetogaster* would not cross a small distance to disperse between two living snails that could not contact (Experiments 1 and 2). There are other examples of symbionts, especially parasites, which predominantly disperse during direct host contacts (e.g., Zohdy et al. 2012). If future models of the spread of *Chaetogaster* through host populations are created, it may therefore be profitable to base them on models of directly transmitted parasites.

However, even when dispersing through non-habitat matrix is strongly avoided, organisms may still cross the non-habitat matrix when conditions on the current habitat patch become extremely poor. For instance, despite the risk of drowning in freezing waters, lemmings may swim to new habitats when food resources are exhausted by high lemming densities (de

Kock and Robinson 1966). For symbionts, host death may be analogous to exhaustion of resources. Additionally, remaining on a recently dead host until a new host appears is unlikely to be a good dispersal strategy for snail symbionts (e.g., Zimmermann et al. 2013), because healthy snails tend to avoid injured or recently dead snails (Dalesman et al. 2006). In Experiment 3, many *Chaetogaster* dispersed across an open distance from a euthanized donor snail to a living receiver snail, even though they would not cross that same distance to disperse between two healthy snails. However, just as many lemmings may drown during their risky matrix dispersal (de Kock and Robinson 1966), we often observed more than 40% *Chaetogaster* mortality when a donor host was euthanized during Experiment 3 (see also Gruffydd 1965). This mortality most likely occurred when *Chaetogaster* became ensnared in degrading snail tissue, which would also explain why there was a significant negative effect of host size on dispersal in that experiment: the tissue of larger snails took longer to degrade, and the oligochaetes may have been stimulated to disperse later on those larger snails. Regardless, the high mortality rates occurred when *Chaetogaster* had to cross a distance of a mere 20 mm in the laboratory, whereas the distance between snails in the pond will often be much longer and the environment will be structurally more complex and will include natural enemies.

Though we expected that symbionts would be more likely to disperse when they were maintained at high densities on the donor host, there was no effect of density on the probability of dispersal in Experiment 4. In that same experiment, we observed an increase in the proportion of symbionts on the receiver snail as the time available for contacts between snails increased. However, even after 24 hours, some receiver snails in each density treatment group had acquired few or no *Chaetogaster*. While we did not quantify the number and duration of snail contacts, the large variation in dispersal rates that we observed among replicates (Figure 2.3) can likely be

explained by variation in snail contacts during each period (e.g., Lloyd-Smith et al. 2005). For instance, the probability of transmission of Sin Nombre Virus between mice depends on both the number and duration of mouse contacts (Clay et al. 2009). We expect that this is an important consideration for the dispersal/transmission of many directly-transmitted symbionts.

Larger hosts may provide greater per capita resources for symbionts (e.g., Castro 1978; Lindberg and Stanton 1989; Lubin et al. 2003; Pap et al. 2005), and snails infected by trematodes often serve as high resource environments for *Chaetogaster* (e.g., Fernandez et al. 1991). Therefore, we predicted that symbionts would preferentially disperse to large snails and infected snails. However, we found the opposite patterns. In Experiment 5, net symbiont dispersal was greatest from large to small snails, and in Experiment 6, net symbiont dispersal was lowest when the receiver snails were infected. Both results warrant further study. However, dispersal to smaller snails may be advantageous if the symbionts need to disperse from the overwintered cohort of older, larger snails to the new cohort of younger, smaller snails each spring (Calow 1978; Dillon 2000). Directly dispersing to the new host generation before the older generation dies could allow *Chaetogaster* to avoid the high symbiont mortality associated with host death (Experiment 3). Other authors have suggested that contacts among old and pre-reproductive snails – which do not copulate - would be too infrequent for effective spring *Chaetogaster* dispersal (Gruffydd 1965). However, younger snails very commonly graze on the shells of older snails (*pers. obs*. SRH), giving *Chaetogaster* frequent opportunities to disperse via direct contact between the temporarily overlapping host generations.

The dispersal behavior of symbionts can help explain the distribution and abundance of symbionts among hosts. Specifically, when symbiont immigration rates vary among hosts and/or when symbionts reproduce directly on their hosts, symbionts will be aggregated among hosts as

long as negative density-dependent death or emigration processes do not overwhelm the aggregating processes (Anderson and Gordon 1982). Here, we found that (1) *Chaetogaster* dispersal rates varied among hosts with different body sizes and infection statuses (Experiments 5 and 6) and (2) *Chaetogaster* dispersal was not density-dependent across a natural range of *Chaetogaster* densities (Experiment 4). Additionally, previous work has demonstrated direct asexual reproduction by *Chaetogaster* on the host snail (e.g., Vaghin 1946). Therefore, we would expect *Chaetogaster* to be aggregated among snails. And in fact, in the natural pond environment, the distribution of *Chaetogaster* among snails is well approximated by a negative binomial distribution (mean=1.02, k=0.14; Hopkins et al. 2013), where most snails have few *Chaetogaster* and a few snails harbor many *Chaetogaster*. This distribution is particularly important because both the intensity of protection against trematodes (e.g., Sankurathri and Holmes 1976, Hopkins et al. 2013) and the costs incurred by hosts when harboring the oligochaetes (Stoll et al. 2013) increase with *Chaetogaster* density, so that some snails receive the bulk of the benefits and costs associated with symbionts. We suggest that by combining our understanding of symbiont dispersal with the existing theoretical framework for the functional responses relating host fitness to symbiont density (Holland et al. 2002), we can now work towards understanding both how symbiont densities are achieved and maintained on individual hosts and what this means for individual host fitness, host population dynamics, and host-symbiont co-evolution.

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Table 2.1. Parameter estimates and P values from the negative binomial generalized linear models with offsets for Experiments 1-6. Parameter estimates are on the log scale.

Experiment	Parameter	Estimate	SE	p value
1	Intercept (No Contact)	-5.088	1.000	<0.001
	Contact Treatment	4.174	1.007	<0.001
2	Intercept (No Contact)	-4.844	1.000	<0.001
	Contact Treatment	3.900	1.010	<0.001
3	Intercept (No Euthanasia)	-1.417	1.545	<0.001
	Euthanasia Treatment	4.166	1.030	<0.001
	Snail Size	-0.382	0.139	0.006
4	Intercept	-2.214	0.405	<0.001
	ln(Time) Treatment	0.584	0.175	<0.001
	Chaetogaster Density Treatment	0.025	0.017	0.138
	Time*Density Interaction	-0.013	0.008	0.092
5	Intercept (Both Uninfected)	-0.714	0.168	<0.001
	Receiver Uninfected Treatment	-0.291	0.231	0.208
	Receiver Infected Treatment	-0.704	0.254	0.006
6	Intercept (Large*-Small)	-0.709	0.125	<0.001
	Small*-Large Treatment	-0.390	0.193	0.044
	Large*-Large Treatment	-0.719	0.315	0.022
	Small*-Small Treatment	-0.439	0.256	0.087

Figure 2.1 a) In Experiment 1, where donor and receiver snails were tethered so that they could (long tethers) or could not (short tethers) contact each other, *Chaetogaster* only dispersed when snails could contact. **b)** In Experiment 2, providing natural substrate did not aide dispersal; *Chaetogaster* still only dispersed when host snails could contact. **c)** However, when the donor snail was euthanized in Experiment 3, *Chaetogaster* dispersed without snail contact. The dashed horizontal line represents a 50/50 distribution between the donor and receiver snails. Each point represents an individual snail, and points are jittered slightly on the X axis to aid visualization. The horizontal bars are the predicted proportions for each group.

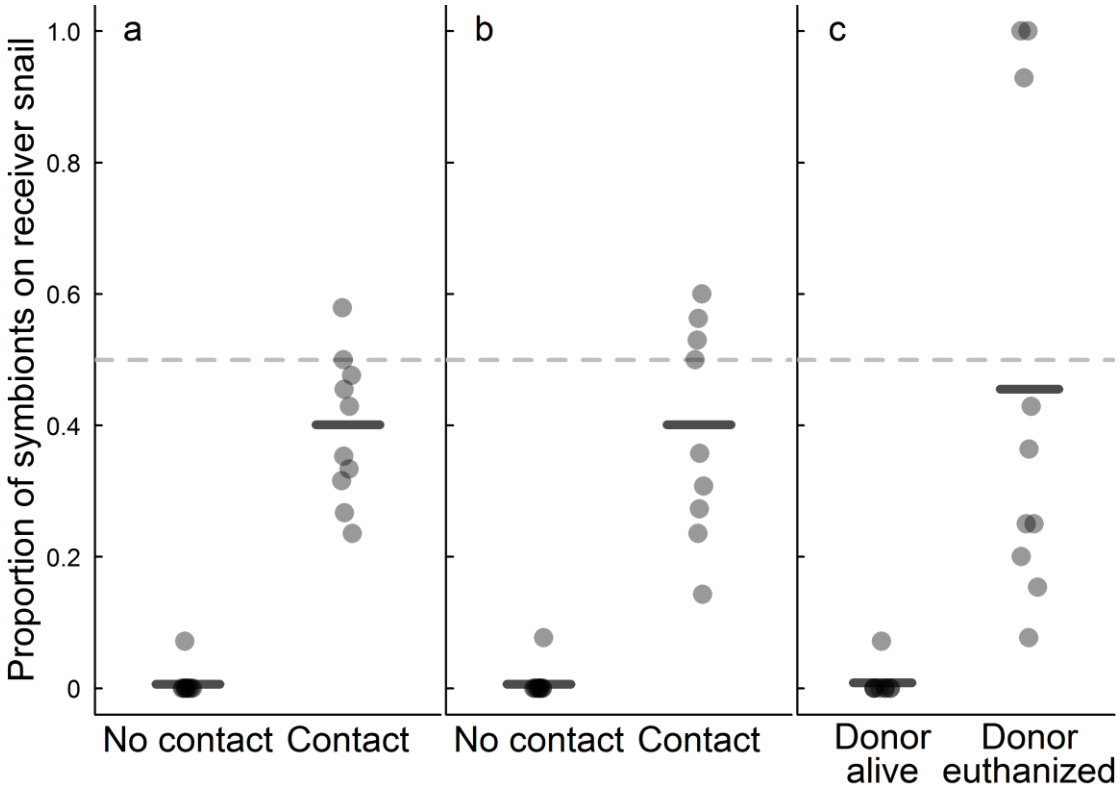


Figure 2.2. (a) Model predictions from the two-patch differential equation dispersal model with density-dependent (dashed lines) and density independent (solid line) emigration and immigration rates for experiment 4. The density-independent model was more parsimonious, because (b) the proportion of *Chaetogaster* found on the receiver snail did not depend on initial *Chaetogaster* density (NB GLM; n = 72; P = 0.14), and there was no interaction between time and *Chaetogaster* density (P = 0.092). Gray points show each receiver snail. Open symbols show the predicted proportion of *Chaetogaster* on the receiver snails in each density treatment at each time point from the NB GLM [five (circles), 15 (squares), and 30 (triangles) *Chaetogaster*-density treatment groups]. Vertical lines delineate 95 % confidence intervals for the parameter estimates from the NB GLM.

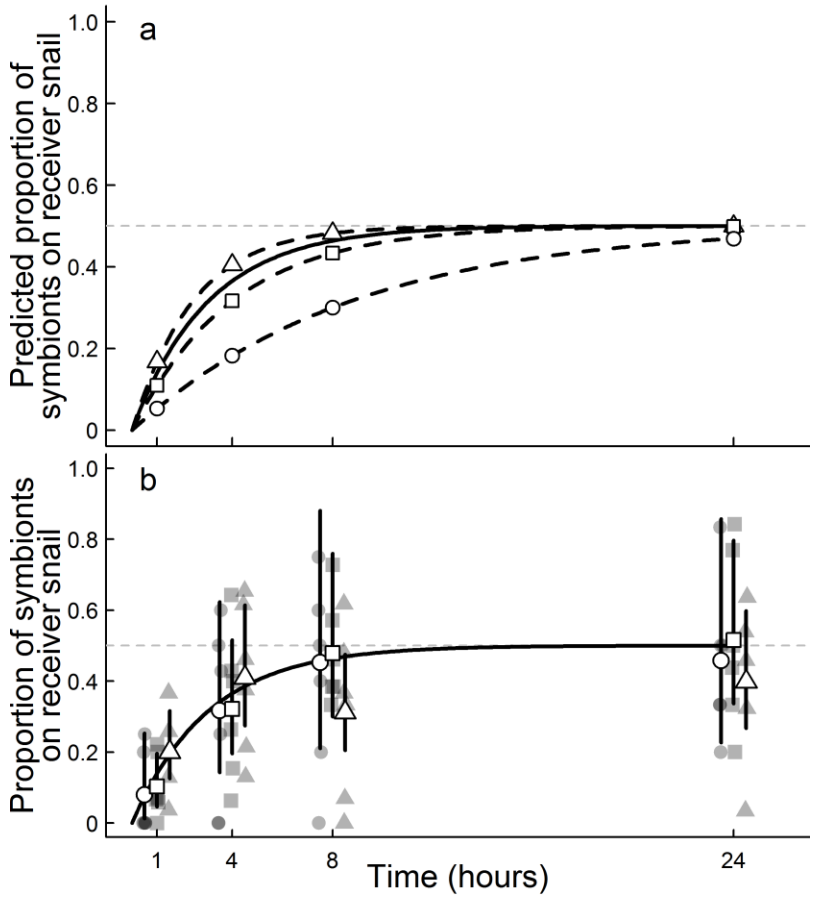


Figure 2.3. a) In Experiment 5, *Chaetogaster* reproduction rates varied by treatment group, so that there were more total *Chaetogaster* in the treatment group where the donor snail was shedding echinostome cercariae and the receiver snail was uninfected (Poisson GLM; n=28; P=0.0027). **b)** Dispersal rates were greatest when both snails were uninfected (i.e., when larval trematodes were absent), and there was significantly less dispersal from uninfected donor snails to infected receiver snails in comparison to controls (NB GLM; P=0.006). The dashed horizontal line represents a 50/50 distribution between the donor and receiver snails. Each point represents an individual snail, and points are jittered slightly on the X axis to aid visualization. The horizontal bars are the predicted proportions for each group.

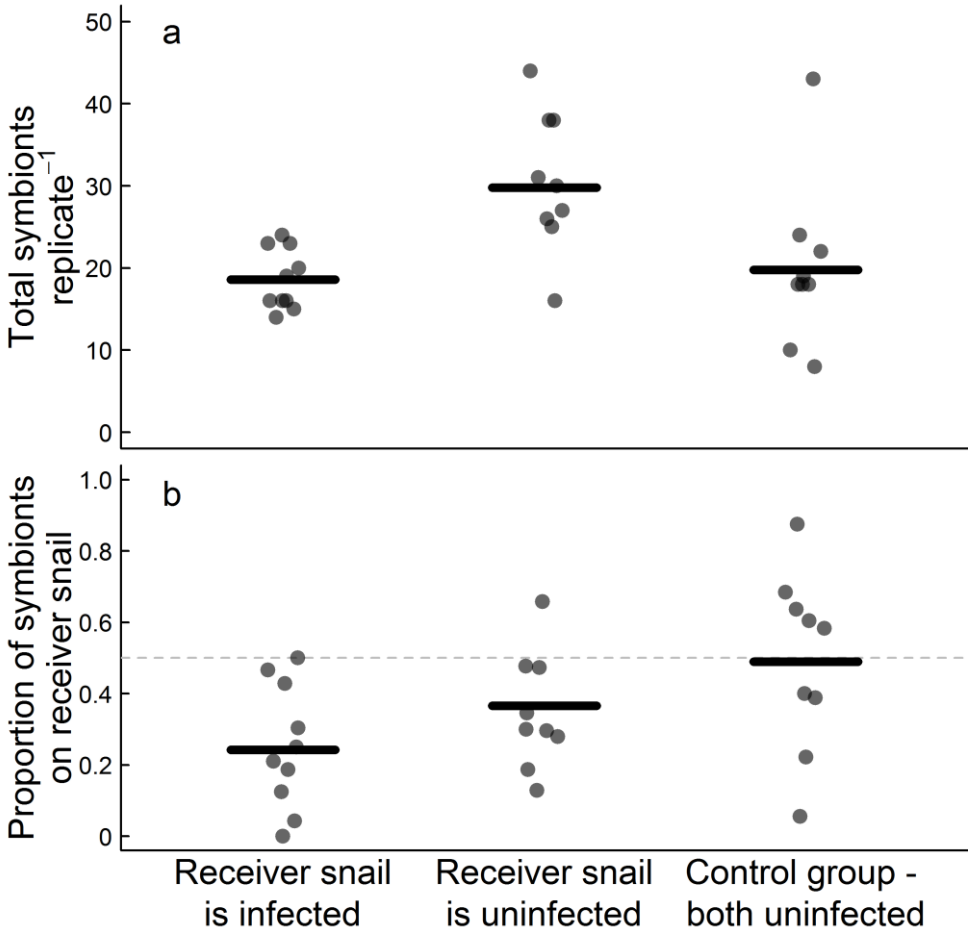
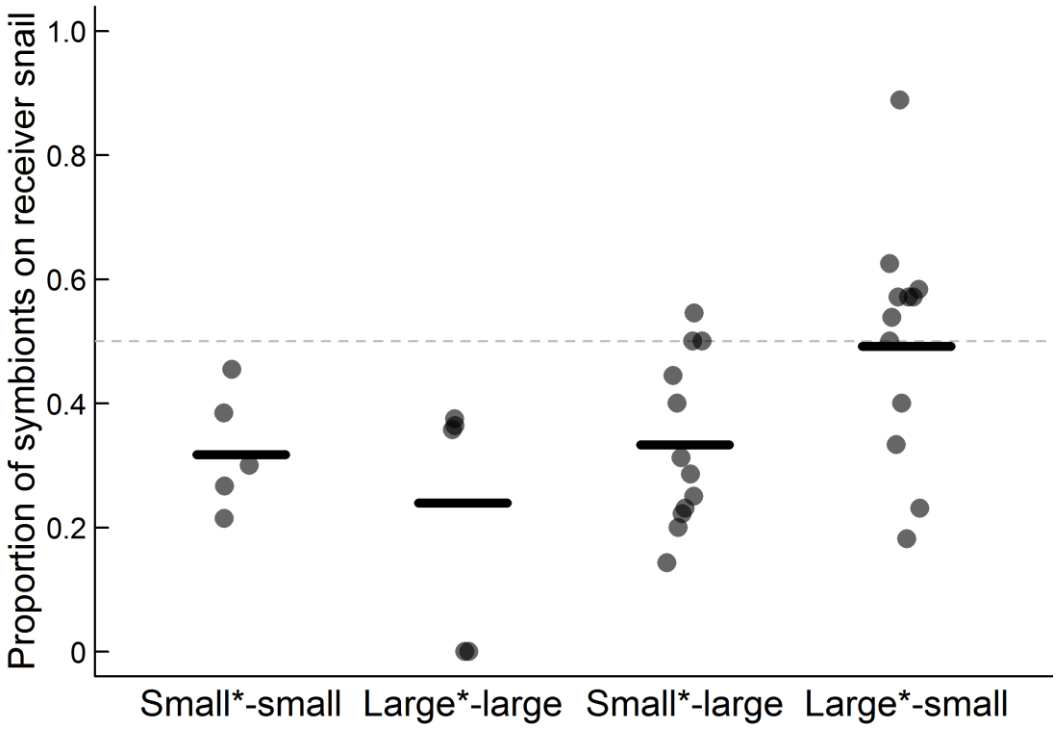


Figure 2.4. In Experiment 6, *Chaetogaster* were more likely to disperse from a larger donor snail to a smaller receiver snail than between snails in any other treatment group (NB GLM; n=34; P=0.044, P=0.022, P=0.087). The dashed horizontal line represents a 50/50 distribution between the donor and receiver snails. Each point represents an individual snail, and points are jittered slightly on the X axis to aid visualization. The horizontal bars are the predicted proportions for each group.



CHAPTER 3: HANDLING TIMES CREATE NONLINEAR RELATIONSHIPS BETWEEN HOST CONTACT RATES, SYMBIONT TRANSMISSION RATES, AND HOST DENSITY IN A COMMON SNAIL-WORM SYMBIOSIS

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Abstract:

All dynamic species interaction models contain an assumption regarding how contact rates scale with population density, which is subsumed within the 'functional response' or 'transmission function.' In host-parasite models, nonlinear transmission functions appear to be more biologically realistic, but there is some reluctance towards using the available nonlinear functions because they are phenomenological; their parameters do not have obvious biological interpretations. Here, we borrow a well-known, mechanistic function from predator-prey models, the Holling Type II functional response, and evaluate its potential utility in epidemiological models. To do this, we first used field sentinel hosts and a typical nonlinear, phenomenological transmission function to describe net nonlinearity in the transmission-density relationship in a common snail-symbiont system. We then performed laboratory contact rate experiments and quantified how well the Holling Type II functional response could describe the nonlinear contact-density relationship that we observed. We found that a nonlinear contact-density relationship can at least partially explain why transmission-density relationships were nonlinear in the field, and contact handling times are responsible for contact rates that saturate with density. The Holling Type II functional response provided better fits to our contact rate data than linear models, and accurately estimated the mean contact durations that we observed, suggesting that this functional response was biologically-relevant to our system. Because most animal

contacts should involve a non-instantaneous handling time, the Holling Type II functional response could be broadly useful in epidemiological models.

Introduction:

The common loon (*Gavia immer*) is not particularly common in Virginia, and thus the probability that an average backyard birder will encounter a common loon in Virginia is small. However, loon population densities vary in space and time, and avid Virginia birders can increase their probabilities of encountering loons by traveling to locations with higher loon population densities. This idea that encounter rates or contact rates depend on population density is so intuitive that all dynamic species interaction models contain a function that describes how contact rates among individuals scale with density (e.g., Lafferty et al. 2015). For instance, in predator-prey models, the contact function is subsumed within the functional response, and in epidemiological models, the contact function is subsumed within the transmission function. If chosen and parameterized properly, these functions can allow ecologists to take a model designed for one population or community and apply it across space or time to populations with variable densities (e.g., McCallum et al. 2001). But choosing an appropriate functional response or transmission function is not as straightforward as it might seem.

Most dynamic species interaction models start with the assumption that contact rates are positive linear functions of population densities (e.g., Holling 1959, Lafferty et al. 2015, Chapter 2). This default assumption has various names in the ecological literature: Holling Type I functional response (predator-prey models), density-dependent transmission function (epidemiological models), and "mass-action" movement assumption. But in 1959, Holling suggested that contact rates that increase infinitely with population densities are not biologically reasonable. In particular, his proposed Type II functional response was designed to describe

saturation in the rate that predators consume prey, which occurs because there is limited time available for predators to hunt (T = total time) and a non-instantaneous time commitment for each contact (H = handling time) (Holling 1959). Holling also proposed a third functional response, which is predominantly used to model predators that only switch or learn to target a prey species when the prey population reaches high densities (Holling 1965). Though there is still some debate in the literature regarding which functional responses should be used in predator-prey models (Abrams and Ginzburg 2000, Abrams 2015), the existing options are mostly mechanistically tied to predator-prey ecology, making it relatively straightforward to choose an appropriate functional response.

In epidemiological models, the guidelines for choosing an appropriate transmission function are not as clear. The two best known transmission functions are linear; they assume that contact rates increase linearly with host density (density-dependent transmission, hereafter DD) or that contact rates are independent of host density (frequency-dependent transmission, hereafter FD) (Begon et al. 2002). For infectious diseases of wildlife, the FD transmission function has historically been recommended for modeling parasites or pathogens that are transmitted via vectors or sexual interactions (Thrall et al. 1993,Antonovics et al. 1995,Anderson and May 1991,Keeling and Rohani 2008) or for modeling systems where population densities do not change with the number of hosts (e.g., fixed territory sizes; Begon et al. 2002,McCallum et al. 2002), and the DD transmission function has historically been recommended for all other parasites and pathogens. However, many recent ecological papers do not follow these suggested guidelines when using the canonical functions (Chapter 2). Furthermore, transmission rates should be not be linear functions of host density whenever there is variation in transmission success among individuals or whenever contact rates are nonlinear functions of density (e.g.,

Antonovics et al. 1995, Dwyer et al. 1997), two common characteristics of host-parasite systems. However, nonlinear transmission functions are rarely used in the ecological literature, even though they are better supported by the majority of existing empirical data than linear functions (Chapter 2). Given these discrepancies, many authors have called for a renewed focus on transmission functions, with a particular emphasis on the potential utility of nonlinear transmission functions (e.g., McCallum et al. 2001, Lafferty et al. 2015, McCallum et al. 2017, Chapter 2).

Ecologists might be under-utilizing nonlinear transmission functions in epidemiological models for several reasons, but one particular complaint is that the existing nonlinear functions are predominantly phenomenological; their parameters do not have obvious biological interpretations. This parallels a broader trend in the literature where disease ecologists have been "unpacking" or "deconstructing" the transmission rate (Beta) into smaller mechanistic parts, especially by separating contact rates and host susceptibility (e.g., Civitello and Rohr 2014, Antonovics 2017, McCallum et al. 2017). In this paper, we evaluate whether the well-known, mechanistic Holling Type II functional response developed for predator-prey models can also serve as a theoretical backbone for epidemiological models. The theoretical utility of the Holling Type II functional response within epidemiological models was proposed several decades ago by Antonovics et al. (1995), and more recently a universal Type II functional response has been proposed for all consumer-resource models, including host-parasite models (Lafferty et al. 2015). However, to our knowledge, the Holling Type II functional response has never been empirically evaluated in a real host-parasite system, and empirical tests of all transmission or contact functions are generally needed (Chapter 2). Therefore, we first quantified whether the transmission of a directly-transmitted ectosymbiotic oligochaete (*Chaetogaster limnaei*, hereafter

Chaetogaster) was a linear or nonlinear function of host snail density in the field, and then we "unpacked" the nonlinearity in the transmission-density relationships by using the Holling Type II functional response to provide a mechanistic description of just one piece of β: the contact rate function.

Methods

Brief overview of contact rates, transmission functions, and forces of infection

In a typical Susceptible, Infected, Recovered (SIR) epidemiological model for directly-transmitted parasites and pathogens, the transmission function is given by g(N)*v*(I/N)*S, where g(N) is the contact rate function, v is the probability of transmission success given a contact (sometimes called host susceptibility), I is the density of infected hosts, S is the density of susceptible hosts, and N is the total host density. The prevalence of infection (I/N) can be interpreted as the probability that a given contact involves an infected host. The transmission rate (β) described above is the product of g(N) and v. Additionally, the rate at which susceptible hosts in the population become infected (the force of infection, FOI), is also a conglomerate parameter, where FOI = g(N)*v*(I/N).

As is often the case in ecological studies, we first described a pattern in the field using a phenomenological model, and we then tested the ecological mechanisms underlying that pattern using a controlled laboratory experiment. In particular, we first quantified how natural changes in host density in the field affected the FOI in sentinel hosts housed in field enclosures, and we described the net nonlinearity in that relationship using a flexible, nonlinear, phenomenological model. We then quantified the relationship between host snail contact rates and snail density in the laboratory, to determine whether nonlinear contact rates could explain the nonlinear transmission-density relationship we observed in the field. For our laboratory experiment, we

switched from using a flexible, phenomenological model to using the more mechanistic Holling Type II functional response. For both the field and laboratory studies, we also considered two alternative hypotheses: that transmission (or contact) rates were linear, increasing functions of host density (DD transmission) and that transmission (or contact) rates were independent of host density (FD transmission).

Quantifying symbiont FOIs in the field:

Every two weeks between 20 April and 15 September 2013, *Helisoma trivolvis* and *Physa gyrina* snails were sampled from a pond in Montgomery County, Virginia. This provided cross-sectional surveys of the resident snail populations at eleven time points. After eight of those eleven cross-sectional surveys, lab-reared, symbiont-free *Helisoma* snails ("sentinel" hosts) were placed in field enclosures in the pond for approximately one week (Table 3.1) to quantify the rates at which susceptible *Helisoma* became infested by *Chaetogaster* (=weekly force of infection). Together, the cross-sectional surveys and field enclosure trials were used to determine the roles of host density and host infestation prevalence in symbiont transmission (FOIs) in the field.

The focal pond had an area of 2.3ha and an approximate perimeter of 935m, and was originally formed by damming a small creek. We focused our studies in eight randomly selected sites ($1m^2$ each) within a 145m reach from the head of the pond, where the water was not too deep for stovepipe sampling (<61cm) or too shallow for field enclosures (>10 cm). All cross-sectional surveys and field enclosure experiments occurred at these permanent $1m^2$ sites. However, early in the season, we only worked in a subset of five of the eight permanent sites (Table 3.1).

Cross-sectional field surveys:

Using a modified stovepipe sampler (a plastic trash bin with the bottom cut out; volume = 41L, cross sectional area = 0.075m^2), haphazardly-placed quadrats were sampled from around the perimeter of each 1m^2 site. Within each quadrat, all aquatic vegetation, leaf litter, sticks, and rocks were visually inspected for snails, and then three handfuls of the mud substrate (~2 passes over entire substrate) were visually inspected for snails. Most snails were found on the mud substrate, on the lily pads at the surface, or foraging in the waters' surface tension, such that the approximate surface area with snails foraging per quadrat was 0.15m^2. All collected snails were placed in individual 50mL centrifuge tubes containing 30mL spring water, and then snails were returned to the laboratory and dissected within 24 hours (see dissection procedure below).

To balance sampling effort among sites within the pond and across sampling dates, and to limit the impact of weekly sampling on each site, two rules determined the number of quadrats sampled per site: approximately 15-20 total snails (*Helisoma trivolvis* or *Physa gyrina*) were sampled per site, and up to five quadrats were sampled per site. If 15 snails were collected prior to finishing the five quadrat samples, then the full five quadrats were not sampled at that site; therefore, pond-level prevalence estimates were not unevenly weighted towards high density sites. If the five quadrat samples did not result in 15 snails, additional snails were collected haphazardly from the vegetation within the site, and those "extra" snails were used to estimate infection prevalence, but not snail densities.

Within the pond, *Helisoma trivolvis* and *Physa gyrina* were by far the most numerous snail species; of the 1062 snails sampled in 2013, only 11 individuals were other species. For this study, we focus specifically on intraspecific *Chaetogaster* transmission among *Helisoma* snails, and thus refer to *Helisoma* as the "snails" hereafter. Note that because only 52% of the total snails were *Helisoma*, less than 15 *Helisoma* were typically collected per site, especially early in

the season when *Helisoma* were still emerging from torpor (Table 3.1). During those weeks, prevalence estimates had low precision, but density estimates were still based on 25 quadrat samples.

Field enclosure trials:

Field enclosures were cylindrical bags (total volume ≈ 4000 cm^3) constructed out of 1mm fiberglass window screen, with inner rings of PVC that maintained the cylindrical shape. These bags were attached to wooden dowels that were fixed in the mud at each $1m^2$ site, so that individual bags were ~30 cm apart near the center of the site. Each bag contained five sentinel *Helisoma* and one dried maple leaf. Sentinel *Helisoma* could easily contact field snails through the mesh bags, thereby allowing for the direct snail contacts that are necessary for *Chaetogaster* transmission (Hopkins et al. 2015). As with the cross-sectional surveys, the early field enclosure trials were conducted at five sites, while the later trials were conducted at all eight sites (Table 3.1). Ten sentinel snails (two field enclosures) were placed at each site per time point, except for one time point where we increased the precision of our estimates by placing 20 sentinel snails (four field enclosures) per site (Table 3.1).

For all enclosure trials, symbiont-free *Helisoma* raised from eggs in stock cattle tanks were brought to the laboratory the day before the trial, separated in individual centrifuge tubes containing 30mL spring water, and randomly assigned to sites and bags within sites. The morning after randomization – which was also the morning after the corresponding cross-sectional survey – sentinel snails were placed in their bags in the pond. Snails were left for 5-8 days (Table 3.1) to become infested by *Chaetogaster*. Then snails were removed from their bags, placed in individual centrifuge tubes containing 30mL spring water, returned to the laboratory, measured (shell diameter) and dissected within 24 hours under a dissecting microscope to

quantify *Chaetogaster* infestation intensities (*sensu* Hopkins et al. 2015). A few snails died during the enclosure trials (17/570 snails), and those snails were excluded from our analyses.

Model fitting procedures for cross-sectional field surveys and field enclosure trials:

We used the cross-sectional surveys and the associated field enclosure trials to evaluate how intraspecific host density and infestation prevalence influences the force of infection in sentinel *Helisoma* snails; in particular, we evaluated whether transmission dynamics were best described by linear (density-dependent or frequency-dependent) or nonlinear transmission dynamics. Because our permanent sites were placed randomly in the pond, we combined data from all *Helisoma* snails from all sites for each sampling date and considered this a representative sample of the *Helisoma* population in the pond. Cross-sectional surveys provided eleven pond-level estimates of total *Helisoma* density (N), total susceptible *Helisoma* density (*Chaetogaster*-free snails; *S*), total infested *Helisoma* density (*Chaetogaster*-infested snails; *I*), and the prevalence of *Helisoma* infestation (infested density divided by total density; *I*/*N*). For modeling fitting procedures, we limited our focus to the pond-level estimates (8/11) that occurred at the times of the eight field enclosure trials. The sentinel snails from the field enclosures provided us with estimates of the proportion of susceptible *Helisoma* that became infested with *Chaetogaster* in a given enclosure trial (=force of infection). We treated *Chaetogaster* infestation as a binomial presence vs. absence variable, because *Chaetogaster* abundance data would be confounded by the fact that *Chaetogaster* can rapidly asexually reproduce on the host snail (Hopkins et al. 2013).

We fit three alternative models to the field transmission data: the density-dependent transmission function, the frequency-dependent transmission function, and a flexible, phenomenological, nonlinear transmission function (Table 3.2). The general model formulation

for the ordinary differential equation describing the transmission process is given in Eq. 1, where λ is the force of infection equation given by each model and S_0 is the number of initially susceptible snails in field enclosures. From Eq. 1, we obtained a solution describing the predicted proportion (p) of field enclosure snails that are infested by the end of an enclosure trial (Eq. 2), where t is the number of days for which the enclosure trial lasted (Table 3.1). That predicted proportion served as the probability parameter (p) in the binomial distribution that described the probability of observing the number of infested enclosure snails each week ($1\text{-}S_t$) given the initial number of susceptible enclosure snails each week (Eq. 3).

$$\text{Eq. 1:} \quad \frac{dS}{dt} = -\lambda S_0$$

$$\text{Eq. 2:} \quad p = 1 - \frac{S_t}{S_0} = 1 - e^{-\lambda t}$$

$$\text{Eq. 3:} \quad y_i \sim \text{binomial}(p, S_0)$$

We estimated model parameters from the three force of infection models (Table 3.2) in a Bayesian framework with Markov chain Monte Carlo (MCMC) methods executed using the package R2WinBUGS (Sturtz et al. 2005). We used uninformative uniform priors constrained between 0 and 10 for all parameters; we also tried larger and smaller ranges, but posterior distributions were not sensitive to the range of the prior distributions. We ran three MCMC chains for each model, with 10000 draws from the posterior distribution, a thinning rate of 1, and a burn-in period of 5000 draws. This resulted in a final sample of 5000 draws from the posterior distributions. Convergence in each analysis was assessed visually and using the Gelman-Rubin statistic. Model fits were assessed by examining plots of the Pearson residuals and model predictions.

Laboratory contact rate experiment:

To test the mechanisms underlying the force of infection models applied to our observational data, we performed a laboratory experiment to quantify how intraspecific *Helisoma* contacts rates varied with *Helisoma* density. The experiment took place in plastic containers containing 2 L of well water (28x15cm, ~0.1m^2 surface area), with 12 density treatments (2-13 individually marked *Helisoma*). We chose this density range to reflect the natural range that we saw in 0.1m^2 of surface area in our field survey. There were 4 replicates of each of the 12 density treatments, for an initial total of 360 *Helisoma* snails. We performed each of these replicates on a different day, so that the whole experiment was completed during four days. In a few cases (4/360 snails), a snail was found to be unusually lethargic or dead on the morning of their contact trial. When that occurred, the snail was removed from the experiment, and the density treatment was adjusted accordingly.

All snails for the experiment were collected in 2015 from the same sites in the pond described above. Snails were maintained in individual 50 mL centrifuge tubes for up to three days before being used in the contact rate experiments. During that time they were fed Spirulina pellets *ad libitum*. Snails that were shedding trematode cercariae on the day that they were brought into the laboratory were excluded from the experiment, to reduce the probability that parasites would be present in the containers during behavior trials. The remaining, non-shedding snails were randomly assigned to treatment groups and trial days. Then snails were painted with unique two dot color codes for individual identification (Sally Hansen's insta-dry nail polish *sensu* Hopkins et al. 2015a). Snails were left overnight after painting before their trial.

To facilitate normal snail foraging and movement, periphyton was added to all experimental containers. Periphyton was grown on 50 10x10cm ceramic tiles maintained in similar light and nutrient environments for 10 days prior to the experiment. Each trial container

received one-tile's worth of periphyton the night before the container was used in the experiment.

The morning of each trial, snails were added to the appropriate container 1.5 hours before the observation period to acclimate. Each observation period lasted for 44 minutes (45 minutes with the last minute removed to exclude contacts that had not finished by the end of the trial), during which one person would watch two containers (one higher density and one lower density) at a time. Each time a contact was made, the identities of all snails involved in the contact and the time that the contact was initiated and terminated was recorded. If a snail left a contact and reinitiated contact seconds later, the second contact was recorded as a new contact. Occasionally, three snails would join together into one cluster, and in these cases all unique combinations were recorded as new contacts (i.e., A-C and B-C are new contacts when Snail C joins pre-existing A-B contact). All observation periods were recorded using Logitech web cameras and ISpy video software, and these videos were used to confirm the identities of snails or the start or end times of particular contacts when necessary.

Model fitting procedures for contact experiment:

The primary goal of the contact rate experiment was to quantify the relationship between average per capita intraspecific contact rates and host density. To that end, we fit three contact rate models, including the nonlinear Holling Type II functional response used in dynamic predator-prey models (Figure 3.1). Individuals within a tank were not independent, because contacts require at least two individuals, by definition; and if one individual was particularly active, it could increase the contact counts for all individuals in the tank. Therefore, we included the tank as a random effect, where the continuous, zero-truncated encounter rate for each tank was drawn from a gamma distribution (Figure 3.1). Even after including the random effect, there

was more variation in contact counts than would be expected based on a Poisson distribution, so we used a Poisson-Gamma mixture model (equivalent to a negative binomial model) to account for the overdispersion. The three hierarchical models were fit to the contact rate data in a Bayesian framework following the procedures outlined above. Finally, to evaluate how biologically relevant the Holling Type II function was for our system, we compared the 95% credible interval for the handling time estimated from our model fitting procedures to the mean observed contact duration from our experiment.

Results:

Cross-sectional surveys and field enclosure trials:

We observed 60 infestation events in 570 sentinel snails across our eight field enclosure experiments. The FOI was very low early in the season, when *Helisoma* snails were nearly impossible to find, and peaked late in the season before declining (Figure 3.2). The flexible, phenomenological, nonlinear transmission function provided a better fit to the force of infection data than either of the linear transmission functions, as indicated by comparing model DICs. Additionally, the 95% credible interval for the density-dependence parameter (k) in the nonlinear model did not include 0 (FD) or 1 (DD) (Table 3.2, Figure 3.2).

When comparing just the two linear functions, the FD function performed poorly in comparison to the DD function, because the FD function could not predict the decline in FOI late in the season when infestation prevalence remained high, but snail densities declined (Tables 3.1-2, Figure 3.2).

Contact rate experiments:

We observed 612 unique contacts among 356 snails across all treatment groups and trial days. The relationship between per capita intraspecific contact rates and snail density (Figure

3.3) was consistent across days, eliminating the need for a trial day effect, whereas the random tank effect was justified by the particularly high and low contact rates observed in some tanks (Figure 3.3).

As with the field FOI data, the nonlinear contact rate function outperformed both linear contact functions for our laboratory contact rate data (Table 3.2, Figure 3.3); but in this case, the nonlinear function was the mechanistic Holling Type II functional response. Despite the notable difference in DICs, the best-fit lines of the density-dependent function and Holling Type II functional response were qualitatively quite similar. However, if we were to extend the range of snail densities past N=13 snails per $0.1m^2$, we would expect the qualitative differences in the model fits to increase; the linear DD model would predict that the contact rate would continue to increase, whereas the contact rate actually appears to saturate near N=6-10 snails per $0.1m^2$ (Figure 3.3). When comparing just the two linear contact functions, the DD function outperformed the FD function, furthering mirroring the results from the field FOI data (Table 3.2, Figure 3.3).

Intraspecific conduct durations ranged from 1 to ~24 minutes (1431 seconds) and were approximately exponentially-distributed, with many short contact durations and a few long contact durations. Across all contacts made by all snails in all density treatments, the mean contact duration was 1.6 minutes. The estimated handling time using the Holling Type II functional response was 3.6 minutes, and the 95% credible interval for that estimate included the independently-observed mean contact duration (Table 3.2, Figure 3.3).

Snails spent an average of 5.5 of the 44 minutes engaged in intraspecific contacts, with the total time that individual snails spent in contacts increasing to saturation with snail density (Figure 3.3). But even at saturation, snails did not engage in contacts during the entire time (44

minutes). For instance, snails in the highest density treatment spent an average of only 4.3 total minutes engaged in contacts. Note that there is some double-counting in these total duration data (i.e., two snails had total contact durations > 44 minutes), because snails occasionally engaged in multiple contacts at once, but this does not alter the qualitative results in Figure 3.3.

Discussion:

Measuring contact and transmission rates is notoriously difficult, especially in the field, and thus there are few empirical tests of transmission functions in wild populations (McCallum et al. 2001,Antonovics 2017, Lello and Fenton 2017,McCallum et al. 2017). But in accordance with the majority of those existing empirical studies (Chapter 2), we found that symbiont transmission rates in the field were nonlinear functions of host density. That net nonlinearity in the transmission-density relationship could be explained by the nonlinear relationship between snail contact rates and snail density that we observed in our laboratory experiment. In particular, the mechanistic Holling Type II functional response provided a better description of the contact-density relationship that we observed than either of the linear, canonical functions. Furthermore, the Holling Type II functional response could estimate the mean contact duration that we independently observed during our laboratory experiment. Therefore, though the Holling Type II functional response was initially proposed for predator-prey interactions, we found that the same underlying logic could be re-appropriated for our host-symbiont system, as earlier authors had suggested might be theoretically possible (Antonovics et al. 1995, Lafferty et al. 2015).

For brevity, we have used the terms “handling time” and “contact duration” mostly interchangeably throughout this paper, but there is an important distinction between the terms: handling times can include time when one individual is not contacting another individual. For instance, when predators are satiated, they might stop searching for prey and even avoid prey

contacts, and thus the handling time is longer than the contact during which the predator ingests the prey. This difference between handling times and contact durations might explain why our estimated handling time from the Holling Type II functional response was nearly twice as large as the observed mean contact duration, even though the 95% credible interval for our handling time estimate still included the observed mean contact duration. Though we did not quantify snail avoidance behavior, we did observe many instances where two snails appeared to be headed for a certain contact, but then veered off course at the last moment and did not contact. Concordantly, we found that few snails spent the entire 44 minute trial engaging in contacts with other snails, and the average total time budget for intraspecific contacts was only 4.3 minutes per snail (~10% of the available time) in the highest density treatment. Therefore, it seems plausible that snails reached some kind of contact satiation, such that handling times could have been longer than observable contacts.

Though we were somewhat surprised to find that snails only spent ~10 % of their time engaging in intraspecific contacts, we are also unsure why snails spent so much time in contacts, because we never observed snails mating during our trials; it is difficult to say whether most contacts were accidental or antagonistic, or perhaps whether snails were evaluating potential mates. *Chaetogaster* transmission does occur during non-mating snail contacts (Hopkins et al. 2015), so the non-mating laboratory contacts that we observed were relevant to *Chaetogaster* transmission dynamics in the field. However, it occurs to us that we might have found a stronger or weaker saturating effect if we had run our laboratory experiment during peak snail mating periods, depending on if snails budget more time for intraspecific contacts and/or alter their mean contact durations during those periods. Context-dependent changes in contact rates due to changes in host densities (e.g., when animals aggregate to breed or forage) are widely

documented in disease systems (e.g., Becker et al. 2015), but future work should consider whether those changes are solely due to changing encounter rates, or whether context-dependent handling times are equally important to contact and transmission dynamics.

Nonlinear contact functions are not the only way to create nonlinear transmission/FOI functions, because the latter are a combination of the contact rate function [$g(N)$] and the probability of transmission success (v); if the probability of transmission success is not a simple constant, it can give rise to additional nonlinearity in the transmission function (e.g., McCallum et al. 2017). For instance, even if contact rate functions are linear, transmission functions could be nonlinear if the probability of transmission success varies among hosts, as it does for *Chaetogaster* (Hopkins et al. 2015). One benefit of using the Holling Type II functional response is the ability to estimate both $g(N)$ and v separately – a feat that is usually not possible when $g(N)$ and v are combined in a single transmission term (β) and/or structurally unidentifiable (e.g., Civitello and Rohr 2014,McCallum et al. 2017). But this can also be a downside of the Holling Type II functional response, because it requires estimating three model parameters (v, e, and H) to fit an FOI model, instead of the two parameters required for the flexible, phenomenological, nonlinear function (β, k). Because we did not have the power to tease apart the roles of nonlinear contact rate functions versus variation in the probability of transmission success in our field force of infection data, we simply note that the net nonlinearity that we described using the flexible, phenomenological, nonlinear transmission function can be at least partially explained by nonlinear contact rates, but is probably also affected by variation in transmission success.

Because intraspecific and interspecific host contact rates are probably not instantaneous in most animal systems, we suggest that the Holling Type II functional response might be broadly applicable to epidemiological models. In the disease ecology literature alone, there are

several carefully-collected datasets documenting exponentially-distributed, non-instantaneous contact durations for host-host contacts, similar to those described here (Figure 3.4), for systems where the hosts are as diverse as gopher tortoises and mice (Clay et al. 2009, Aiello et al. 2016). Additionally, for trophically-transmitted parasites where transmission occurs via predation, the classic Holling Type II functional response in predator-prey models *is* the transmission function. Therefore, in accordance with the recent theoretical work of Lafferty et al. (2015), we suggest that the Holling Type II functional response be widely considered for use in host-parasite models.

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Table 3.1. In 2013, we quantified *Helisoma* densities and *Chaetogaster* infestation prevalences at 11 time points in a pond in Riner, Virginia via sampling snails at five or eight permanent $1m^2$ sites. Though *Physa* snails were also collected, we only summarize the *Helisoma* data here. After eight of those cross-sectional surveys, we also quantified the rates at which uninfected, lab-reared sentinel *Helisoma* became infested during 5-8 day exposures in field enclosures.

Date	Cross-Sectional Surveys				Field Enclosure Experiments			
	Snails per site (min : max)	Number of sites	Total quadrats sampled	Total snails sampled	Sentinel hosts per site	Number of sites	Total sentinel hosts	Length (days)
20-Apr	0 : 2	5	25	3	10	5	50	6
14-May	0 : 2	5	25	3	10	5	50	5
27-May	0 : 3	5	25	9	10	5	50	5
9-Jun	1 : 6	5	25	14	10	5	50	7
23-Jun	0 : 7	5	8	11	10	5	50	6
7-Jul	2 : 13	8	26	69	10	8	80	6
23-Jul	3 : 19	8	17	102	-	-	-	-
3-Aug	6 : 20	8	32	109	10	8	80	7
17-Aug	11 : 15	8	40	106	-	-	-	-
31-Aug	5 : 16	8	40	76	20	8	160	8
15-Sep	4 : 11	8	36	52	-	-	-	-

Table 3.2. Given the density and prevalence data from the cross-sectional surveys, a flexible, phenomenological, nonlinear transmission performed better when describing the force of infection in sentinel snails than either of the linear, canonical transmission functions, despite the cost of the extra density-dependence parameter (k). Similarly, the mechanistic Holling Type II functional response performed better when describing the laboratory contact rate data than either of the linear, canonical transmission functions. The effective number of parameters is given by pD, and the end-points of the 95% credible intervals for the parameters are given on either side of the mean estimates. Deviance Information Criterion (DIC) differences of greater than two between models indicate that the model with the lower DIC has substantial support.

Model		Parameter	2.5%	Mean	97.5%	pD	DIC
Field force of infection models							
Density dependent (k=1):	$\lambda_{DD}=\beta_{DD}*(I/N)*N^1$	β_{DD}	8.81E-06	1.15E-05	1.46E-05	1.0	41.4
Frequency dependent (k=0):	$\lambda_{FD}=\beta_{FD}*(I/N)*N^0$	β_{FD}	2.37E-05	2.37E-05	3.97E-05	1.0	50.9
Flexible nonlinear function:	$\lambda_{NL}=\beta_{NL}*(I/N)*N^k$	β_{NL}	1.17E-05	1.89E-05	2.78E-05	2.0	38.7
		k	0.246	0.577	0.919		
Laboratory contact rate models							
Density dependent:	$\lambda_{DD}=e_{DD}*T*N$	$1/\mu_{eDD}$	82.37	115.19	152.37	162.9	1416.8
Frequency dependent:	$\lambda_{FD}=e_{FD}*T$	$1/\mu_{eFD}$	10.72	14.80	19.44	162.6	1417.8
Holling Type II function:	$\lambda_{NL}=\frac{(e_{NL}*T*N)}{(1+e_{NL}*H*N)}$	$1/\mu_{eNL}$	41.97	70.78	109.80	156.0	1411.0
		H	1.263	3.606	5.725		

Figure 3.1. Directed acyclic graph of the hierarchical Bayesian Poisson-Gamma (negative binomial) mixture model with a random tank effect. The square nodes represent known quantities (data = y_{ij}, snail density = N_i, and total time = T) and the circles represent unknown model parameters. The solid and dashed arrows indicate deterministic and stochastic dependencies, respectively. The large, gray boxes indicate whether the variable varied at the level of the individual snail or the tank.

Noninformative priors
Encounter rate (e_j) ~ Gamma(1, μ)
Handling time (H) ~ Uniform(0, T)
ρ ~ Gamma(α,α)

Noninformative hyper priors
μ ~ Uniform(0, 1000)
Dispersion (α) ~ Uniform(0, 5)

Poisson-Gamma mixture model
y_{ij} = Poisson(Λ_{ij})
$\Lambda_{ij} = \lambda_{ij}\ \rho_i$
Type II functional response
$\lambda_{ij} = e_j T N_i/(1+e_j H N_i)$
Density dependent model (H=0)
$\lambda_{ij} = e_j T N_i$
Frequency dependent model
$\lambda_{ij} = e_j T$

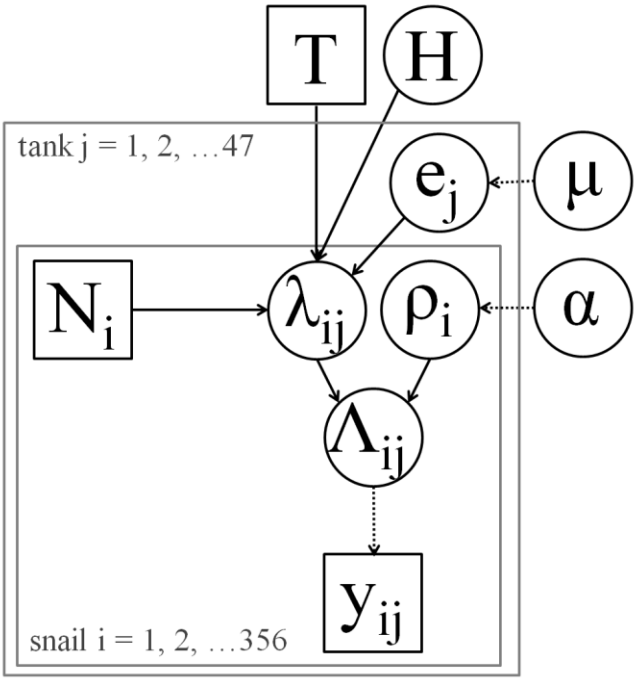


Figure 3.2. The observed weekly probability that an uninfected sentinel snail would become infected during each of the eight field enclosure trials, where the vertical bars delineate 95% asymmetrical binomial confidence intervals for each estimate (top). The colored polygons show the best-fitting force of infection models while including estimate uncertainty for the transmission rate (β) (Table 3.2). The models were fit to the eight FOI samples, but we show the predictions based on all eleven of the observed weekly densities of infected (I) and total (N) snails (bottom).

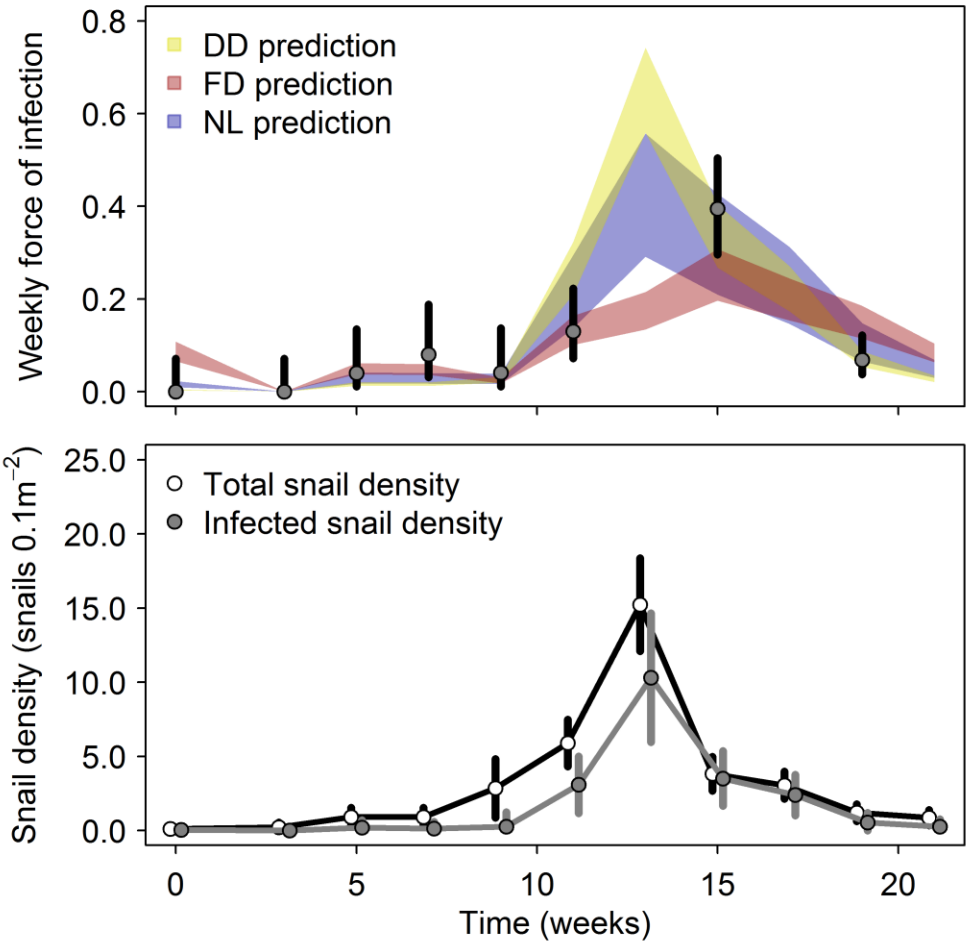
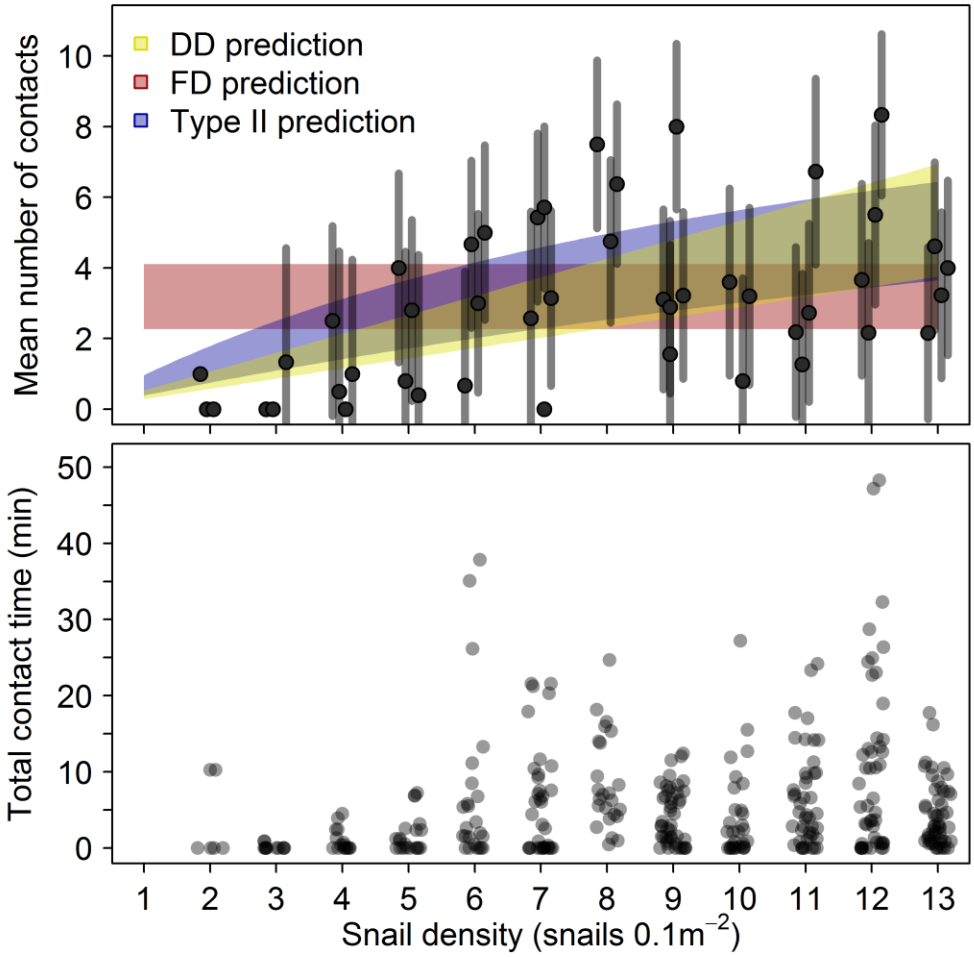


Figure 3.3. The mean observed number of contacts per snail per tank during the laboratory contact rate experiment saturated with snail density (top), where the vertical bars delineate 95% negative binomial confidence intervals for the means. Points are offset on the X axis to aid visualization. The colored polygons show the best contact rate models while including estimate uncertainty for the mean encounter rate (μe) (Table 3.2). The total time that each snail spent engaging in contacts was typically much less than the total 44 minutes available for contacting (bottom), but note that because snails occasionally had contacts with two partners at once, the sum of all contact durations was sometimes greater than 44 minutes.



CHAPTER 4. QUANTIFYING THE BARRIERS AND BRIDGES TO INTERSPECIFIC TRANSMISSION IN A COMMON SNAIL-SYMBIONT SYSTEM

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Introduction:

Many parasites and pathogens infect multiple host species (Cleaveland et al. 2001, Taylor et al. 2001). For instance, West Nile virus infects hundreds of bird and mammal species (Marra et al. 2004), the fungus that causes white nose syndrome infects dozens of bat species (e.g., Langwig et al. 2015, Hoyt et al. 2016), and the trematodes that cause schistosomiasis in humans infects dozens of mammalian definitive host species (e.g. Rudge et al. 2013). This complexity can be daunting for ecologists and epidemiologists tasked with predicting or controlling the spread of important parasites and pathogens (hereafter parasites). But fortunately, many host species do not actually contribute substantially to parasite transmission, even though they can become infected (e.g., Kilpatrick et al. 2006, Rudge et al. 2013). When host-parasite systems have just one key host species amidst many peripheral host species (‘apparent’ or ‘spillover’ multi-host systems), rather than many key host species (‘true’ multi-host systems), predicting and managing parasite transmission should be more straightforward (Fenton and Pedersen 2005). However, differentiating between true and apparent multi-host systems can be challenging, and thus one pressing goal in disease ecology is to develop and test theories and methodologies for identifying key host species versus peripheral host species (Buhnerkempe et al. 2015, Fenton et al. 2015) .

One recent theoretical framework described three possible characteristics of key host species (Streicker et al. 2013): key hosts are very abundant and thus more likely to make many interspecific contacts (super-abundant host species); key hosts have high prevalence of

infection, so that any given contact with that species has a high probability of being with an infected individual (super-infected host species); or key hosts produce many infectious stages, thereby increasing parasite transmission success given a contact (super-infectious host species). Using a similar framework, we suggest that host species that create strong barriers to interspecific transmission have characteristics opposite to those of the super host species. In particular, peripheral host species should be super rare, super uninfected, or super uninfectious.

Though it is easier to consider the three (or six) 'super' or 'inferior' characteristics of key or peripheral hosts separately, the relevant processes underlying those characteristics (i.e., contact rates, infection prevalence, and probability of transmission success) are all multiplied together within epidemiological models, and thus they should act collectively. For this reason, any one of the 'inferior' characteristics that reduce interspecific transmission might turn a potential key host into a peripheral host. For instance, the mosquitoes that vector West Nile virus have a strong preference for feeding on crows, potentially turning crows into super-infected and/or super-infectious hosts, but the relative rarity of crows prevents them from playing an important role in community-level West Nile virus transmission (Kilpatrick et al. 2006). But this West Nile virus study is one of just a handful of case studies that have quantified the relative roles of multiple host species to community-level transmission and identified the characteristics that determine those relative roles (e.g., LoGiudice et al. 2003, Searle et al. 2016). Hence, it is unclear how often host characteristics work additively versus orthogonally to determine the host species' overall role in interspecific transmission. For instance, are super-abundant hosts usually also super-infected and/or super-infectious? Therefore, in this study, we quantified interspecific contact rates, infection prevalence, and interspecific transmission success, as well as how those

processes interacted to influence net interspecific transmission in a common host-symbiont system.

In our study system, ectosymbiotic oligochaete worms (*Chaetogaster limnaei*, hereafter *Chaetogaster*) live on the headfoot of aquatic snails and are predominantly transmitted among hosts via direct snail contacts (Hopkins et al. 2015). *Chaetogaster* is remarkably cosmopolitan, with hosts in 16 snail genera in 10 families (see review in Smythe et al. 2015). But within a given snail community, there can be substantial variation in the prevalence and/or infestation intensities among potential host species, including cases where some snail species do not harbor *Chaetogaster* in snail communities where the worms are otherwise present (e.g., Ibrahim 2007). This variation among potential hosts makes this system a good one for investigations regarding the characteristics of key host species. To that end, we first quantified net rates of interspecific *Chaetogaster* transmission in the field, as well as natural snail densities and prevalences of *Chaetogaster* infestation. We then investigated interspecific contact rates and transmission success rates experimentally to understand why there is no detectable interspecific transmission between two common snail host species, even though they have high population densities, prevalences of infestation, and infestation intensities.

Methods:

The empirical work presented here consists of three components: quantifying (1) interspecific transmission rates in the field, (2) interspecific contact rates in the laboratory, and (3) interspecific transmission success rates in the laboratory. For all field and laboratory studies, we used *Helisoma trivolvis* snails as our focal hosts, so interspecific transmission refers to *Chaetogaster* transmission from *Physa gyrina* to *Helisoma trivolvis* (P-H transmission) and intraspecific transmission refers to *Helisoma-Helisoma* transmission (H-H).

Interspecific Chaetogaster *transmission in the field:*

To understand interspecific transmission dynamics in the field, we quantified how *Helisoma* infestation risk varied with natural variation in the density and prevalence of infested *Physa*. We quantified *Helisoma* infestation risk (force of infection) by placing lab-reared, uninfested *Helisoma* in field enclosures for approximately one week, where they could acquire *Chaetogaster* infestations via direct contact through the mesh enclosures with *Physa* and *Helisoma* living in the pond. We conducted eight of those enclosure trials between April and September 2013 in a pond in Montgomery County, Virginia. Concurrent with the enclosure trials, we surveyed the resident snail community, and we used multi-host epidemiological models to relate the density estimates obtained in the surveys to the forces of infection observed in the field enclosures. Some of these data were used in a separate study describing how intraspecific *Helisoma-Helisoma* transmission depends on *Helisoma* density (Chapter 3).

All cross-sectional surveys and field enclosure trials occurred within eight randomly-selected, $1m^2$ sites within a 145m reach in the pond where the water was a reasonable depth for field enclosures. Each field enclosure (~4000 cm^3) contained five randomly assigned sentinel *Helisoma* snails and one dried maple leaf. Two enclosures (10 snails) were placed at each site for each trial, except for one trial where we placed four enclosures (20 snails) at each site (Table 3.1). After 5-8 days, sentinel *Helisoma* were collected from their enclosures and dissected to quantify the number of *Chaetogaster* per snail (*sensu* Hopkins et al. 2013). The force of infection (FOI) was quantified as the number of newly infested sentinel *Helisoma* divided by the total number of sentinel *Helisoma*.

The day before each enclosure trial, *Helisoma* and *Physa* snails were sampled from each $1m^2$ site using a modified stove pipe sampler. Because snail densities vary across time, we

developed a set of sampling procedures that would allow us to compare across weeks without biasing our estimates towards high density weeks and/or sites. In particular, we tried to sample ~15 snails per site per week, and we never sampled more than five haphazardly placed quadrats per site. The number of snails of each species sampled per week is summarized in Table 3.1 and further details on the sampling procedure are available in Chapter 3. All snails collected during cross-sectional pond surveys were individually housed, returned to the laboratory, measured (snail diameter to the nearest 0.1mm), and dissected to quantify *Chaetogaster* infestation intensities.

To quantify the contributions of intraspecific and interspecific transmission to *Helisoma* force of infection, we fit a multi-host transmission function to the data: $FOI_H = \beta_{HH}N_H^{kHH}(I_H/N_H) + \beta_{HP}N_P^{kHP}(I_P/N_P)$, where β_{HH} and β_{HP} are the intra- and interspecific transmission rates, N_H and N_P are the total *Helisoma* and *Physa* densities, and I_H and I_P are the infested *Helisoma* and *Physa* densities. We were not sure whether transmission was a linear or nonlinear function of host density, so we tried multiple options, where k_{HH} and k_{PH} are flexible, unitless density-dependent parameters that allow intra- and interspecific transmission to be linear increasing functions of host density (k=1, density-dependent transmission), nonlinear functions of host density (0<k<1), or independent of host density (k=0, frequency-dependent transmission). Using a Bayesian framework with non-informative uniform priors, we fit the integral of this model to our paired field enclosure and cross-sectional survey datasets, which created an eight-point time series summarizing data from 1600 snails. All analyses were conducted using R2WinBUGS (Sturtz et al. 2005), and model fits were assessed by examining plots of Pearson's residuals, model predictions, and convergence plots and Gelman-Rubin statistics. Comparing β_{HP} to β_{HH} allowed us to compare the relative roles of inter- and intraspecific transmission to net *Helisoma*

infestation risk. However, when fitting multi-host epidemiological models to this short time series, the number of parameters approached the number of data points. Therefore, while we believe that the results of this model fitting exercise are insightful, we recommend that quantitative estimates be evaluated conservatively.

Interspecific snail contact rates in the laboratory:

Though *Physa* were super abundant, *Physa-Helisoma* contact rates might still have been low if the two species avoided interspecific contacts, and thus we performed a laboratory experiment to determine whether low rates of interspecific contact could explain the low interspecific transmission rates that we observed in the field. We previously performed a similar experiment to quantify the relationship between intraspecific *Helisoma* contact rates and *Helisoma* density (see detailed methods in Chapter 3). For this study, we wanted to quantify how the number of interspecific contacts that individual *Helisoma* snails had with *Physa* snails varied with *Physa* density. Therefore, we varied *Physa* densities along a realistic gradient (1,2,3,…14,16,18 *Physa*) in 28x15cm plastic containers containing 2 L of well water and lose periphyton, and we added three focal *Helisoma* snails to each container. There were three replicates of each of the 16 *Physa* density treatments (48 tanks; 417 *Physa* and 144 *Helisoma* snails), and we performed these replicates over six trials of eight containers each, with even representation of high and low treatment groups in each trial. A few *Physa* (3/417) were lethargic or dead on the morning of their trials, and we excluded those individuals and adjusted the density treatments to reflect their absence.

All experimental snails were collected in 2015 from the same $1m^2$ sites described above. We excluded any snails that were shedding trematode cercariae on the day that they were brought into the laboratory from the study, and the remaining snails were randomly assigned to

treatment groups and trials. All snails were painted the day before being used in a trial with a unique two dot color code (Sally Hansen insta-dry nail polish; *sensu* Hopkins et al. 2015).

During each trial, snails were added to their containers 1.5 hours before the observation period to acclimate, and then each observation period lasted for 45 minutes. Each time there was a contact, we recorded the start time of the contact, the individuals involved in the contact, and the end time of the contact. If three or more snails joined together into one cluster, we recorded all unique pair combinations as contacts. All trials were also video recorded so that we could confirm details, as needed.

We previously found that a Holling Type II functional response [number of contacts = (encounter rate*total time*density)/(1+encounter rate*handling time*density)] provided a good description of the relationship between intraspecific *Helisoma* contact rates and *Helisoma* density, where the mean encounter rate was 0.014 contacts/minute (95% CI 0.009-0.024 contacts/minute) and the mean handling time was 3.6 minutes (Chapter 3). To determine whether interspecific *Helisoma-Physa* contacts were substantially less frequent than intraspecific *Helisoma* contact rates, we fit a similar Holling Type II functional response to the interspecific *Helisoma-Physa* contact rate data, and then we compared the best-fitting inter- and intraspecific models. We performed the model fitting procedures in a Bayesian framework using MCMC methods similar to those described above and in Chapter 3, where we used a Poisson-Gamma mixture model to account for overdispersion in our contact count data and a random tank effect to account for the non-independence of snails within the same container.

Interspecific transmission success experiments in the laboratory:

We also evaluated whether the low interspecific transmission rates estimated from our field force of infection models could be explained by very low interspecific transmission success,

rather than (or in addition to) low interspecific contact rates. In particular, we quantified the rates at which *Chaetogaster* would disperse from *Helisoma* to *Helisoma* snails versus the rates at which *Chaetogaster* would disperse from *Physa* to *Helisoma* snails given certain contacts between individuals, with the prediction that dispersal from *Physa* to *Helisoma* would be very low. In both cases, *Chaetogaster* were added to 'donor' *Helisoma* or *Physa*, and then the donor snail was placed with an uninfected 'receiver' *Helisoma* in a cup small enough to ensure contacts. We did not know whether the source host (*Helisoma* or *Physa*) of the *Chaetogaster* would affect our results, so we performed this experiment in two trials: once with *Chaetogaster* sourced from field *Helisoma* (H-source), and once with *Chaetogaster* sourced from field *Physa* (P-source). Because we had performed intraspecific transmission success experiments with *Helisoma*-sourced *Chaetogaster* in the past (Chapter 3), we performed more replicates of the interspecific transmission treatments (especially for *Physa*-sourced *Chaetogaster*) in this experiment, with 20, 21, 40, and 49 replicates in the H-source H-H, H-source P-H, P-source H-H, and P-source P-H treatments, respectively.

For all trials, we separated lab-raised, *Chaetogaster*-free *Helisoma* and *Physa* into individual 150mL plastic cups containing ~50mL of well water two days before the experiment. *Helisoma* were randomly assigned to treatment groups and replicates, and then *Helisoma* in the H-H treatment were randomly assigned to be donor versus receiver snails. *Physa* were randomly assigned to replicates in the P-H treatment group. The receiver snail in each replicate was painted with a single dot (*sensu* Hopkins et al. 2013). The same day as painting, 10 *Chaetogaster* were added to each of the donor *Helisoma* and *Physa* from the two treatment groups, following the methods of Hopkins et al. (2015). The *Chaetogaster* were sourced from field *Helisoma* or *Physa*

taken from the $1m^2$ sites described above. All snails were fed Spirulina fish flakes and left overnight.

On the day of each experiment, the donor and receiver snails from each replicate were combined in a single 150 mL plastic cup containing 50 mL of well water (hereafter "experimental cup"). Snails were initially placed such that they were not touching. In the first trial, where *Chaetogaster* were sourced from *Physa*, replicates were observed for one hour after being placed together in experimental cups. During that time, all donor-receiver pairs had contacted at least once. Because all snail pairs had contacted, and because we had found that ~16% of *Chaetogaster* disperse from donor *Helisoma* to receiver *Helisoma* in one hour in a previous experiment (Hopkins et al. 2015), we assumed that one hour was enough time for transmission. We therefore separated the donor and receiver snails into their individual containers after one hour and began dissections. However, after dissecting all snails from eight replicates in each of the H-H and P-H treatment groups, we were surprised to find that out of 230 total recovered *Chaetogaster*, only one was recovered from a receiver snail. We therefore placed the remaining donor and receiver snails together in their experimental cups for an additional 17 hours. We discuss the initial eight replicates in our results, but we only included the 73 18-hour replicates in our models. When we ran the second trial, where *Chaetogaster* were sourced from *Helisoma*, we paired all donor and receiver snails in their experimental cups for 18 hours prior to separating and dissecting all snails within 12 hours of ending the experiment. In addition to dissecting the donor and receiver snails for *Chaetogaster*, we also checked the individual donor cups, receiver cups, and experimental cups for any *Chaetogaster*. These *Chaetogaster* could have (1) failed to add to the donor snail during set-up (donor cups), (2) dispersed from the donor

to the receiver snail and then fell off after the receiver snail was placed back in its individual cup (receiver cup), or (3) could have fallen off either snail during the experiment (experimental cup).

We used binomial generalized linear models with logit links to evaluate whether the proportion of *Chaetogaster* that dispersed from the donor to receiver snail was affected by the donor species (H-H and H-P), using two separate GLMs for each trial (*Helisoma* vs. *Physa* source of *Chaetogaster*). We also used Poisson GLMs with log links to determine whether the total number of *Chaetogaster* varied across treatment groups, as might happen if *Chaetogaster* experienced higher mortality or asexual reproduction rates in one treatment (e.g., Hopkins et al. 2013). All models were run in R V3.3.2 using package 'MASS' (Venables and Ripley 2002), and model fits were assessed visually by examining predictions and Pearson's residuals plots.

Results:

Interspecific Chaetogaster *transmission in the field:*

In the field, *Helisoma* and *Physa* densities varied over time, with *Physa* densities peaking earlier than *Helisoma* densities (Figure 4.1). The prevalence of *Chaetogaster* infestation also varied over time for both species, but more synchronously, with a peak late in the season followed by a subsequent decline. When *Physa* densities were high, estimated sentinel *Helisoma* forces of infection were very low, and when *Helisoma* densities were high, estimated sentinel *Helisoma* forces of infection were high (Figure 4.1).

Correspondingly, the multi-host models including *Physa-Helisoma* transmission had higher DIC values than if we had only included intraspecific *Helisoma-Helisoma* transmission (Chapter 3) – whether we allowed transmission to be a linear (density or frequency dependent) or nonlinear function of host densities (Table 4.1) – indicating that interspecific transmission did not improve model fits. In fact, our model fits and intraspecific transmission rate estimates are

nearly identical between the single host models that we fit in Chapter 3 and the multi-host models that we fit here, suggesting that interspecific transmission plays no discernible role in net *Helisoma* force of infection in the field. Furthermore, for all models, the estimated interspecific transmission rate was at least one order of magnitude smaller than the estimated intraspecific rate (Table 4.1).

Interspecific contact rate experiment in the laboratory:

We observed 559 interspecific contacts between *Helisoma* and *Physa* snails, where individual *Helisoma* had anywhere from 0 to 16 contacts with *Physa*. As with the field *Helisoma* force of infection data, we fit three contact rate models to the laboratory interspecific contact data, where *Helisoma-Physa* contacts could be linear (density or frequency dependent) or nonlinear (Holling Type II) functions of *Physa* density. The Holling Type II model was best supported by DICs (Table 4.1) and visual assessment of Pearson's residual plots. When comparing 95% credible intervals, the interspecific *Helisoma-Physa* encounter rate (0.014 encounters/minute) estimated here by the Holling Type II functional response was no different from the intraspecific *Helisoma-Helisoma* encounter rate (0.016 encounter/minute) that we previously published (Table 4.1 vs. Table 3.2). In contrast, the interspecific Holling Type II functional response estimated a relatively large handling time (~17 minutes; Table 4.1), reflecting that the interspecific contact-density relationship saturated at a lower contact rate than the intraspecific contact-density relationship (Figure 4.2). However, the mean duration of an interspecific *Helisoma-Physa* contact was 1.08 minutes, similar to the mean duration of an intraspecific *Helisoma-Helisoma* contact (1.6 minutes; Chapter 3).

Interspecific transmission success experiment in the laboratory:

The outcome of the transmission success experiment depended on whether the *Chaetogaster* used in the trial were sourced from *Physa* snails (P-source) or *Helisoma* snails (H-source). In the *Helisoma* source experiment, a greater proportion of the *Chaetogaster* dispersed from a *Physa* donor snail (interspecific transmission) than from a *Helisoma* donor snail (intraspecific transmission; 83% vs. 32%; binomial GLM, $p<0.001$, df=39; Figure 4.3A). In contrast, in the P-source experiment, there was a lower proportion of *Chaetogaster* that dispersed from a *Physa* donor snail than a *Helisoma* donor snail (binomial GLM, $p=0.045$, df=68; Figure 4.3B). Additionally, in the P-source experiment, the proportions of *Chaetogaster* that dispersed were very small; on average, just 3% and 8% of the *Chaetogaster* dispersed during the 18-hour trial (Figure 4.3B).

At the end of each trial, we removed paired donor and receiver snails from their experimental cups and returned them to their individual donor and receiver cups until dissection. In contrast to our previous experimental work in this system, we recovered many *Chaetogaster* that were not attached to snails. Of the total recovered *Chaetogaster*, in the P-source trials <1% (3/396 worms) were in the cups and in the H-source trials 24% (38/158 worms) were in the cups. In the latter case, the majority of the *Chaetogaster* were recovered from the donor cup (87%; 33/38 worms), as opposed to the experimental cup (8%; 3/38 worms) or the receiver cup (5%; 2/38 worms). This indicates that free-living *Chaetogaster* were not on a host snail because they failed to add to the donor snail (donor cup), as opposed to leaving the donor during the experiment (experimental cup) or dispersing to the receiver snail and then leaving the receiver snail after the experiment (receiver cup).

In both trials, each donor snail was originally infested with 10 *Chaetogaster*, for a total of 730 and 410 *Chaetogaster* in the P-source and H-source trials, respectively (excluding the 1-hour

replicates). After accounting for all of the *Chaetogaster* recovered from snails or cups, 40.5% and 14.1% of the *Chaetogaster* were missing across both treatment groups in the P-source and H-source trials, respectively. These worms either died and degraded beyond recognition within the 18-hour period, or they were ingested by snails after they died. These mortality events were evenly distributed among treatment groups in the H-source experiment (Poisson GLM, $p=0.377$, $df=39$), with a mean of 8.6 *Chaetogaster* per replicate at the end of the trial. However, in the P-source experiment, where total mortality rates were higher, mortality events were twice as likely in the H-H treatment as in the P-H treatment (4 vs. 8 remaining *Chaetogaster*, on average; Poisson GLM, $p<0.001$, $df =87$; Figure 4.3A).

Discussion:

In the *Chaetogaster*-snail system, we expected substantial interspecific transmission among two of the most abundant pond-dwelling snail species in our region (*Helisoma trivolvis* and *Physa gyrina*), because we often find those species in close proximity or engaged in interspecific contacts in the field, and we find high *Chaetogaster* infestation intensities and prevalences in both species. But when we quantified the rate that sentinel *Helisoma* snails became infested in the field, our models attributed the majority of infestation risk to intraspecific *Helisoma-Helisoma* transmission, with little to no detectable role for interspecific *Physa-Helisoma* transmission. *Physa* might have been 'inferior' in terms of interspecific transmission due to very low interspecific contact rates (similar to super rare), infection prevalence (super uninfected), or probability of transmission success (super uninfectious), which are all multiplied together in the transmission function. From our field data, we could rule out the possibility that *Physa* were 'super rare' in the sense of low abundance or 'super uninfected,' because *Physa* densities and prevalences of infection matched or exceeded that for *Helisoma*. That left us with

two, non-mutually exclusive hypotheses for the lack of interspecific transmission: (1) despite being abundant, *Physa* rarely contacted *Helisoma* (and thus were super rare in this sense) or (2) there was low *Chaetogaster* transmission success from *Physa* to *Helisoma* given a direct contact (super uninfectious). Though interspecific *Physa-Helisoma* contacts were less common than intraspecific *Helisoma-Helisoma* contacts, they were still frequent. In contrast, interspecific *Physa-Helisoma* transmission success was extremely low, and that particularly strong barrier to interspecific transmission, so much so to potentially divide this potential multi-host system into discrete single host systems.

To our surprise, the results of our transmission success experiment depended on whether the *Chaetogaster* used in a trial were originally from *Helisoma* or *Physa* snails: when the source snails were *Helisoma*, there was actually slightly higher *Chaetogaster* dispersal between interspecific P-H than intraspecific H-H snail pairs, and when the source snails were *Physa*, there was lower *Chaetogaster* dispersal between P-H than H-H pairs. An even greater surprise was that after 18-hours together in a small cup, where donor and receiver snails would have contacted dozens of times, there was little intra- or interspecific transmission in the *Physa*-source experiment (3 and 8% worm dispersal) in comparison to the *Helisoma*-source experiment (32% and 83% worm dispersal). The lowest transmission success rate was therefore seen in the treatment that best represented interspecific transmission in the field, where *Chaetogaster* from a *Physa* snail were moving from a *Physa* to a *Helisoma* snail (3% dispersal), and overall, *Chaetogaster* seemed to preferentially stay on, or move to, hosts of their origin. It is possible that these results do not reflect host preferences in a single cosmopolitan *Chaetogaster limnaei* species, but rather the different life cycles of multiple cryptic symbiotic *Chaetogaster* species. However, Buse (1974) found that *Chaetogaster* preferences were ‘trainable’, where oligochaetes

that were forced to occupy a new host species eventually preferentially chose the new host species over the old host species, after a long enough acclimation period. Additionally, preliminary genetic work on the oligochaetes in our focal pond did not reveal differences among the oligochaetes on *Helisoma* and *Physa* (A. Smythe, *unpublished data*).

If there is a single *Chaetogaster limnaei* species, as we suspect, it is unclear why *Chaetogaster* strongly prefer their current host species. In previous studies on the host preferences of free-living *Chaetogaster* that were forcibly removed from hosts, researchers left the oligochaetes for several days in the choice chambers, which could have obscured any mortality of initial worms if there was rapid asexual reproduction by surviving worms (e.g., Hopkins et al. 2013). In this study, we did not give the oligochaetes a choice of hosts when we placed free-living *Chaetogaster* in the donor cups with the donor snails. And under those conditions, 50% of the initial *Chaetogaster* in the intraspecific H-H treatment died in the *Physa*-source experiment, and 21% of the total remaining *Chaetogaster* were recovered from the bottom of the donor cup, because the *Chaetogaster* probably never added to the donor *Helisoma* in the first place. We do not know why a large proportion of the oligochaetes did not add to the novel host species. But given that *Chaetogaster* have difficulty foraging and moving while free-living (e.g., Gruffydd 1965), which probably explains the high mortality risk associated with being free-living even under predator-free laboratory conditions (this study and Hopkins et al. 2015), there might be strong selection for affinity towards host-finding cues, so that *Chaetogaster* can rapidly relocate their host if they are accidentally dislodged. That strong affinity for the current host might mean that oligochaetes do not immediately recognize novel hosts as potential hosts. If so, this is an interesting example of symbiont life history tradeoffs, where short-term survival is favored over high interspecific transmission rates.

Physa snails were abundant, at least during part of the season, and interspecific contact rates were not rare in the laboratory contact rate experiment. In fact, at the average rate of ~2 interspecific contacts per 45-minute trial, the sentinel *Helisoma* snails in the field would have experienced hundreds of interspecific *Physa* contacts during a week-long enclosure trial. However, interspecific contact rates were still lower than previously observed intraspecific contact rates (Chapter 3), and that likely contributed to the relatively low interspecific transmission rates in the field. But because *Physa* tend to be more active than *Helisoma* snails, mass-action movement assumptions would predict interspecific contact rates should have been higher or equivalent to intraspecific contact rates, so why were interspecific contact rates lower? Given that densities of the two host species were comparable in the field and between laboratory experiments, we can rule out differences in host species density. And given that that the inter- and intraspecific encounter rates estimated by Holling Type II functional responses were roughly equivalent, we can rule out differences in the rate that *Helisoma* come into close proximity with other *Helisoma* versus *Physa* snails. Instead, what differed between the two models were the estimated handling time for an intraspecific (~4 minutes) versus an interspecific contact (~17 minutes), where longer handling times relative to the time available for contacts (45 minutes) caused interspecific contact rates to saturate more rapidly at a lower contact rate than intraspecific contacts. However, despite the differences in *estimated* handling times, the average durations of *observed* inter- and intraspecific contacts in our contact rate experiments were similar (~1 minute), and much smaller than the estimated interspecific handling time. This might suggest that *Physa* and/or *Helisoma* avoided interspecific contacts, causing overall handling times to be longer than actual contact durations (also see Chapter 3). Other aspects of snail behavior might also explain why mass-action movement assumptions could not predict why

Physa-Helisoma contact rates were relatively low, but contact avoidance is one particularly feasible explanation.

In this study, we quantified the relative contributions of intra- and interspecific transmission to infection risk in our focal host species (*Helisoma*), rather than the relative contributions of *Physa* and *Helisoma* to overall persistence of *Chaetogaster* within the host community (the basic reproductive rate, R_0). With respect to our focal species (*Helisoma trivolvis*), interspecific transmission rates were low and intraspecific transmission rates were high, and thus our system would be classified as a 'potential emerging infectious disease' within the Fenton and Pedersen (2005) community epidemiology framework. But the *Helisoma* snails living in our focal pond had high *Chaetogaster* prevalences and intensities for several years before our field study (S. Hopkins, unpublished data), so the 'potential' for *Chaetogaster* to emerge in this population was already met: either interspecific 'spillover' from *Physa* to *Helisoma* occurred previously, or exogenous *Chaetogaster*-infested *Helisoma* were introduced to the pond previously. Though we did not quantify whether transmission is symmetric in *Physa* and *Helisoma,* we predict that intraspecific *Physa-Physa* transmission rates are as high as intraspecific *Helisoma-Helisoma* transmission rates, and that interspecific *Helisoma-Physa* transmission rates are as low as interspecific *Physa-Helisoma* transmission rates, making *Chaetogaster* a facultative multi-host pathogen that could persist in the absence of either host species (*sensu* Fenton et al. 2015). Future work should more thoroughly evaluate the roles of all snail species to *Chaetogaster* persistence, especially with regards to whether the many rarer aquatic snail species that we did not study are only infested during spillover events (i.e., low intra- and interspecific transmission rates). Given the variation in *Chaetogaster* infestation prevalence and intensity among host species that have been documented from pond surveys (e.g.,

Ibrahim 2007), this system appears to be ideal for future exploration regarding the ecological conditions that favor 'disease' emergence.

By breaking the transmission process down into its component parts, we found that a host species that had the potential to form strong transmission bridges among species (super abundant and super infected) was not a major source of interspecific transmission because there was a strong barrier to transmission (super uninfectious). Similar orthogonal effects of contact rates, transmission success, and infection prevalence have been documented in other systems. For instance, invasive *Daphnia* were highly susceptible hosts for a fungal pathogen, which might best classify them as 'super infected,' but invasive *Daphnia* were too rare to amplify fungal infection in native *Daphnia* ('super rare'; Searle et al. 2016). In a different example, squirrels were abundant and heavily infested by ticks, but low transmission success of the Lyme spirochete from squirrels to ticks could dilute Lyme disease risk in communities with many squirrels (i.e., LoGiudice et al. 2003), demonstrating an opposing interaction between 'super abundant' and 'super uninfectious' characteristics. Similar studies that carefully parse the relative contributions of multiple host species and identify the characteristics causing these important or peripheral roles are still needed. As we continue to quantify the relative contributions of each host species to parasite maintenance in host communities, it is important to remember that host and parasite characteristics can interact to increase or decrease a host species' overall contribution to interspecific transmission.

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Table 4.1. Model output from the linear (density-dependent or frequency-dependent) and nonlinear multi-host force of infection models that were fit to the field enclosure and field survey data (top) and interspecific *Physa-Helisoma* contact rate models that were fit to the laboratory data (bottom), where β_{ii} is the relevant transmission rate, k_{ii} is the relevant density-dependent parameter, λ is the interspecific P-H contact rate, e is the interspecific encounter rate, T is the total time available for contacts, H is the interspecific contact handling time, and N is *Physa* density. The effective number of parameters is given by pD, and the end-points of the 95% credible intervals for the parameters are given on either side of the mean estimates.

Model		Parameter	2.5%	Mean	97.5%	pD	DIC
Field force of infection models							
Density dependent (k=1):	Intraspecific	β_{HH}	8.22E-06	1.09E-05	1.39E-05	1.5	42.1
	Interspecific	β_{HP}	2.94E-08	4.54E-07	1.22E-06		
Frequency dependent (k=0):	Intraspecific	β_{HH}	1.85E-05	2.75E-05	3.64E-05	1.1	52.5
	Interspecific	β_{HP}	9.49E-08	3.42E-06	1.12E-05		
Flexible nonlinear function:	Intraspecific	β_{HH}	9.44E-06	1.89E-05	2.60E-05	2.4	40.0
		k_{HH}	0.268	0.600	1.004		
	Interspecific	β_{HP}	3.74E-08	2.15E-06	7.83E-06		
		k_{HP}	0.017	0.451	1.275		
Laboratory contact rate models							
Density dependent:	$\lambda_{DD}=e_{DD}*T*N$	$1/\mu_{eDD}$	153.80	219.80	296.70	234.7	1608.4
Frequency dependent:	$\lambda_{FD}=e_{FD}*T$	$1/\mu_{eFD}$	21.73	30.27	40.35	231.9	1607.5
Holling Type II function:	$\lambda_{NL}=\frac{(e_{NL}*T*N)}{(1+e_{NL}*H*N)}$	$1/\mu_{eNL}$	41.97	61.14	106.60	198.0	1573.5
		H	12.570	17.040	21.440		

Figure 4.1. The observed weekly probability that an uninfected sentinel snail would become infected during each of the eight field enclosure trials, where the vertical bars delineate 95% asymmetrical binomial confidence intervals for each estimate (top; also see Chapter 3). The colored polygons show the best-fitting multi-host force of infection models while including estimate uncertainty for the interspecific transmission rate (β_{HP}) (Table 4.1). The models were fit to the eight FOI samples, but we show the predictions based on all eleven of the observed weekly densities of *Physa* and *Helisoma* snails (bottom).

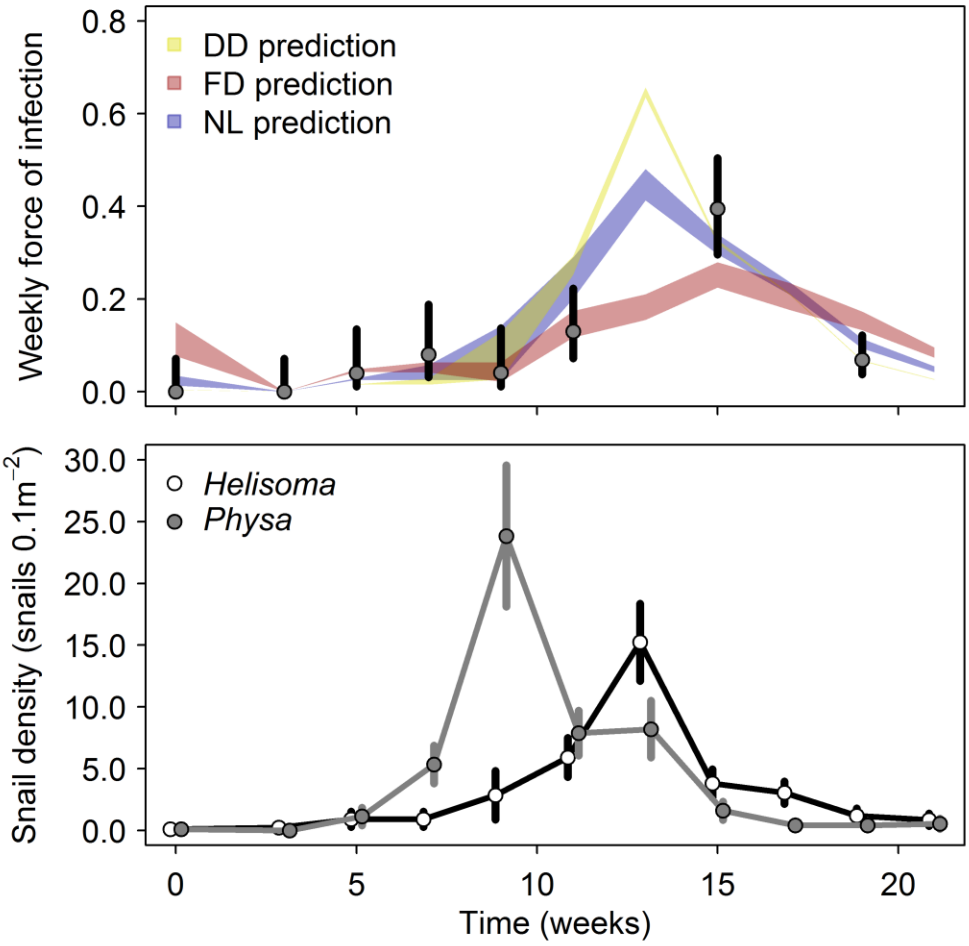


Figure 4.2. The mean observed number of contacts with *Physa* per *Helisoma* per tank during the laboratory contact rate experiment (top), where the vertical bars delineate 95% negative binomial confidence intervals for the means. Points are offset on the X axis to aid visualization. The colored polygons show the best contact rate models while including estimate uncertainty for the mean encounter rate (μ_e) (Table 4.1). The solid line is the best-fitting Holling Type II functional response for intraspecific contact rates that we previously published in Chapter 3.

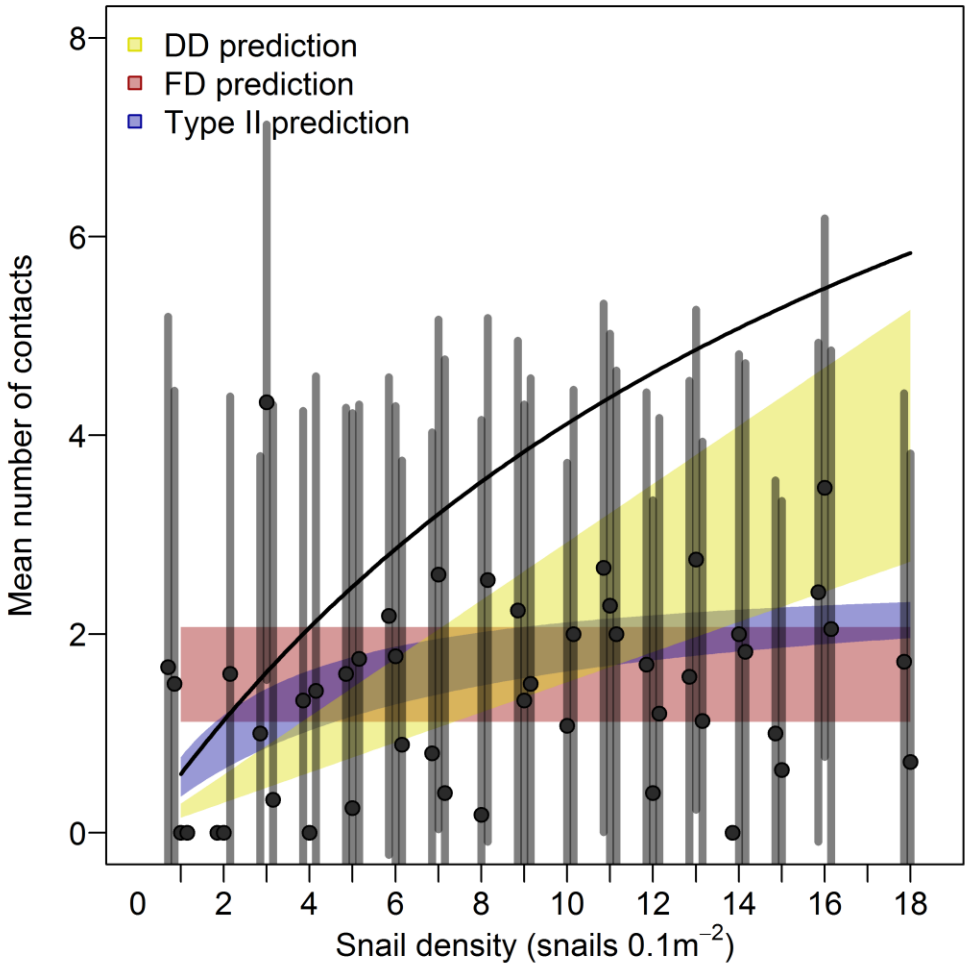
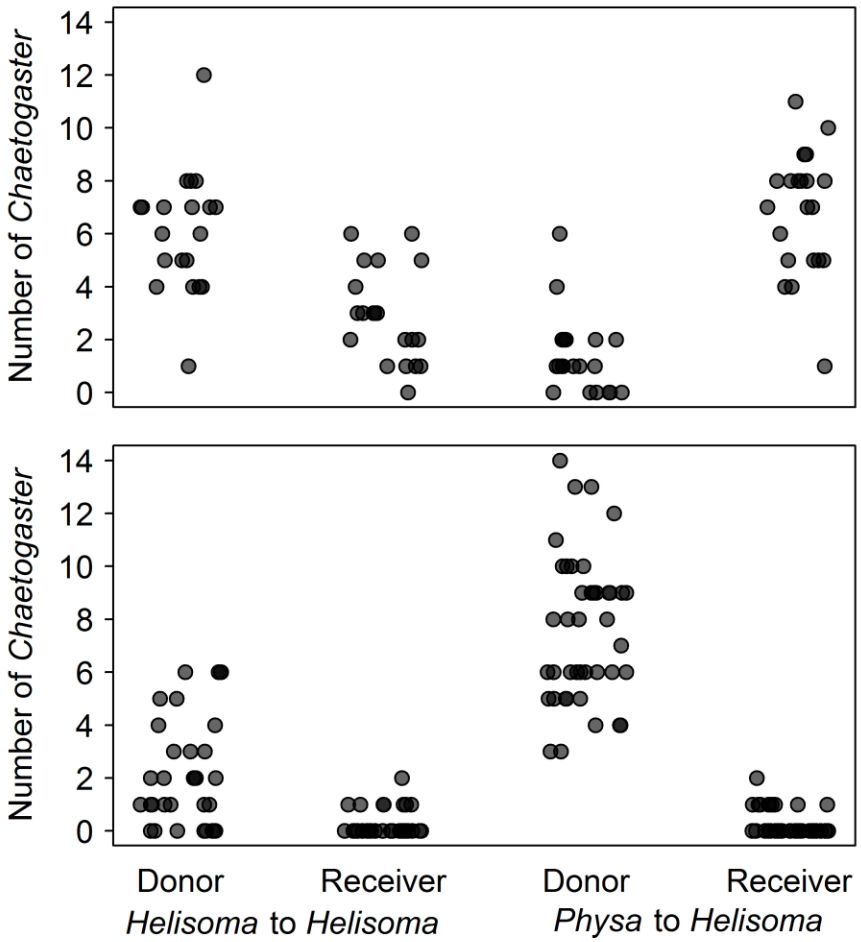


Figure 4.3. When *Chaetogaster* were sourced from *Helisoma* snails, there was more interspecific (*Physa-Helisoma*) than intraspecific (*Helisoma-Helisoma*) *Chaetogaster* dispersal from the donor to the receiver snails (top). In contrast, when *Chaetogaster* were sourced from *Physa* snails, there was less interspecific (*Physa-Helisoma*) than intraspecific (*Helisoma-Helisoma*) *Chaetogaster* dispersal, but in both cases dispersal rates were very low (bottom).



CONCLUSION

During the course of this dissertation research, untold numbers of parasites and pathogens (hereafter "parasite") species spread to new geographic regions or new host species, or switched from relatively benign endemic transmission dynamics to epidemic dynamics within their normal host communities (e.g., Daszak et al. 2000,Taylor et al. 2001, Jones et al. 2008, Wiethoelter et al. 2015). In wildlife, these emerging infectious diseases (EIDs) can be a threat to population persistence and biodiversity (De Castro and Bolker 2005, Smith et al. 2006), as we have seen with white nose syndrome in bats (Frick et al. 2015), chytridiomycosis in amphibians (Kilpatrick et al. 2010), and facial tumor disease in Tasmanian devils (McCallum et al. 2009). And spillover of parasites from wildlife can cause EIDs in human populations, as we have seen with rabies, highly pathogenic avian influenza, and Ebola (Jones et al. 2008, Wood et al. 2012, Liu et al. 2014). In this era of global change, where EIDs are expected to continue emerging at an increasing rate (e.g., Jones et al. 2008), we need epidemiological models to aid us in understanding, predicting, and managing the transmission of these important parasites. The same models can also help us to understand parasites and other symbionts that are not of conservation, medical, or economic concern, but are of interest due to their prevalent and important positions in ecosystems.

But 'transmission' is a multi-faceted process that is difficult to define and measure (e.g., Lello and Fenton 2017), and thus a difficult entity to study. Ecologists usually subsume many ecological and physiological processes into a single linear transmission parameter within epidemiological models (e.g., Lello and Fenton 2017, McCallum et al. 2017), with the hope that these simplifying assumptions will turn complex systems into tractable entities for study, without losing any vital real-world details. But "unpacking" the transmission rate can break a

complicated process down into measurable biological processes at discrete scales and create a more nuanced, mechanistic understanding of host-parasite systems (e.g., Civitello and Rohr 2014, McCallum et al. 2017). Therefore, the overarching goal of this dissertation work was to describe transmission dynamics at multiple scales, and then to identify and quantify how contact rates and transmission success rates work independently and collectively to influence net transmission dynamics at multiple scales.

Summary:

The goal of most epidemiological models is to use population-level average rates to describe and/or predict epidemic dynamics in single host and single parasite populations (e.g., Anderson and May 1991, Keeling and Rohani 2008). Therefore, my first chapter was a quantitative literature review of the transmission functions used in single host population-level models. I found that most published epidemiological models in the ecological literature assume that transmission is a simple linear function of host density, whereas most empirical studies have found that transmission is a nonlinear function of host density. I used model simulations to show that using simple linear transmission functions when the true underlying relationship is nonlinear can substantially bias parameter estimation and compromise understanding and predictions based on epidemiological models.

Though most empirically derived transmission-density relationships in my literature review were nonlinear (Chapter 1), few studies attempted to determine what caused the nonlinearities. This paucity of data is likely caused by the logistical and conceptual difficulties associated with measuring contact rates and transmission success in wildlife systems (McCallum et al. 2017). Therefore, in Chapter 2, I tested the mechanisms underlying nonlinear transmission functions in an empirically tractable system where ectosymbiotic oligochaete worms

(*Chaetogaster limnaei*) live on snail hosts. I found that *Chaetogaster* is primarily transmitted via direct snail contacts, revealing that the *Chaetogaster*-snail system is a good model system for testing the mechanisms underlying typical SIR-type epidemiological models. I then ran a variety of transmission success experiments to determine how individual-level host characteristics influence *Chaetogaster* transmission/dispersal. I found that transmission success varied among host snails according to snail size and infection status, and this variation is one individual-level mechanism that could explain why *Chaetogaster* transmission rates were nonlinear functions of density in the field (see next - Chapter 3).

In Chapter 3, I used a combination of field surveys, field enclosure trials with sentinel hosts, and mathematical modeling to describe the nonlinear relationship between intraspecific *Chaetogaster* transmission and intraspecific host density in the field. I then used a laboratory contact rate experiment to show that intraspecific contact rates were also nonlinear functions of host density. Contact rates saturated with host density because each contact required a nonzero handling time, and during that time snails missed out on other contact opportunities. This individual-level behavioral mechanism could be modeled using the Holling Type II functional response, and helped to explain the nonlinear population-level transmission-density relationship observed in the field.

After I 'unpacked' population-level transmission dynamics into individual-level mechanistic processes that likely acted together to produce nonlinear transmission-density relationships in Chapters 2-3, I took this same approach to a higher level of ecological organization to describe the mechanistic underpinnings of interspecific or community-level transmission in the *Chaetogaster*-snail system in Chapter 4. I found that even though *Physa* snails are abundant, have frequent contacts with *Helisoma* snails, and have a high prevalence of

Chaetogaster infestation, there is little to no *Chaetogaster* transmission from *Physa* to *Helisoma* snails in the field. Instead, strong *Chaetogaster* host preferences cause very low interspecific transmission success, negating any characteristics that might cause *Physa* to have high interspecific transmission rates.

Conclusions:

Identifying which host species have high versus low intraspecific and/or interspecific transmission rates is important for predicting the spread and maintenance of parasites in host communities (Fenton and Pedersen 2005, Streicker et al. 2013), and thus recent theoretical frameworks have been proposed to aid these endeavors (Fenton et al. 2015). But determining *why* intra- and interspecific transmission rates are high or low is equally important (e.g., Streicker et al. 2013), and this has rarely been accomplished because quantifying contact rates and transmission success is typically logistically infeasible (e.g., Fenton et al. 2015). Taken together, this dissertation research quantified net intra- and interspecific transmission, and then unpacked those processes into their mechanistic components to understand why intraspecific transmission was a saturating function of intraspecific density (i.e., lower than predicted at high densities) and why interspecific transmission rates were nearly zero. Because contact rates and transmission success rates are multiplied together in the transmission function, these processes can act additively or orthogonally to affect net transmission dynamics. Correspondingly, saturating contact-density relationships caused by contact handling times and heterogeneity in transmission success both could have explained the nonlinear intraspecific transmission-density relationships observed in the field, and the two mechanisms probably acted together to create the observed nonlinearity. In contrast, low interspecific transmission success rates opposed frequent interspecific contacts and high infection prevalence to create low net interspecific transmission

rates. Unpacking transmission in this way resulted in one of the most detailed empirical studies of transmission dynamics in a wildlife system, and yielded many surprising new insights into *Chaetogaster* ecology that would not have been discovered with a purely phenomenological or holistic view of transmission.

Throughout this dissertation, I critically evaluated existing modeling practices that have been used extensively and with great success in the ecological and epidemiological literature for decades. In some cases, I believe that existing practices could be substantially compromising understanding and prediction or reproducibility; for instance, most studies do not clarify whether their models tracked host densities or numbers, or whether study areas were constant (Chapter 1). However, I do not mean to imply that all simple linear transmission functions and/or all phenomenological treatments of the transmission rate are detrimental to the field. Dynamic species interactions models require a three-way trade off between model generality, realism, and precision (Levins 1966), and generality/simplicity is typically favored over biological realism and precision during the early stages of model development and when available data are too limited to add in additional complexity. As we continue to accumulate large, high-quality datasets, and as we work towards improving the predictive capabilities of epidemiological models, 'unpacking' transmission rates and adding heterogeneity and nonlinearity to models will become increasingly important (Buhnerkempe et al. 2015, Lello and Fenton 2017, McCallum et al. 2017). Still, the simplest models proposed by epidemiology giants like Anderson and May (e.g., Anderson and May 1991) will always have a place in the field.

Future directions:

Most ecological studies of host-parasite systems focus on one host species and one parasite species, whereas most parasites infect multiple host species (Buhnerkempe et al. 2015).

Understanding how parasites are spread and maintained in multi-host species remains a pressing challenge (Dobson 2004, Buhnerkempe et al. 2015). In this dissertation, I quantified intraspecific *Helisoma-Helisoma* transmission and *Physa-Helisoma* transmission, which was a good start, but a great deal more empirical work waits within the *Chaetogaster*-snail system. For starters, it would be illuminating to determine whether transmission is symmetrical in this system; is interspecific *Helisoma-Physa* transmission as low as *Physa-Helisoma* transmission, as strong host preferences would predict? Additionally, I only focused on the two most common snail species in southwestern Virginia, but *Chaetogaster* infests upwards of 15 known snail genera in 10 families (Smythe et al. 2015), and it is still unclear which species are most important for maintenance of *Chaetogaster* in larger multi-host communities. Are the rarer species only infected during spillover events, or do they play important roles as *Chaetogaster* reservoirs? This common, experimentally-tractable study system could still provide many more novel insights into the factors that determining which host species serve as key host species and why.

In addition to focusing on single host-parasite pairs, most ecological studies – including ecological studies of infectious diseases – also focus on a single scale, ignoring or phenomenologically-treating dynamics at higher and lower scales (Johnson et al. 2015). In this dissertation work, I considered transmission from the individual to community-level scales, obtaining good breadth across levels of the ecological hierarchy. But I limited my field work to a single pond, so there might be important dynamics at the landscape level that my work did not capture. Additionally, though I have referred to my host preference studies as focusing on 'individual-level' host characteristics, I actually considered the slightly higher-level of 'functional group' organization by lumping snails of similar size or infection status together and working with their group means (Chapter 2). A great deal more work could be undertaken at the

true individual-level; for instance, contact networks and more nuanced transmission success studies might better explain why some snails have many *Chaetogaster* while most have low infestation intensities, leading to a system where some snails are likely *Chaetogaster* 'superspreaders' (e.g., Lloyd-Smith et al. 2005).

Today, the *Chaetogaster*-snail system is poorly known, despite being extremely cosmopolitan and easy to work with. I hope that this dissertation work not only advances our understanding of epidemiological models, but also encourages other researches to consider working in this valuable and experimentally-tractable system.

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