Epidemic dynamics in metapopulations: The role of heterogeneity in the dissemination of disease

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ABSTRACT

Understanding infectious disease dynamics is of fundamental concern to human health and conservation, particularly in the context of globalization, habitat fragmentation and climate change. Although connectivity may increase species persistence over a wider metapopulation, it may also allow for the spread and persistence of parasites, either by introducing them to new, susceptible populations or providing an influx of susceptible hosts to an endemic population. It is therefore important to understand how heterogeneity among populations may impact host-parasite relations. A large body of theoretical and observational work on this topic exists, but laboratory experiments are still lacking. This thesis uses the well-studied model system of guppies and their ectoparasite, *Gyrodactylus turnbulli* to investigate how heterogeneity at multiple levels of interest may influence host-parasite dynamics in metapopulations using a series of laboratory experiments bridged with theoretical work. It specifically examines three types of heterogeneity at the individual, population and metapopulation level that may affect parasite dynamics at those levels and demonstrates theoretically the importance of heterogeneity in individual resistance.

At the metapopulation level the effect of variability in initial parasite distribution among tanks and the presence or absence of connectivity on host and parasite outcomes was investigated using isolated tanks into which either a high or low number of parasites were introduced, and groups of tanks connected by host migration into which a high number of parasites were introduced to one tank or a low number of parasites were introduced to all tanks. It was found

that, in accord with basic metapopulation and epidemiolocal theory, connectivity increased parasite persistence in metapopulations compared to isolated tanks. It was hypothesized that a concentrated (high loads into one tank) parasite introductory distribution would further prolong parasite persistence in a metapopulation by forcing asynchrony in dynamics among subpopulations, however this effect was not detected, indicating that connectivity is the most important factor to parasite persistence. Importantly an interactive effect of connectivity and parasite load on host outcomes was detected: at low parasite introductory loads, connectivity had no impact on parasite mean intensity, however at high loads connectivity lowered the mean intensity, indicating an importance of considering parasite distribution when determining disease mitigation and conservation strategies.

Moving one level down, heterogeneity at the population level was investigated specifically in the form of sex and sex ratio and group size. As guppies are sexually dimorphic, whether a difference in resistance exists between the sexes and whether that difference impacts parasite dynamics in single-sex or mixed sex populations was of interest. It was found that females endured higher parasite loads than males in isolation, and that parasites persisted for longer in groups than on isolated fish, regardless of whether they were all-male, all-female or mixed-sex, again highlighting the importance of connectivity to disease persistence. Again, an important interactive effect of heterogeneity and connectivity was observed, as no difference in parasite burdens was observed among groups, indicating a benefit of connectivity for female hosts.

At the individual level, data from the first metapopulation experiment were re-examined in the

context of individual host competence, and the role that heterogeneity in migrating individuals may have on disease incidence. Fish were categorized as having intense infections, prolonged infections, both or neither, and it was found that fish with prolonged infections, regardless of parasite intensity were responsible for the most transmission, indicating that tolerance may be an important and overlooked mechanism in the spread of infectious disease and that individual heterogeneity in resistance and tolerance can impact metapopulation outcomes.

Finally, data obtained from these experiments were used to develop a mathematical model that describes this system. The model can also be used to predict longer-term dynamics and to estimate the effects of various parameters that we have not been able to effectively test in the laboratory, as well as identify parameters whose heterogeneity have the greatest impact on host-parasite dynamics through sensitivity analysis. Outbreak peak magnitude and timing were most sensitive to parameters relating to host resistance and parasite virulence, indicating that heterogeneity in these traits has the potential to impact epidemic dynamics. Ideally, this model can be applied to other directly transmitted, directly reproducing parasites for which parasite burden impacts host-parasite relations.

Together, these projects offer further insight into epidemic dynamics in metapopulations and the role of host heterogeneity and connectivity in the dissemination of disease. They particularly highlight the importance of scale when investigating these effects, since important effects at one scale may sometimes be undetectable at another or may interact with and influence higher-level dynamics.

RESUME

Comprendre les dynamiques des maladies infectieuses représente un enjeu central pour la santé humaine et la conservation, particulièrement dans le contexte de la globalisation, la fragmentation des habitats et les changements climatiques. Bien que la connectivité puisse favoriser la persistance des espèces à travers une plus grande méta-population, cela peut également faciliter la propagation et la persistance des parasites, que ce soit en les introduisant dans de nouvelles populations susceptibles ou en offrant un influx d'hôtes susceptibles à une population endémique. Il est donc important de comprendre comment l'hétérogénéité entre les populations peut influencer les relations hôte-parasite. Une quantité appréciable d'études théoriques et observationnelles existent sur ce sujet; toutefois, les expériences en laboratoire sont très limitées. Cette thèse se concentre sur un système d'étude bien établi, les guppies et leurs ectoparasites (*Gyrodactylus turnbulli*), pour investiguer comment l'hétérogénéité à plusieurs niveaux d'intérêt peut influencer les dynamiques hôte-parasite dans les méta-populations à partir d'une série d'expériences en laboratoire ainsi qu'un modèle théorique. Cette thèse examine spécifiquement comment l'hétérogénéité à trois niveaux d'organisation (c-à-d, au niveau de l'individu, de la population et de la méta-population) peut affecter les dynamiques de parasites et démontre théoriquement l'importance de l'hétérogénéité de la résistance individuelle.

Au niveau de la méta-population, les effets de la variabilité de la distribution initiale des parasites entre les habitats (aquariums) et de la présence ou absence de la connectivité entre les hôtes ou les parasites ont été investigués. Pour ce faire, nous avons utilisé des aquariums isolés à

l'intérieur desquels un nombre faible ou élevé de parasites ont été introduits; des groupes d'aquariums ont également été connectés soit par la migration d'hôtes sur lesquels un nombre élevé de parasites ont été introduits dans un aquarium, ou par l'introduction d'un faible nombre de parasites dans tous les aquariums. Conformément aux théories de base pour les dynamiques de méta-populations et en épidémiologie, nous avons trouvé que la connectivité favorisait la persistance des parasites dans les méta-populations comparativement aux aquariums isolés. Nous avons émis l'hypothèse qu'une introduction concentrée de parasites (charge élevée de parasites dans un aquarium) pourrait davantage prolonger la persistance des parasites dans une méta-population en désynchronisant les dynamiques entre les sous-populations. Toutefois, cet effet n'a pas été détecté, suggérant ainsi que la connectivité est le facteur le plus important pour déterminer la persistance des parasites. Un effet interactif entre la connectivité et la charge de parasites sur l'état des hôtes a cependant été détecté : à une charge faible de parasites introduits, la connectivité n'avait pas d'impact sur l'intensité moyenne des parasites, mais à charge élevée, la connectivité diminuait l'intensité moyenne. Ces résultats suggèrent qu'il est important de prendre en compte la distribution de parasites lorsque la migration de maladies et les stratégies de conservation sont discutées.

L'hétérogénéité au niveau de la population a été investiguée spécifiquement en ce qui a trait aux sexe, ratio de sexe et taille du groupe. Considérant que les guppies sont sexuellement dimorphiques, la question à savoir s'il existe une différence de résistance entre les sexes et si cette dernière influence les dynamiques des parasites dans les populations à un seul ou plusieurs

sexes est particulièrement d'intérêt. Nous avons trouvé que les femelles toléraient une charge plus élevée de parasites que les mâles en isolation, puis que les parasites résistaient pour une plus longue période sur les poissons au sein des groupes que sur ceux isolés, indépendamment du fait qu'ils soient tous des mâles, femelles ou de sexe mixte. Ceci met encore une fois l'emphase sur l'importance de la connectivité concernant la persistance des maladies. Ici aussi, un effet interactif important entre l'hétérogénéité et la connectivité a été observé, avec aucune différence observée dans les infections parasitaires entre les groupes, suggérant que la connectivité favorisait les hôtes femelles.

Au niveau individuel, les données de la première expérience de méta-populations ont été ré-examinées dans le contexte de la compétence des hôtes individuelles ainsi que le rôle que l'hétérogénéité des individus migrateurs peut avoir pour l'incidence des maladies. Les poissons ont été catégorisés selon leur type d'infection : intense, prolongée, intense et prolongée ou aucun des types précédents. Nous avons trouvé que les poissons avec les infections prolongées, indépendamment de l'intensité des parasites, étaient responsables pour la plupart de transmission, indiquant que la tolérance peut être un mécanisme important, bien qu'inconsidéré, pour la propagation des maladies infectieuses et que l'hétérogénéité individuelle de la résistance et la tolérance peuvent influencer les méta-populations.

Finalement, les données obtenues à partir de ces expériences ont été utilisées afin de développer un modèle mathématique pour décrire ce système et faire des prédictions. Sur la base d'une analyse de sensibilité, ce modèle peut notamment être utilisé pour projeter les dynamiques à long

terme, estimer les effets de paramètres qui n'ont pas été testés dans nos expériences en laboratoire, de même que pour identifier les paramètres dont l'hétérogénéité pourrait influencer les dynamiques hôte-parasite. La magnitude et le moment du point culminant de l'épidémie étaient les aspects les plus sensibles aux paramètres liés à la résistance des hôtes et la virulence des parasites, suggérant que l'hétérogénéité dans ces traits a le potentiel d'influencer les dynamiques épidémiques. Idéalement, ce modèle pourrait être appliqué à d'autres parasites à transmission directe et reproduction directe pour lesquels le fardeau parasitaire pourrait affecter les relations hôte-parasite.

Cette collection d'études offre une perspective nouvelle sur les dynamiques épidémiques dans les méta-populations ainsi que sur le rôle de l'hétérogénéité et la connectivité dans la dissémination des maladies. En son tout, cette thèse souligne l'importance de l'échelle dans l'étude des effets de l'hétérogénéité et de la connectivité puisque ceux observés à une échelle peuvent parfois être indétectables à d'autres, tout en pouvant influencer ou interagir avec les dynamiques à des niveaux d'organisation plus élevés.

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PREFACE

THESIS FORMAT

This manuscript-based thesis is composed of a general introduction, followed by four manuscripts—all of which have been published, or are in review for publication in peer-reviewed journals—and a general discussion. The thesis conforms to the Thesis Specifications and Thesis Formatting Guidelines of McGill University. The corresponding bibliographic information for the manuscripts is as follows:

Chapter 2:

Tadiri, C.P., Scott, M.E, Fussmann, G.F. 2018 "Microparasite dispersal in metapopulations: A boon or bane to the host population?" *Proceedings of the Royal Society B: Biological Sciences* 285 (1885), 20181519.

Chapter 3:

Tadiri, C.P., Scott, M.E., Fussmann, G.F. 2016. "Impact of host sex and group composition on parasite dynamics in an experimental metapopulation." *Parasitology* 143 (4), 523-531.

Chapter 4:

Tadiri, C.P., Scott, M.E., Fussmann, G.F. "Parasite spread in experimental metapopulations: Resistance, tolerance and host competence" *In review: Journal of Animal Ecology.*

Chapter 5:

Tadiri, C.P.*, Kong, J.D.*, Scott, M.E., Fussmann, G.F., Wang, H. 2019. “A data-validated host-parasite model for infectious disease outbreaks.” *Frontiers in Ecology and Evolution* 7, 307.

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CONTRIBUTION TO ORIGINAL KNOWLEDGE

This body of work makes several contributions to knowledge, in the form of both original findings and methodological innovation. To my knowledge, this work is the first to use experimental metapopulations of vertebrates and to conduct a multilevel analysis of experimental epidemic dynamics, thus representing an original experimental design and method for Chapters 2 and 4. Each chapter also includes original key findings: Chapter 2 expands on the generally accepted idea that connectivity increases parasite persistence in the metapopulation by demonstrating an interactive effect of parasite introductory distribution and connectivity. I demonstrate that connectivity lowers parasite mean intensity but only when parasites are introduced at high levels, thereby indicating that whether hosts or parasites benefit from connectivity may be a function of initial parasite distribution among sub-populations. Chapter 3 broadens our understanding of sexual dimorphism in resistance to infection in guppies, by showing that there is an interaction with grouping that impacts parasite dynamics as females in isolation had more parasites than males, but their burden was lowered by being in a group while males did not benefit from grouping. Chapter 4 demonstrates that infection duration at an individual level is crucial to spreading and maintaining parasite metapopulations, implicating the importance of tolerance, a factor often overlooked experimentally in vertebrates and in the context of host competence. Chapter 5 improves upon previous attempts to mathematically model this system by incorporating a specific deterministic equation for parasite population size and an equation for host immune response with distributed delay to more accurately describe the waning of host immunity and indicates that heterogeneity in parasite virulence and host

resistance would have the greatest influence on outbreak time and intensity compared to other parameters for both initial and long-term dynamics. Together, these manuscripts advance our understanding of host-parasite dynamics.

CONTRIBUTION OF AUTHORS

Each chapter was written by me, with editing input from my supervisors/co-authors and based on experimental work and analysis that I performed myself. I break down in detail the division of labour for each project and resulting manuscripts below, particularly for the final, collaborative manuscript for which I share first authorship with Dr. J.D. Kong.

Experimental design for Chapters 2-4 was developed by CPT, GFF and MES. CPT conducted all experimental data collection and lab maintenance of fish and parasites for Chapters 2-4. CPT wrote the first drafts of manuscripts for Chapters 2-4 with editing input from GFF and MES. For Chapter 4, the mathematical model was developed at a meeting between CPT, JDK, GFF and HW. CPT collected the data (either experimentally or from published literature) used for parameter estimates and model fitting, while JDK validated the model and conducted the sensitivity analysis. CPT wrote the initial draft of the manuscript, with editing input from all co-authors. This manuscript was not used as a chapter in JDK's doctoral thesis.

1 CHAPTER 1: GENERAL INTRODUCTION

My objective is to investigate how ecological dynamics interact at multiple scales by determining how heterogeneity may impact epidemic dynamics in metapopulations using the host-parasite relationship between the guppy and *Gyrodactylus* spp. as a model system. I specifically examine three types of heterogeneity at three different levels of analysis that may impact resistance to parasites and therefore host-parasite dynamics: connectivity within the metapopulation and initial distribution of parasites throughout the metapopulation, sex and sex-ratio and group size, and individual host competence, and how these factors influence parasite dynamics at the metapopulation, population and individual level, respectively.

1.1 HOST-PARASITE DYNAMICS IN METAPOPULATIONS

Human society and health are currently at the best, and worst of times. People are living longer than ever before, and many infectious diseases that used to wipe out populations are now preventable or curable thanks to modern advances in medicine and technology. However, these advances are also coupled with increases in climate change, habitat degradation/fragmentation, globalization and broader social networks, all of which have been shown to contribute to an increase in disease transmission and to emergence and re-emergence of infectious diseases (1-6). Thus, although we now know more than ever about infectious disease prevention and treatment, it is also more important now than ever to understand disease dynamics, particularly in the context of changing landscapes and connectivity.

Generally, habitat connectivity among populations to form a metapopulation is seen as an

advantage to species conservation (7, 8), particularly in the context of preserving populations fragmented or degraded due to human activity. Connectivity of patches and migration among populations may provide refuge for individuals and thus promote species persistence over a larger metapopulation (7, 9). However, dispersal among patches may also act as an agent for disease transmission and/or persistence, either through continued introduction of susceptible hosts to infected populations or the introduction of parasites to naïve populations (10, 11). Though disease transmission throughout metapopulations has been modeled (10-12), as well as the role of spatial heterogeneity among patches and individual hosts (13-15), few studies have looked at the role of host heterogeneity in host-parasite metapopulation dynamics, and there is a need for more experimental work to better understand these theoretical dynamics.

1.2 Conceptual Mechanisms of Host-Parasite Dynamics

According to Anderson and May's (1979) definition, all infectious disease-causing agents can be considered as pathogens, and they can be generally classified as either microparasites or macroparasites. Microparasites are microscopic, such as viruses, protozoa and bacteria. They replicate rapidly to high numbers within a host, are often transmitted directly between hosts or via a vector, and typically produce some form of lasting immunity (16). Macroparasites tend to be larger, such as nematodes, tapeworms, trematodes and parasitic arthropods, and often have more complex life cycles involving different stages of development in multiple host species or free-living stages. Macroparasites usually have a longer generation time than microparasites, and rarely elicit lasting immunity (16). For macroparasites, intensity of infection—the number of

parasites infecting a host (17)—may also play a large role in dynamics, since they are often quantifiable. Microparasites on the other hand are often difficult to quantify and infection intensity is often assumed to be irrelevant and the host infection status is most important. Due to these various differences in characteristics, disease dynamics tend to differ between microparasites and macroparasites, with microparasites tending to cause periodic epidemics and macroparasites tending to persist endemically (16, 18), making this distinction helpful to understanding disease dynamics in host populations.

Within a host population infected with a microparasite, hosts are typically classified as susceptible, infected or recovered individuals (SIR) (19-21). The most important factor to epidemic spread is the transmission rate, the rate at which susceptible individuals become infected. For directly transmitted diseases (those with no vector or intermediate host), transmission is a function of the probability of contact between a susceptible and infected individual and the probability of that contact resulting in parasite establishment on a new host. The probability of contact between infected and susceptible individuals is mostly based on demographics, most importantly the host population density (19) and the proportions of susceptible, infected, and resistant individuals in a population (16, 20, 21), but host and/or parasite behaviours may also promote or prevent contact in some cases (22-24). Parasite establishment (17) on a new host is determined by the interplay between the pathogen's ability to infect (infectivity) and host's susceptibility to infection (25), traits that may co-evolve over generations (26-29). Changes in current environmental conditions may also influence gene

expression for parasite reproduction (and therefore virulence) or host susceptibility or resistance to infection (30, 31).

Resistance, the inverse of susceptibility, is a host's ability to resist parasite infection. It can be reflected in terms of preventing parasite establishment entirely or limiting parasite growth and clearing infection rapidly and is therefore an important factor to determining infectious disease dynamics (32). Resistance may be innate, as demonstrated in an individual's ability to fight initial infection or acquired post-infection as is the case for many microparasite infections that induce lasting immunity (26, 29, 33). Due to the presumed costs associated with investing in immunological defences (34, 35), a wide variability in resistance to parasites may exist within a host population (25, 36). Understanding how heterogeneity in host resistance and other population-level factors may impact parasite establishment and growth is therefore important to understanding host-parasite dynamics.

Heterogeneity in resistance is known to play an important role in metapopulation disease dynamics in plants (36-38), but few studies have experimentally examined this relationship with vertebrates. In this thesis I specifically examine how host heterogeneity, and its relation to parasite resistance impacts parasite dynamics at a larger, multilevel scale using the Trinidadian guppy-*Gyrodactylus turnbulli* host-parasite system as a model system. Although numerous studies have examined how ecological and evolutionary factors may contribute to an individual's resistance to parasites in this system (29, 39-41), few have examined how this heterogeneity among hosts impact parasite dynamics at a larger, population or metapopulation-level scale.

1.3 THE GUPPY-*GYRODACTYLUS* SYSTEM AS A MODEL FOR EPIDEMIC BEHAVIOUR

Guppies (*Poecilia reticulata*) are a common ovoviviparous tropical fish that, due to their rapid generation time, have served as a model species in numerous eco-evolutionary studies (42). Due to variation among their natural habitats, particularly the presence of predators and availability of food, guppies exhibit a wide range in several life-history traits, many of which have been shown to evolve rapidly (43, 44). Guppies also vary significantly in their resistance to *Gyrodactylus* spp. infection (39, 45, 46), and some studies have shown *Gyrodactylus* spp. may exert selective pressure on guppies (47, 48) and that guppies may rapidly evolve increased or decreased resistance to parasites (45, 49).

Although *Gyrodactylus* spp. are flatworms, and therefore technically macroparasites in the most traditional sense of this classification, they behave more like microparasites. They are able to rapidly reproduce on the host, their infections are generally short-lived, they are directly transmitted by contact, and usually induce some host immunity; thereby causing epidemics but offering the advantage that they can be quantified, making them an ideal model species for microparasite epidemiology (50). Due to the numerous conveniences of working with both species, considerable research has been done using the guppy-*Gyrodactylus* system as a model for epidemic behaviour. Intrinsic population dynamics of *Gyrodactylus* sp. on isolated fish have been identified under standardized environmental conditions (51, 52). Both long and short-term dynamics of *Gyrodactylus* sp. within laboratory populations of guppies have also been observed, finding oscillating cycles of infection based on the temporary refractory periods of the fish and

the rate of introduction of new susceptible hosts (53, 54), that parasites tend to be over-dispersed in a host population and that aggregation may also oscillate over time (53, 55), and that parasites may persist at low levels in a populations without going extinct due to the host refractory period (56). However, investigation of the influences of population connectivity and host heterogeneity upon these population dynamics has been limited, which obscures our ability to fully understand host-parasite dynamics.

1.3.1 GUPPY BIOLOGY

Guppies are native to Trinidad, Venezuela and Guyana, although they have been introduced worldwide (57). They have been found in a wide range of freshwater habitats, and exhibit tolerance for pollution in some (42). Their diet largely consists of unicellular algae, benthic invertebrates and zooplankton (58). They are also well-known to exhibit density-dependent cannibalism of young (59). Their abundance, hardiness and ubiquitous distribution have made them ideal subjects for research in various disciplines.

Guppies exhibit strong sexual dimorphism, with males being much smaller than females and brightly coloured. Though guppy morphology and life-history traits vary considerably with ecological conditions (44), averages for the wild populations are described as follows: mature adults range in size from about 13mm to 20mm in length (60). After a gestation period of about 25 days, adult females give birth to around 2-8 fry per brood (60). Growth and maturation takes about 1.5-3 months, after which guppies may live for up to 3 years in the laboratory, giving birth to a large number of broods (61). This rapid reproduction rate makes guppies an excellent model

species, and they are used particularly often in evolution research.

1.3.2 *GYRODACTYLUS* BIOLOGY

Gyrodactylus spp. (Monogenea) are ectoparasitic flatworms which feed on the epithelial cells and mucus of many marine and freshwater teleost fish species (50). They attach to the epidermis of their host via specialized hooks and are directly transmitted primarily by jumping to a new host during skin-to-skin contact (54, 62). Some laboratory studies have suggested that they may also be transmitted from dead fish, or if dislodged may float to the surface of the water and reattach to new hosts when fish come up to feed (22), but this mechanism seems unlikely in the wild with running water. *Gyrodactylus* spp. have evolved host-specificity among the broad range of teleost fish that they infect, and are primarily identified by the size and shape of their attachment hooks (50, 62). *Gyrodactylus bullatarudis* and *Gyrodactylus turnbulli* are the two most prevalent *Gyrodactylus* species that infect *P. reticulata* in the wild (63). More recent work has also demonstrated evidence for cryptic speciation of *G. bullatarudis* and recorded *Gyrodactylus poeciliae* in wild guppies (64). Although it has been demonstrated in the laboratory that *G. turnbulli* and *G. bullatarudis* are capable of infecting other teleost species when directly transferred to a new host (65, 66), they are considered to be specific to guppies as other species are not known to sustain or transmit them.

Gyrodactylus spp. are viviparous, with an unusual method of reproduction: the developing embryo contains within itself a second developing embryo which also contains a third-generation embryo, which allows for rapid population growth of the parasite on an infected host (50, 62).

Both their rapid generation and their external location on the fish make them an ideal subject for research, as infection can be monitored in a non-invasive manner and information can be gathered over a relatively short period of time (50, 54).

Upon establishment, *Gyrodactylus* spp. elicit several responses in guppies. Physical manifestations of infection begin with mucus secretion. This non-specific immunological reaction is thought to physically aid in the shedding of ectoparasites, by making attachment more difficult and by displacing the parasites as they move around on the fish (67). It also aids in defence at the molecular level, containing a non-specific immune complement protein which kills gyrodactylids (50, 68-70). Weight-loss, often severe if the infection persists long-term or if the infection intensity is high, can often occur (*pers. obs.*). Fins often clamp shut, which causes guppies to exhibit a characteristic "shimmying" swimming pattern due to constrained fin movement (22, 71). In extreme cases, fins (particularly the caudal fin) and the epidermis may deteriorate, resulting in increased susceptibility to secondary fungal or bacterial infections (68). Behavioural changes also take place, such as changes in food intake and frequency of sexual displays (39, 72, 73).

Infection-induced mortality in laboratory-reared guppies is estimated at about 0.7% per day, nearly twice the estimated background mortality rate in the laboratory of about 0.4% per day (54). In wild guppies, infection has also been shown to cause severe mortality particularly for males, with a 19% decrease in recapture rate with each additional parasite observed on a fish (47). Mortality of wild-caught guppies in laboratory infections has been as high as 70-100%,

depending on the population of fish and the strain of *Gyrodactylus* sp. used (41). On fish that survive infection, infections usually last for about 11.5 days, during which *Gyrodactylus* sp. grow at an exponential rate until a peak where the fish immune system is able to overcome infection, at which point intensity drops dramatically (39, 54). Upon recovery, guppies will exhibit resistance to further infection for a period of about 4-6 weeks, with immunity gradually waning over time (52, 56).

1.4 METAPOPULATION HETEROGENEITY IN CONTEXT

The nature through which parasites spread throughout a host population exhibits many parallels to traditional metapopulation theory (74) where the host is the equivalent of a patch. *Gyrodactylus* spp. in particular provide a good example of these parallels in that parasite population growth and persistence is dependent on host ("patch") size and quality (41, 75), migration to another patch is determined by host rate of contact (connectivity) (76), overall parasite population is dependent on the number of patches (76), and patches may appear (birth or immigration) or disappear (death or emigration). Also important is the concept that *Gyrodactylus* spp. are able to persist in a host population, periodically causing epidemics, due to fluctuations in the availability of naïve or susceptible hosts, despite local extinction of infected hosts. Although it has been shown that immigration of new susceptible hosts is necessary for *Gyrodactylus* spp. persistence in a local host population (53), and some field studies have shown that parasite burden may influence individual guppy migration (47), the effects of host dispersal or heterogeneity on disease dynamics at the metapopulation level have not been examined in this

host-parasite system.

Previous work with this system has shown that many factors may affect an individual's resistance to parasites, for example larger fish tend to harbour more parasites (75), females tend to have more parasites than males (49, 77), resistance may vary among populations due to differing evolutionary pressures (39, 45) and genetic MHC profiles (78). However, few have expanded these factors to the larger population- or metapopulation-level dynamics. Given the structure of guppy populations in the wild, and the ubiquity of migration and rapid evolution among these populations (42, 43), it is important to acknowledge the potential of these factors to influence host-parasite ecology on a broader scale and longer time-period.

In the wild, guppies inhabit streams which are punctuated by "pools" separated by waterfalls, thus creating a network of populations among which only unidirectional migration of hosts (and potentially parasites) downstream is possible (47, 79). I recreated a modified situation in a laboratory setting, setting up tanks of fish and establishing unidirectional but looped migration among them, manipulating specific population structural characteristics such as connectivity and introductory parasite distribution. By controlling dispersal among populations, as well as other environmental (such as temperature and humidity, water quality and food supply and the removal of all other species in the community) and demographic variables (such as initial population size and density, and the number of parasites introduced), I was able to determine the influence of our variables of interest on host-parasite dynamics in the multilevel context of a metacommunity where parasites infect individual guppies (host patch) that are themselves grouped into

subpopulations that are connected to the larger metapopulation through dispersal.

1.5 CHAPTER OVERVIEW

In this thesis, I investigate different forms of heterogeneity and their impacts on metapopulation epidemic dynamics in guppies using a multilevel and integrative approach. In Chapter 2, I first explore the metapopulation concept in the context of a host-parasite system and examine how connectivity and heterogeneity in parasite introductory intensity and initial distribution influence temporal heterogeneity and metapopulation epidemic dynamics by comparing parasite dynamics in connected versus unconnected systems. Here, spatial heterogeneity is incorporated at the system scale via initial parasite burden and distribution, with the intention of forcing asynchrony or synchrony within metapopulations. In Chapter 3, I then explore within-population heterogeneity and investigate how a simple host characteristic (sex) influences host susceptibility to parasites at the individual level by determining whether males or females experience infection differently, and at the population level by determining whether those differences still exist when grouped and how sex ratio in a host population influences parasite population dynamics. In Chapter 4, I explore the ways in which individual host heterogeneity may be driving broader metapopulation epidemic dynamics by identifying more competent hosts (those with a higher propensity to transmit infection) and which characteristics they share, particularly in the form of resistance (the ability to avoid/clear infection) and tolerance (the ability to withstand infection), and the extent to which they are responsible for metapopulation outcomes. Finally, in Chapter 5, I develop a mathematical model in collaboration with Dr. Hao Wang and his former student Dr.

Jude Kong combining their expertise in mathematical modelling with our extensive knowledge of this host-parasite system. We use the data collected from my experiments to train and test the model and conduct a sensitivity analysis to predict the factors for which variability may have the greatest influence on dynamics, in order to inform future work. This model will help to better describe our system and predict future parasite behaviour in this and other similar systems and contribute to a better understanding of disease dynamics and offer more concrete predictions than models based on observational data alone. Together, this research contributes to a better understanding of host-parasite dynamics across metapopulations based on heterogeneity at multiple levels of analysis.

1.6 REFERENCES

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2 CHAPTER 2: MICROPARASITE DISPERSAL IN METAPOPULATIONS: A BOON OR BANE TO THE HOST POPULATION?

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2.1 ABSTRACT

Although connectivity can promote host species persistence in a metapopulation, dispersal may also enable disease transmission, an effect further complicated by the impact that parasite distribution may have on host-parasite population dynamics. We investigated the effects of connectivity and initial parasite distribution (clustered or dispersed) on microparasite-host dynamics in experimental metapopulations, using guppies and *Gyrodactylus turnbulli*. We created metapopulations of guppies divided into four subpopulations and introduced either a low level of parasites to all subpopulations (dispersed) or a high level of parasites to one subpopulation (clustered). Controlled migration among subpopulations occurred every 10 days. In additional trials, we introduced low or high levels of parasites to isolated populations. Parasites persisted longer in metapopulations than in isolated populations. Mortality was lowest in isolated populations with low level introductions. The interaction of connectivity and initial parasite distribution influenced parasite abundance. With low level introductions, connectivity helped the parasite persist longer but had little effect on the hosts. With high levels, connectivity also benefited the hosts, lowering parasite burdens. These findings have implications for disease management and species conservation.

Keywords: Metapopulation ecology, asynchrony, parasite distribution, host-parasite dynamics, guppies, *Gyrodactylus*

2.2 INTRODUCTION

Infectious disease (1) and habitat fragmentation (2) both contribute to species decline and therefore have important implications for conservation. Although connectivity and migration among populations promote species persistence in metapopulations (3, 4), dispersal made possible by connectivity may also act as an agent for disease transmission, either through continued introduction of susceptible hosts to infected populations or introduction of parasites to naïve populations (5-7). Therefore, connectivity may also negatively impact host survival, if pathogens are present within the system. Despite a rich theoretical literature on disease dynamics in metapopulations (5, 8-10), few empirical studies test the theoretical predictions (11, 12). To better understand the impacts of host population connectivity on host-parasite dynamics, laboratory experiments that manipulate connectivity are necessary.

Across a metapopulation, asynchrony in population dynamics among patches may further prolong species persistence by reducing the risk of extinction overall (4, 13). For parasitic infections, particularly those that confer immunity, asynchrony in dynamics can be a major contributor to prolonging parasite persistence by allowing parasites to thrive in some patches despite the presence of resistant hosts in others (14). Therefore, within a metapopulation context, the way in which parasites are initially distributed across host populations may impact parasite dynamics.

We set out to determine the impacts of connectivity on parasite-host dynamics, ultimately asking whether connectivity benefits hosts, parasites or both. To tailor this question to different initial

parasite distributions, we additionally considered the effects of either clustered, high local abundance infection where parasites were introduced into a single host sub-population at high levels, or dispersed, low local abundance infection where parasites were introduced evenly to host sub-populations at low levels. To answer these questions, we used the guppy-*Gyrodactylus turnbulli* host-parasite system (15).

Gyrodactylus spp. are monogenean ectoparasites of fish that reproduce rapidly on the surface of the fish via polyembryony. Transmission occurs through direct skin-to-skin contact between live hosts (16). They have no free-living stage, though detached *Gyrodactylus* sp. may survive for up to 12 hours (15) and can potentially reattach to a host (17). Gyrodactylids have been shown to cause severe disease and high mortality rates in aquaculture settings (18, 19), and in the laboratory (20, 21). However, some hosts are able to clear their infection, after which they are refractory to reinfection until their immunity wanes (22-24). Gyrodactylids persist in the wild at low levels periodically causing epidemics with fluctuations in the number of susceptible, infected and recovered hosts in the population (25). They can be counted without destructive sampling of the host and their numbers can be tracked on individual hosts over time (15, 26), making them convenient subjects for the study of host-parasite metapopulation dynamics.

Guppies (*Poecilia reticulata*) are a common freshwater fish and the specific host of *Gyrodactylus turnbulli* (25). Although it has been shown that immigration of susceptible hosts into laboratory guppy populations is necessary for gyrodactylid persistence (15), and that in the field, downstream guppy dispersal is more likely for heavily infected guppies (27), the effects of

host dispersal on disease dynamics at the metapopulation level have not been examined in this host-parasite system. Also, the impact of initial infection burden of *Gyrodactylus* spp. on parasite dynamics on individual guppies has been investigated (28), but not the impact of initial introduction levels or distribution on host-parasite dynamics within a metapopulation.

The goal for this experiment was to test the effects of connectivity and the initial parasite distribution throughout connected metapopulations on parasite persistence, host-parasite population dynamics, and host mortality. We predicted that parasites would persist longer in connected metapopulations than in isolated control populations, as movement through the metapopulations would provide the parasite with access to susceptible fish. We also predicted that focal introduction of parasites into only one subpopulation rather than simultaneous introduction into all the subpopulations would further prolong parasite persistence in the metapopulation by forcing asynchrony in local parasite dynamics among tanks. Finally, we hypothesized that parasite abundance would reach higher peaks in tanks into which more parasites had been initially introduced, resulting in higher host mortality, and that this would be more evident in isolated compared to connected populations because parasites would be unable to spread out and would thus be constrained to a local population.

2.3 METHODS

2.3.1 EXPERIMENTAL DESIGN

This experiment consisted of two types of metapopulations, each containing four tanks of eight fish with two distinct starting conditions: either two parasites introduced into each tank at the

same time (low/dispersed parasites, connected) or eight parasites introduced into only one of the four tanks (high/clustered parasites, connected). Every 10 days, one fish was haphazardly selected from each tank in these connected metapopulations and moved to the next tank in a unidirectional loop (A→B→C→D→A). A diagram of the experimental design can be found in supplemental materials (Figure 2.4). The 10-day interval was chosen to coincide with anticipated major epidemic parameters such as peak prevalence and abundance after 10 days, the end of the epidemic after 20 days (29), and the waning of most acquired immunity after 40 days (22). To control for connectivity, we also included two types of isolated tanks which were not part of any metapopulation (no dispersal was applied), into which either two (low parasites, isolated), or eight (high parasites, isolated) parasites were introduced. The full experiment was replicated four times, in a total of five blocks of trials due to manpower constraints.

2.3.2 BACKGROUND

Guppies were purchased from a Montréal pet store and brought to aquariums in a McGill University laboratory maintained at 26 +/- 1 ^{o}C and a 12-hour light-dark cycle where they were bred for two generations. Upon receipt, fish had low levels of infection (less than 20 parasites on about 20% of the fish), and parasite transmission continued in our breeding stock. *Gyrodactylus turnbulli* were obtained from an infected pet store guppy and cultured in the laboratory by infecting one naïve guppy with one parasite and routinely adding naïve fish. This parasite culture has been maintained for several years and identified as *G. turnbulli.*

Only adult male guppies were used for the experiment. Fish (mean weight 0.125g, standard

deviation 0.043) were haphazardly selected from our breeding stock tank, assigned to groups of 8 and placed in 6L tanks in an Aquaneering, Inc. (San Diego, California, U.S.A.) flow-through system. As transmission rates are affected by host density (26), it is important to note that this density of fish is higher than wild populations (30), but lower than in commercial guppy populations (31), and similar to those used in laboratory epidemic experiments (29, 32). Prior to the experiment, fish were treated twice at a one-week interval with 25g/L salt water for 15 min (33) to eliminate *Gyrodactylus.* They were then anaesthetized in 0.02% tricaine methanesulfonate (MS-222) buffered to a neutral pH with sodium bicarbonate (Sigma-Aldrich, Darmstadt, Germany) and scanned using a dissecting microscope with a cold light-source to confirm the absence of *Gyrodactylus.* Fish were maintained in their group tanks for 8 weeks to ensure that all fish had overcome the refractory period to any potential prior *Gyrodactylus* infection (22, 23, 34). Tanks were haphazardly assigned to one of the four experimental groups. Every day throughout the experiment, fish were fed a controlled amount of TetraMin© Tropical Flakes (Tetra Werke, Melle Germany) mixed with water into a paste that was distributed through a glass precision syringe to each tank.

2.3.3 EXPERIMENTAL PROTOCOL

One week before parasite introductions, anaesthetized fish were injected with visible implant elastomer dye (Northwest Marine Technologies, Shaw Island, WA, U.S.A.) for unique identification. On day 0 of the experiment, all fish were anaesthetized, weighed to 0.001 grams, and measured to 0.1mm. Fish were infected by removing a scale from a heavily infected donor

fish and placing it on the recipient fish until the parasites had transferred to the new host (29, 35). Two parasites per fish was chosen as the infection dose to increase the probability of parasite establishment in the tank and still allow detection of changes in the initial population growth rate over the first several days (28).

Each fish was then anaesthetized and scanned for parasites every other day. Movement in the connected metapopulations occurred after parasites had been counted, according to experimental design. Dead fish were not replaced to avoid altering dynamics by introducing naïve fish. If all fish in a tank died, the tank was left as an unoccupied "patch" that would be "recolonized" by a fish and potentially parasites at the next 10-day interval. Metapopulation trials lasted 120 days (three full cycles of dispersal) or until no parasites were found in any connected tanks for two consecutive counts. Isolated tanks were monitored until no parasites were found for two consecutive counts.

2.3.4 Statistical Analysis

Analyses were conducted at the system level; thus our experimental units were metapopulations and isolated tanks. For each metapopulation, the aggregation of parasites among the four connected tanks was calculated using the variance to mean ratio of the total parasites per tank on each day. For each metapopulation and isolated tank, parasite prevalence (number of infected hosts per total number of hosts) was also calculated for each day. The total duration of parasite persistence in the metapopulation and isolated tank, host mortality (the proportion of fish that died in the metapopulation or isolated tank), the maximum total number of parasites in the

metapopulation or isolated tank, the daily and peak mean abundance (total number of parasites per fish in the metapopulation or isolated tank), and peak prevalence were recorded over the course of the experiment.

All analyses were performed in R v. 3.2.2 (36). To assess the effect of connectivity (connected vs. isolated), parasite introduction (low/dispersed vs. high/clustered) and their interaction on our system-level response variables (persistence, mortality, peak total parasites and peak mean abundance) we used generalized linear mixed-effect models (GLMM) (function *glmer*, package *lme4*), with the block in which a trial was run treated as a random effect, the interaction of connectivity and parasite introduction as a fixed effect, and with different error distributions depending on the nature of the data. We simplified full models first by removing the interaction term if not significant, then each term (connectivity or parasite introduction) individually, comparing AICs with the full model at each step to find the model with the lowest AIC. Absolute goodness-of-fit of the minimal models was assessed as R^2, by calculating the correlation between the observed and fitted values, squared.

Different response variables had different error distributions given the aggregated nature of parasite load among hosts, and the binomial nature of mortality, therefore different error distributions were applied to our models for each response variable. For overall parasite persistence in a system, we used a GLMM with a Poisson error distribution. For mortality, we used a GLMM with a binomial error distribution. For the peak total number of parasites, we used a GLMM with a negative binomial error distribution. For peak mean abundance, we used a

GLMM with a negative binomial error distribution.

Reported results are means associated with standard error. α was set at 0.05. We report the R^2 value of each minimal model and the *P*-value of significant variables.

2.4 RESULTS

Minimal models and their outputs are summarized in supplemental materials (Table 2.2). Within each replicate, fish size did not significantly differ among treatments ($p>0.05$ for all comparisons of weight and standard length among treatments). In all tanks, parasite populations increased and spread throughout the host population, reaching at least one distinct population peak. In all our initially clustered metapopulations, the dispersal of an infected fish to a naïve tank resulted in parasite populations establishing in the new tank, generating asynchrony among tanks during the first 30 days (Figure 2.1). All 8 isolated tanks (both high and low introductions) reached 100% prevalence, two of the four metapopulations with a dispersed parasite introduction reached 100% prevalence (with the other two reaching a maximum of 37.5% and 96.8%), and none of the metapopulations with a clustered introduction reached 100% prevalence (76.9%, 84.3%, 76.1% and 86.3%). For both clustered and dispersed metapopulations, the variance to mean ratio of parasites among tanks reached high levels and fluctuated over time in a similar manner, despite different initial values (Figure 2.2).

No interactions of connectivity and parasite introduction were detected for parasite persistence or host mortality. Connectivity influenced parasite persistence ($p<0.001$, model $R^2=0.9$), with parasite populations lasting an average of 87±13 days in connected metapopulations, compared

to 45±5 days in isolated tanks (Figure 2.3). Parasite introduction (p=0.001) and connectivity (p=0.009) influenced host mortality (model R^2=0.84) with high initial levels leading to greater mortality (61±8%), than low ones (34.8±11%), and isolated tanks having lower mortality (43.7±11%) than connected metapopulations (51.9±10%).

The interaction between connectivity and parasite introduction influenced the peak total number of parasites in the system (p=0.0085, model R^2=0.38), being lower in isolated tanks with low parasite introductions (209±91) compared to all other treatments (dispersed metapopulation: 1412±716; clustered metapopulation: 1376±202; high parasite isolated tank: 1822±486). Both the interaction of connectivity and parasite introduction (p=0.02) and connectivity alone (p=0.04) influenced peak mean abundance (model R^2=0.55). Isolation lowered parasite peak mean abundance at low parasite introductions but increased it at high parasite introductions: the highest mean abundance was observed in high parasite introduction isolated tanks (270±83), followed by high/clustered (71.4±17.8) and low/dispersed (75.3±45) metapopulations which did not significantly differ, and finally low parasite introduction isolated tanks (33.7±14.4) (Figure 2.3).

2.5 DISCUSSION

Consistent with our hypothesis, parasites persisted longer in metapopulations than isolated tanks. These results are also consistent with basic metapopulation (4, 37, 38) and epidemiological theory (7, 39, 40) as well as predictive models of parasites within a metapopulation context (9, 41). However, to our knowledge, this study is the first to demonstrate this pattern experimentally

in vertebrate hosts, in a setting where infection levels on all individuals and subpopulations were quantified and tracked.

Contrary to our expectations, initial parasite distribution did not impact parasite persistence in our metapopulations. We hypothesized that focal introduction of the infection into a single subpopulation would prolong persistence by forcing asynchrony in epidemic dynamics among tanks (14, 42-44), leading to higher numbers of susceptible hosts in the metapopulation at any given time (compared to a metapopulation with initially dispersed parasites, where hosts would be expected to transition from infected to refractory in a more synchronous manner). However, we did not observe significant differences in parasite persistence between our clustered and dispersed metapopulations. Although we observed asynchrony during the first 30 days in the clustered metapopulations, thereafter parasite variance to mean ratios among tanks were similar, which might explain why parasite persistence did not differ. Furthermore, *Gyrodactylus* are often aggregated on individual hosts within the population (45), which in itself is a type of within-population asynchrony in parasite dynamics that could have obscured an effect of asynchrony on persistence. We also observed that aggregation among tanks within metapopulations established shortly after introductions, even when the original parasite introductions were dispersed. Therefore, the only real factor influencing how long gyrodactylids could be sustained was available resources, in this case the number of potentially susceptible hosts to which they had access. Although parasite introduction did not impact persistence within metapopulations, it had a major impact in isolated tanks and on other parameters.

Host mortality and parasite peak loads were higher when more parasites were introduced into a single tank. It makes sense that introducing a higher number of parasites into the tank would lead to higher numbers of parasites per fish and host death, because higher parasite loads increase likelihood of mortality (15, 27, 46, 47). Consistent with this hypothesis, more fish died in both high parasite isolated tanks and clustered metapopulations compared to those in low parasite isolated tanks. Parasite peak was lowest in low parasite isolated tanks but did not differ among our other three treatments despite differences in connectivity or introduction. In response to *Gyrodactylus* sp. infection, fish exhibit both a physical response of mucus production (48), which is thought to cause parasite shedding (49) and a non-specific complement that kills gyrodactylids (16, 50-52). It is possible that if the parasite initial load is high, parasite numbers will increase to fatal levels before the fish immune system responds, whereas at low numbers, the fish has time to mount a reaction and slow/eventually stop parasite population growth, as response time can vary under different conditions (53), and ability to resist parasites can vary among individuals based both on genetic background and past exposure (34, 46, 51, 54, 55). Our results indicate that high initial infection leads to worse host outcomes overall, because the two treatments in which high numbers of parasites were introduced experienced the higher mortality and parasite loads compared to treatments where parasites were introduced at low levels. At these high burdens we also saw that connectivity modulated these effects.

Perhaps our most intriguing result is that the impact of connectivity differed depending on initial parasite burden: when parasites were introduced at low levels, being in a metapopulation helped

the parasite (persist longer) but had little/no effect on host outcomes (parasite burden). However, when introduced at high levels, being in a metapopulation also benefitted he hosts, since they had lower parasite burdens than when the parasites were unable to disperse. Peak parasite load and mean abundance were influenced by the interaction of connectivity and level of parasite introduction, with isolation lowering peak parasite loads per fish when parasites were introduced at low levels but increasing them when parasites were introduced at high levels. We expected, and previous studies in similar systems have shown, that the number of available hosts significantly impacts the parasite population size (56). However, peak parasite numbers were equally high in our clustered metapopulations, dispersed metapopulations and high introduction isolated tanks (which all began with the same total number of parasites), while the number of potential hosts (connectivity) influenced the mean parasite load per fish. Mean abundance was highest when high numbers of parasites were introduced to isolated tanks, and lowest when low numbers were introduced to isolated tanks. In clustered metapopulations the ability to move and spread parasites to other/naïve fish lowered the average burden per host. However, no difference in peak burden was observed between low-parasite isolated tanks and dispersed metapopulations, both of which began with two parasites per tank. This result highlights the importance of studying both hosts and parasites in a metapopulation context.

We identify several strengths and limitations of this study. To our knowledge this study is the first to experimentally test the impacts of connectivity and initial parasite distribution on vertebrate-parasite metapopulation dynamics. We also conducted four replicates of simulated

metapopulation treatments that showed similar and repeatable dynamics. One potential limitation is that dispersal was controlled experimentally, and despite efforts to haphazardly select the dispersing fish, differences in behaviour (shyness/boldness) or health (sicker fish may be slower and therefore easier to catch) may have influenced which fish was selected. However, it has been shown in the wild that sicker fish may also be the ones more likely to disperse downstream (27). Another potential limitation is that we used laboratory, rather than wild-caught fish and parasites for this experiment. Both fish resistance and parasite virulence are likely to differ in laboratory compared with wild populations given wide variability in resistance that has been reported among wild populations (20, 34), presumably due to different selective pressures (47). However, our purpose was to provide a general model for this host-parasite system, rather than to directly compare our results with any specific wild population.

This experiment provided lab-based evidence that both hosts and parasites can coexist in and benefit from a metapopulation setting, and that the initial distribution of parasites among subpopulations in a metapopulation may determine whether the hosts benefit from connectivity. This finding is of particular importance when species conservation and disease management collide: conservation often emphasizes use of corridors to facilitate species persistence over a patchy landscape whereas disease management often focuses on transmission interruption through methods such as quarantine (5). It is also important to recognize that environmental conditions (57, 58), heterogeneity among individuals (29, 32, 46) and other factors may influence disease spread throughout metapopulations. Further investigation under a wider range

of conditions and with a diversity of host-parasite systems will be useful in better informing management practices.

2.6 Ethics Statement

Approval for animal care and research was obtained from the McGill University Animal Care Committee (AUP 2014-7547) in compliance with the Canadian Council on Animal Care.

2.7 Data Accessibility

The primary data set supporting this manuscript and a short readme file explaining each column is available in Dryad database (doi:10.5061/dryad.1d9r423).

2.8 Competing Interests

We have no competing interests.

2.9 Authors' Contributions

All authors contributed to the design of the study. CPT collected and analysed data. CPT wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

2.10 Acknowledgments

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2.13 FIGURES

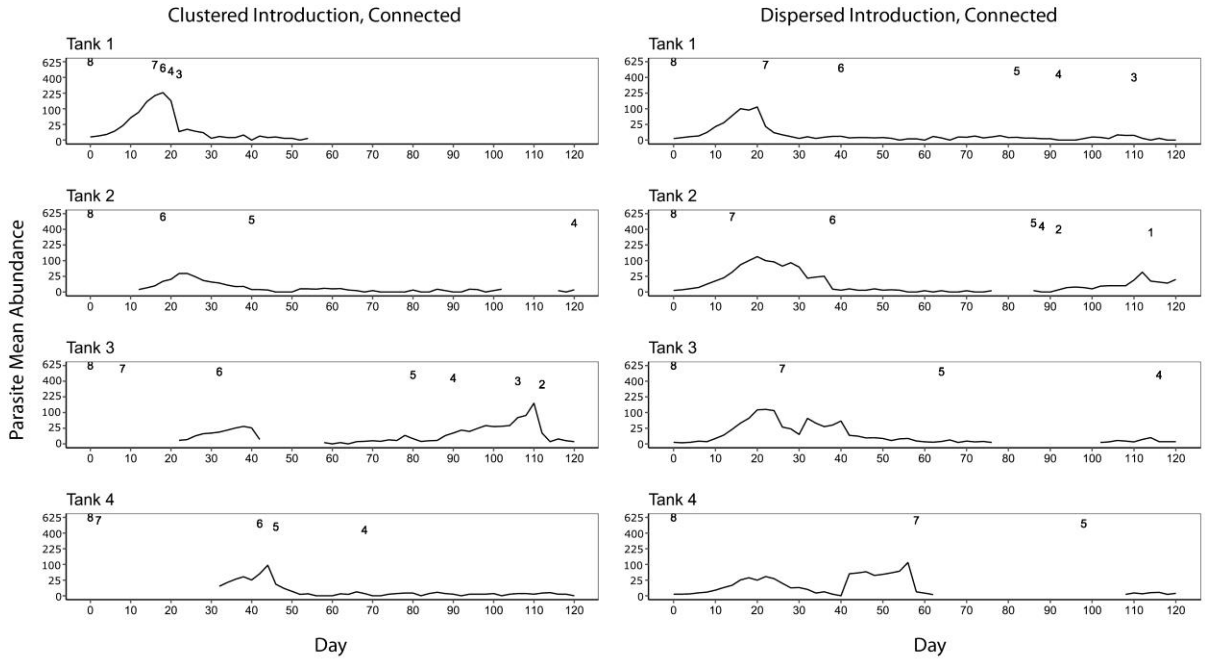

Figure 2.1: Mean Parasite Abundance over Time

Data (square-root transformed for graphing purposes) from one representative replicate of the High Parasite Clustered Metapopulation (left) and Low Parasite Dispersed Metapopulation (right). Fish numbers are recorded when they change across the top of each graph. Note that single instances of zero-values may not represent true zeros for the parasite population, as detection is not perfect, and fish may be cryptically infected from which parasite populations may resurge. Zero-values for two or more consecutive counting days are assumed to be true zeros.

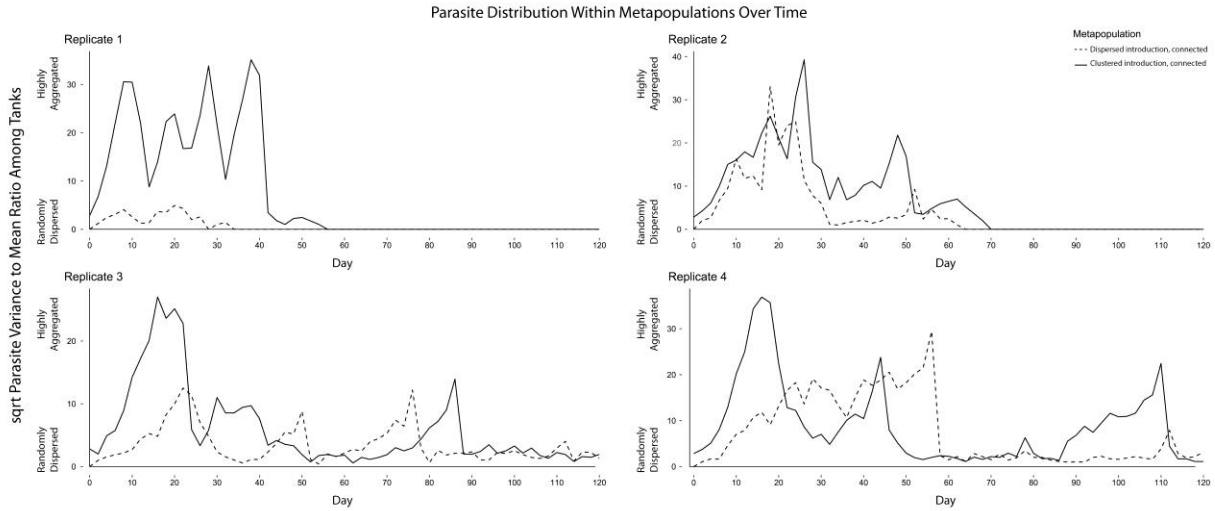

Figure 2.2: Variance to Mean Ratio of Total Parasite Numbers among Tanks
Each replicate set of clustered (bold) and dispersed (dashed) metapopulations, square-root transformed for graphing purposes.

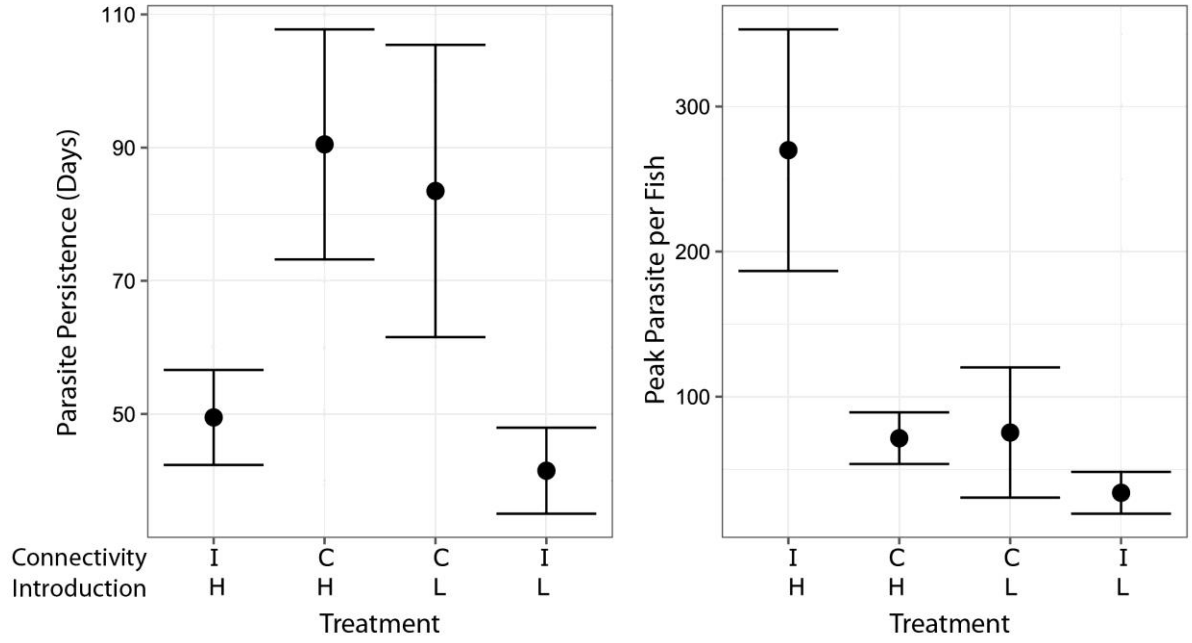

Figure 2.3: Effects of Connectivity and Parasite Introduction ("C" = connected, "I" = isolated) ("H" = high/clustered, "L" = low/dispersed) on parasite persistence (left) and mean parasite abundance per host (right).

2.14 SUPPLEMENTAL MATERIALS

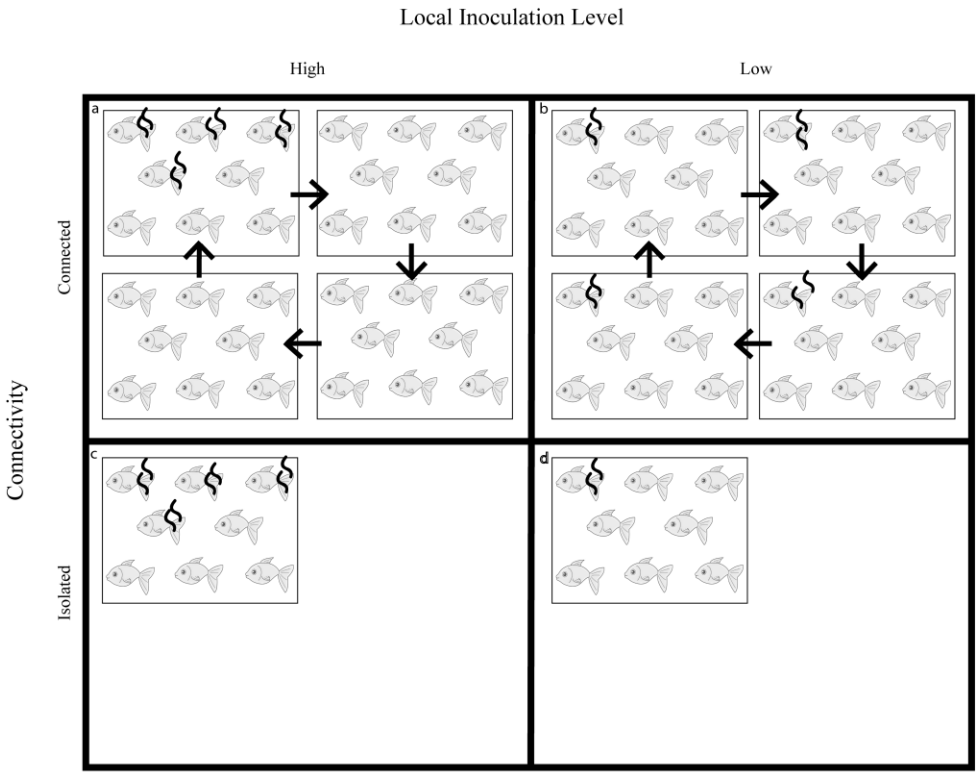

Figure 2.4 Diagram of Experimental Design

Table 2.1: Results of Epidemic Parameters by Treatment

All values are means associated with standard errors

	Parasite Persistence (days)	**Host Mortality (% dead)**	**Peak Total Parasite Load**	**Peak Parasite Mean Abundance**
High Introduction Isolated	49.5 (±7.13)	62.5 (±11.41)	1822 (±485.69)	269.94 (83.28)
Clustered Metapopulation	90.5(±17.27)	59.36 (±18.8)	1375.75 (±201.91)	71.36 (17.8)
Dispersed Metapopulation	83.5 (±21.94)	44.5 (±15.75)	1412.5 (±715.82)	75.28 (±44.9)
Low Introduction Isolated	41.5 (±6.44	25.0 (±15.3)	209.5 (±90.83)	33.75 (±44.92)

Table 2.2: Output of Generalized Linear Mixed-Effects Models

Minimal generalized linear mixed effects models for all response variable (persistence, mortality, peak total parasites, peak parasite mean abundance. Models differed in their error distributions (shown in table). R^2 noted at top of model with variable. All models included the trial replicate/block as a random effect.

Persistence; Error Distribution: Poisson; Model R^2:0.92				
	Estimate	**Standard Error**	**Z**	**P**
Connectivity (Isolated)	-0.8	0.08	-9.15	<0.001 ***
Mortality; Error Distribution: Binomial; Model R^2: 0.84				
	Estimate	**Standard Error**	**Z**	**P**
Parasite Intro (Low)	-0.86	0.26	-3.27	0.001**
Connectivity (Isolated)	-1.21	0.47	-2.6	0.009**
Peak Total Parasites; Error Distribution: Negative Binomial; Model R^2: 0.38				
	Estimate	**Standard Error**	**Z**	**P**
Parasite Intro (Low)	0.03	0.59	0.05	0.96
Connectivity (Isolated)	0.28	0.59	0.48	0.63
*Interaction (Connectivity*Intro)*	-2.19	0.8	-2.63	0.009**
Peak Parasite Mean Abundance; Error Distribution: Negative Binomial; Model R^2:0.55				
	Estimate	**Standard Error**	**Z**	**P**
Parasite Intro (Low)	-0.05	0.65	0.082	0.93
Connectivity (Isolated)	1.33	0.65	2.04	0.04 *
*Interaction (Connectivity*Intro)*	-2.13	0.92	-2.3	2

CONNECTING STATEMENT 1

In this next chapter, I further explore the role of connectivity and asynchrony due to heterogeneity in guppy-*Gyrodactylu*s metapopulations, this time at a smaller scale. In Chapter 2, connectivity among host subpopulations played a large role in persistence of parasites within the metapopulation compared to parasite persistence in isolated host populations. In Chapter 3, I move one level down, to explore connectivity among individual hosts. I compare parasite population dynamics between isolated populations of hosts, each of the same size as the groups from Chapter 2, and isolated individual host patches.

The experiment described in Chapter 3 used F3 lab-reared fish from wild populations as opposed to the previous study which used feeder guppies. Fish were bred keeping track of maternal lines and assigned to tanks so that maternal lines and population of origin were evenly distributed among treatments. Maternal line/population of origin did not significantly impact population-level outcomes and was removed from analysis. To avoid effects of gestation on health, fish were sexed before reaching maturity and isolated by sex so that only virgin females were used in this experiment.

Both of these experiments explore how the addition of multiple patches, either in the form of individual hosts or groups of hosts further prolongs parasite persistence, consistent with basic metapopulation and epidemiological theory. They also investigate the interaction of connectivity with heterogeneity, by comparing the effects of heterogeneity on isolated and connected patches. Together these two experiments explore various forms of heterogeneity and asynchrony at

multiple levels of analysis, further contributing to our understanding of host-parasite dynamics.

3 CHAPTER 3: THE IMPACT OF HOST SEX AND GROUP COMPOSITION ON PARASITE DYNAMICS IN AN EXPERIMENTAL METAPOPULATION

Tadiri, C.P., Scott, M.E., Fussmann, G.F. 2016. "Impact of host sex and group composition on parasite dynamics in an experimental metapopulation." *Parasitology* 143 (4), 523-531.

3.1 SUMMARY

To better understand the spread of disease in nature, it is fundamentally important to have broadly applicable model systems with readily available species which can be replicated and controlled in the lab. Here we used an experimental model system of fish hosts and monogenean parasites to determine whether host sex, group size and group composition (single-sex or mixed-sex) influenced host-parasite dynamics at an individual and group level. Parasite populations reached higher densities and persisted longer in groups of fish compared with isolated hosts and reached higher densities on isolated females than on isolated males. However, individual fish within groups had similar burdens to isolated males regardless of sex, indicating that females may benefit more than males by being in a group. Relative condition was positively associated with high parasite loads for isolated males, but not for isolated females or grouped fish. No difference in parasite dynamics between mixed-sex groups and single-sex groups was detected. Overall, these findings suggest that while host sex influences dynamics on isolated fish, individual fish in groups have similar parasite burdens, regardless of sex. We believe our experimental results contribute to a mechanistic understanding of host-parasite dynamics, although we are cautious about directly extrapolating these results to other systems.

Keywords: epidemic dynamics, host-parasite dynamics, guppies, *Gyrodactylus*

3.2 KEY FINDINGS

- *Gyrodactylus* populations reached higher densities and persisted for longer on groups of guppies compared to isolated guppies

- Host relative condition and sex influenced *Gyrodactylus* dynamics on isolated fish
- *Gyrodactylus* populations reached higher densities on isolated females than on isolated males
- Unexpectedly, neither sex nor relative condition had an impact on parasite dynamics for grouped fish
- Mixing male and female fish had no impact on parasite dynamics on groups of guppies

3.3 INTRODUCTION

Infectious diseases are important drivers of ecological interactions and evolution (1, 2), and are of general concern in the context of disease mitigation and conservation biology (3-5). Traditional microparasite (SIR) models focus on infectious disease from the host point of view by dividing hosts into susceptible, infected and recovered sub-populations (6-10). Although these models effectively describe epidemics/epizootics of those microparasites for which population size per host is irrelevant and/or difficult to quantify, they are less applicable to those microparasites where the size of parasite population within a host is key to understanding host-parasite population dynamics. Recently, a metapopulation framework has been applied to disease dynamics in order to incorporate spatial structuring of the host population (11-13), but in such approaches the unit of the patch is a host population, and the parasite population per host is still overlooked even though dynamics of infection within a host can be affected by individual host characteristics and can have direct impacts both on individual host health, on host movement, and on the rate of transmission. Macroparasite models, on the other hand, directly consider the

parasite population but even these models often do not capture the dynamics in parasite numbers within individual hosts (14-16). Furthermore, not all parasites fit neatly into the micro- or macroparasite conceptual framework. Together, these limitations have led to the call for a unifying framework which considers both host and parasite populations (17). One possible approach applies traditional metapopulation theory to parasite population dynamics, but views individual hosts (rather than local host populations) as patches that can be colonized by the parasite (7). To our knowledge, this approach has not yet been developed theoretically nor investigated experimentally, perhaps because very few parasites allow for the possibility of tracking their dynamics over time without destructive sampling. The use of model systems which can experimentally test how characteristics of individual hosts can influence parasite populations at both the individual host and host population levels are thus of fundamental importance.

Gyrodactylus spp. (Monogenea) are ectoparasites which feed on the epithelial cells and mucus of many marine and freshwater teleost fish species (18). They attach to the epidermis of their host via specialized hooks and are directly transmitted primarily by jumping to a new host during contact (19, 20). *Gyrodactylus* spp. are viviparous, with an unusual method of reproduction: the developing embryo contains within itself a second developing embryo, which allows for rapid population growth of the parasite on an infected host (18, 19). Gyrodactylid infection can result in high rates of mortality (21), and induce a temporary refractory period in surviving hosts (22, 23). As such, gyrodactylids cause epidemic outbreaks, making their population dynamics typical of microparasites (6, 14) despite being helminth parasites. Furthermore, because they are

ectoparasites they can be observed over time without sacrificing the host. Thus, this model system has been useful for studying parasite dynamics on individual hosts within a host population (24-28), and holds potential for furthering our understanding of host-parasite population dynamics.

The guppy (*Poecilia reticulata*) is the host for *Gyrodactylus turnbulli* (see 29). Guppies are a common sexually dimorphic ovoviviparous tropical fish, used as a model species for many ecological studies including exploration of male-female interactions, mate-choice and parasitism (30, 31), and shoaling behaviour (26, 32). In many guppy populations, females harbour more parasites than males (33-35), and the tendency of females to shoal more tightly together than males may facilitate parasite transmission in grouped fish (26, 32). Also, guppy populations vary widely in their ability to resist parasites (24, 36). Thus, the guppy-gyrodactylid system provides a unique opportunity for experimentally testing how heterogeneity among hosts can influence parasite population dynamics both at the individual host level and at the host and parasite population level. Although the effects of sex and number of guppies on parasite population growth have been studied in separate experiments (25, 26, 33, 35), the direct comparison between parasite dynamics on isolated hosts and groups has not been made, nor have the combined effects of grouping and sex on parasite epidemic dynamics been investigated.

The goals for this experiment were to determine whether host sex, group size and group composition influenced host-parasite dynamics at the level of individual and grouped hosts. We expected parasite populations to reach higher numbers and persist for longer in groups of fish

when compared to isolated fish due to greater availability of hosts. We also expected higher parasite burdens on females than males, both on isolated fish and in single-sex groups due to greater size and possibly lower resistance of females (35). For mixed-sex groups, however, our null expectation was that heterogeneity among fish would have an averaging effect on parasite population growth. Although we found that parasites reached higher densities on isolated females than males, this difference did not persist in groups, and heterogeneity in group composition did not influence parasite dynamics.

3.4 MATERIALS AND METHODS

3.4.1 SOURCE AND MAINTENANCE OF FISH

Animal Care Approval was obtained per McGill University Ethics Guidelines (AUP 2009-5759). Guppies obtained from the Guanapo River and Lower Lalaja tributary in Trinidad (10º38'23" N, 61º14'54" W and 10º39'14" N, 61º15'18" W) were bred to the F3 generation, keeping track of maternal lines, in the McGill University Phytotron. The room was maintained at 27 ±1 ºC with a 12-hour light-dark cycle and the fish were raised in common-garden conditions in an Aquaneering Inc. (San Diego, California, U.S.A.) flow-through system. Fish were raised on controlled amounts of TetraMin© Tropical Flakes (Tetra Werke, Melle Germany). In order to mimic a history of natural infection, F3 fish were exposed to our isogenic lab culture of *G. turnbulli* (identified by S. King) from birth.

3.4.2 EXPERIMENTAL DESIGN

The experiment consisted of two parts, conducted simultaneously. The first part was a 2x2

factorial design used to test the effects of host sex (male vs. female) and host group size (1 vs. 8) on parasite dynamics. As treatments, we established groups of 8 males (4 replicates), groups of 8 females (4 replicates), isolated males (8 replicates), and isolated females (8 replicates). The second part of the experiment tested the effect of host heterogeneity in sex on parasite dynamics. This part consisted of 4 replicates each containing a group of 8 fish (4 males and 4 females), and data were compared with the homogenous sex groups from the first part of the experiment.

3.4.3 EXPERIMENTAL PROTOCOL

In order for fish to overcome infection-acquired resistance and regain susceptibility to *Gyrodactylus* spp. (22-24) parasites were eliminated from adult F3 fish by treating them in a 25g/L salt water bath for 15 minutes (37) two months before the start of the experiment. One week later, fish were anaesthetized in 0.02% Tricaine methanesulfonate (MS-222), buffered to a neutral pH with sodium bicarbonate and scanned using a dissection microscope to confirm the absence of parasites. Seven weeks later, adult F3 fish were again scanned for parasites and weighed to the nearest 0.001g, measured for standard length (SL) to the nearest 0.01cm with a calliper, and marked for identification with visible implant elastomer dye (Northwest Marine Technologies Inc., Shaw Island Washington, U.S.A.) which has been shown to have no impact on fish health or behaviour (32, 38). Fish were then assigned to treatments/replicates in a way that would distribute size, population of origin and maternal lines evenly across treatments/replicates and groups of fish were acclimated with one another for one week prior to infection.

A total of 112 fish (56 males and 56 females) were used for this experiment, with an SL of 2.34±0.03 cm for females and 1.61±0.01 cm for males and weights of 0.29±0.01g for females and 0.08±0.002g for males. Each group of 8 fish was housed in a tank with 6L of water and each isolated fish was housed in a tank with 1.8L of water. Each tank was considered an experimental unit for analyses at the population level. Fish were fed daily with TetraMin© Tropical Flakes mixed with conditioned water into a paste and delivered through a glass precision syringe to each tank according to the number and sex of fish in each tank. A low food availability regime was used to prevent compensation of innate resistance through additional food acquisition (28, 39).

To begin infections on isolated fish, a heavily infected fish was taken from our isogenic lab culture of *G. turnbulli* and anaesthetized in 0.02% MS-222. Scales with parasites were removed from the donor fish and placed on an anaesthetized recipient until 3 parasites had transferred to the recipient fish (40). To introduce infection to a group of fish, a juvenile pet-store guppy (sex undetermined) from a naïve laboratory stock was infected with three parasites as above and added to the experimental tank for 4 or 6 days when three parasites had naturally transferred to at least one of the experimental fish in the group, at which time the juvenile pet-store guppy was removed (defined as “Day 0” for each tank). This procedure eliminated the potential bias that might have occurred by initiating the epidemic on a male or a female in the mixed groups.

Parasites on each fish were counted every second day for 36 days or until no parasites were found in a tank on two consecutive counting days. In groups of fish, the first day of infection was noted separately for each group (Day 0 in all cases) and for each individual within the group,

based on the day that it was first infected. If a fish in a group died, it was left in the tank for one day in order to allow transmission to other guppies (20, 41) and then removed.

3.4.4 INDEPENDENT VARIABLES

Our independent test variables were sex (male vs. female), group size (isolated vs. grouped), and group composition (homogenous vs. heterogeneous).

In addition, to account for variability in the size of fish at the beginning of the experiment (28, 42), we calculated the relative condition index (*Kn*) of each guppy based on its weight (*W*) and standard length (*SL*) relative to all other fish of the same sex in the experiment. For each sex, a least squares regression of Log(*SL*) and Log(*W*) was performed, and the slope (*b*) and intercept (log(*a*)) for the line of best fit were obtained. *Kn* was then calculated for each individual fish as $Kn=W/(a * SL^b)$ (43, 44) using the sex-specific parameters. Average *Kn* was also calculated for each group of fish.

3.4.5 DEFINITION AND CALCULATION OF DEPENDENT VARIABLES

Peak parasite burden (maximum number of *G. turnbulli*), time to peak parasite burden, persistence of infection (last day of infection minus first day of infection) and host mortality were recorded for isolated fish, for each individual in a group, and for the population of grouped fish. In addition, asynchrony in when individual fish within groups became infected was recorded as the delay from when infection was introduced into the population. Maximum prevalence (percent of infected fish in groups), time to maximum prevalence and over the course of the experiment were also recorded for groups of fish.

3.4.6 STATISTICAL ANALYSIS

All analyses were done using R Language and Environment for Statistical Computing version 3.1.0 (45). Generalized Linear Mixed-Effects Models (GLMMs) were constructed to determine the effects of host sex, host group size (isolated vs. group of 8), fish size (either *W* and *SL* or *Kn,* and average of the group for group-level response variables) and group composition (homogeneous or heterogeneous) and the interactions thereof on host mortality, peak parasite burden, time to peak, and persistence on isolated fish, on individuals in groups and in the group as whole. For each response variable, models were fitted to the distribution of the variable and models for individual fish-level response variables were nested within the random variable tank. Models using *SL* and *W* as metrics for size were not significant, so all final models used only *Kn.* Final models were produced using the stepAIC function to select the combination of factors which produced a model with the lowest AIC. In all cases, the level of significance was set at $p < 0.05$, and all values reported are means and standard errors.

3.5 RESULTS

3.5.1 BASIC PARASITE DYNAMICS

A total of 28 fish (13.2%) died over the course of the experiment, and mortality did not significantly differ between group sizes ($p=0.271$) or between sexes ($p=0.433$).

In all but two grouped tanks, parasites reached 100% prevalence within 14 days as additional fish became infected asynchronously (Figure 3.1b, 1c, 1e, 1f). In tanks, parasite numbers increased and reached distinct population peaks (Figure 3.2). The rate at which fish became infected (delay

to infection) did not significantly differ among groups (data not shown). Group composition (females, males, mixed sex) had no impact on peak prevalence, time to infection or time to peak prevalence (data not shown).

Table 3.1 gives a full overview of the outcomes of our GLMMs and results are explained in detail below.

3.5.2 Individual vs. Grouped Fish

Peak total parasite population on groups of fish was higher (123.5 ± 40.0) than on isolated fish (26.4 ± 6.0) ($p<0.001$). Parasite populations also persisted longer ($p=0.001$) on groups of fish (24.5 ± 1.3 days) than on isolated fish (17.0 ± 1.5 days). Overall, isolated fish had lower peak burdens than individual fish in groups ($p=0.015$), but there was an interaction between sex and grouping, with isolated females having higher peak burdens (34.9 ±10.1) than individual females within groups (17.5 ± 2.6) (Figure 3.3). No difference in parasite time to peak or persistence on an individual fish was found between isolated fish or individual fish in single-sex or mixed groups.

There was a significant interaction of *Kn* and grouping (isolated vs. in a group of 8) ($p=0.01$), with the effect of *Kn* on parasite burden being stronger on isolated fish than on individual fish within single-sex or mixed groups (Figure 3.4).

3.5.3 Male vs. Female Hosts

Parasites reached higher peak burdens on isolated females (34.9±10.1) than on males (15.4±5.0)

(p=0.006). There was an interaction of sex and *Kn* (p=0.038) on peak parasite burden both for isolated fish and individual fish in groups, with *Kn* having a positive impact on parasite load for males but not for females (Figure 3.4). Parasite numbers peaked later (p=0.001) (Figure 3.3) on females (9.4±0.7 days) than on males (6.6±0.5 days) and the infection persisted longer (p=0.033) on females (17.4±0.9 days) than on males (13.6±0.7 days). Infection also persisted longer on fish with a higher *Kn* (p=0.0479), regardless of sex.

At the group level, there was no difference in time to peak prevalence, parasite population peak burden, time to peak population burden or parasite persistence in a tank between male and female groups.

3.5.4 GROUP COMPOSITION: SINGLE-SEX VS. MIXED SEX GROUPS

We found no differences between mixed-sex groups and single-sex groups (or individual fish within them) for any of the response variables.

3.6 DISCUSSION

Our investigation of parasite dynamics on isolated (single host patch) and grouped (multiple host patches) fish confirms that metapopulation theory is compatible with our model system (7, 46), as the presence of multiple patches and connectivity among them allowed the parasite total population to grow larger and persist longer than on single isolated fish. There was no difference in time between when fish first became infected and when parasite burden peaked or in duration of infection between isolated fish and individual fish in a group, but time to peak parasite numbers in the tank and duration of infection in the tank were prolonged in groups compared to

isolated fish. In this aspect, dynamics on each fish were similar but occurred asynchronously due to consecutive infection, leading to longer persistence of the overall parasite populations. We found that fish characteristics in the form of sex and *Kn* impacted parasite dynamics in isolation, but that these differences were not observed in grouped fish.

Although peak parasite total populations were higher on groups than on isolated fish, they were not eight times higher, and the existence of additional hosts lowered the average parasite burden per fish for female hosts. The addition of multiple hosts presumably provided the parasite with more options if their host mounted an immune response, died, or became overcrowded with parasites (47), and thus allowed it to reach a population growth rate closer to the parasite's innate reproductive potential. However, parasite population growth and dispersal were likely constrained due to trade-offs between carrying capacity, reproductive potential and the cost of migrating. Our study would indicate that the costs of transmission and the parasite's own reproductive potential may have had a greater impact on parasite dynamics than overall quality of the host (carrying capacity). Of course, these inferences are limited by the fact that our epidemics were run in a highly controlled, experimental setting and began with only three parasites. It is possible that host abundance and sex could have a greater impact if more parasites had been introduced.

Consistent with our hypothesis, parasites reached higher burdens and persisted longer on isolated female guppies compared with isolated male guppies. One reason could be that females from the populations we used have been shown to be less resistant to parasites than males (35). Another

reason could be that the larger size of females compared with males provided more resources for the parasite in terms of food, space and ability to move to another region of the host to avoid local defence reactions (48). Previous work has shown that larger guppies harbour more gyrodactylids than smaller ones (42) and that the parasites disperse more rapidly through a group of fish when introduced on a fish with a higher *Kn* (28). In this study, we found a positive relationship between *Kn* and peak parasite burden on isolated males, but not on isolated females (which were overall larger than males). However, despite differences in parasite dynamics between the sexes observed at the individual level, we did not find a difference in parasite burden between individual grouped males and grouped females, nor did we find any effect of *Kn* on parasite burden for grouped fish, indicating that there was also an effect of group size on individual burden.

In contrast to previous reports of higher transmission in female than male groups (Richards et al 2010) and higher transmission in male than female groups (Richards et al 2012), we did not observe any differences in peak prevalence, time to first infection or time to peak prevalence between our single-sex groups. In both previous studies, the measure of transmission was the number of non-focal fish that became infected within 3 days of introduction of a focal fish infected with either 30 (Richards et al 2012) or 100 gyrodactylids (Richards et al 2010). This contrasts with our protocol in that we explored transmission from an initial population of three parasites to the time of peak prevalence in populations of smaller feeder guppies at higher density compared with the larger ornamental guppies kept at lower density. Richards et al (2012)

suggested that transmission may be a function of initial parasite load and the impact it has on shoaling behaviour or courtship displays but given the number of differences between our experiment and the two previous studies, it is difficult to attribute the different findings to a single factor.

We found that parasites peaked earlier on males than females, despite having similar burdens in groups. One possibility for the lower parasite growth rate in grouped females could be that females increase investment in parasite resistance (rather than growth) when grouped at a high density, where infection is more likely to occur, an effect observed in many invertebrate systems (49), and potentially also in ours (50). While we did not find a significant difference in somatic growth between isolated and grouped females as Pérez-Jvostov *et al.* (50) did, this could have been an issue of power, since there were only 8 isolated females and changes in weight were much less drastic than differences in parasite loads.

We also found no effect of group composition (homogenous vs. heterogeneous) on parasite dynamics, as our mixed-sex groups did not differ from all-male or all-female groups, nor did individuals within these groups. This finding is inconsistent with theoretical work that suggests heterogeneity would promote asynchrony in local population dynamics and therefore parasite persistence (10, 13, 51). However, since parasite dynamics were similar between single-sex groups of males and females in our study, mixing the sexes in our system may not have generated the heterogeneity in individual hosts that we had expected and can thus explain why we found no influence of heterogeneity on parasite dynamics. Similar results have also been

reported in mice (52), where grouping susceptible and resistant strains together resulted in similar nematode burdens among mice of both strains, but that increasing transmission rates effected a distinction between the two strains (53). However, those studies did not investigate parasite dynamics in single-strain groups, and our results indicate that grouping, rather than group composition, has the greatest impact in homogenizing parasite dynamics.

Although this study set out with the intention of determining how host heterogeneity may influence parasite population dynamics, we found that group composition and factors which influenced parasite dynamics on fish in isolation (*Kn* and sex) had almost no effect on parasite dynamics on fish in groups or at the group level. These findings indicate that factors associated with grouping fish become more relevant than the effects of the individual host characteristics sex and *Kn* of individual hosts for both individual and group-level outcomes, but we are cautious about over-generalizing these interpretations, given that our study comes with the limitations of using a specific experimental system.

Our ability to detect some biologically important differences may have been limited by having only four replicates per treatment. The relatively small size of the fish tanks probably limited our ability to detect differences in parasite dynamics that would have been driven by host behaviours including shoaling of females but not males. We did not know the infection history of individual fish, other than the fact that they had been previously exposed to parasites, and as such could not explore any possible impact of differences in acquired resistance to parasites (22, 23, 54) or of an interaction between sex and acquired resistance. Finally, this study only looked at two host traits

(sex and size) and it is possible that other host characteristics, such as MHC profiles (55), colour (31) or population of origin (21, 36), could have a stronger impact on parasite dynamics.

Metapopulation theory, while compatible in our system in the sense that additional hosts allowed for asynchronous dynamics to promote parasite persistence, predicts that heterogeneity in patch quality prolongs persistence due to greater asynchrony in local patch dynamics (56-61). Our study has shown that the ability of a parasite to move from host to host (connectivity) may override individual host differences in the absence of connectivity, thus rendering the expectation of persistence over heterogeneous patches weaker for our system. This study served as the first steps towards conceptualizing a theory that incorporates dynamics within individual hosts rather than focusing solely on infection status of individuals (like microparasite models) or the total parasite populations (like macroparasite models), and further investigation into these dynamics is necessary to develop a more unifying framework for parasite population growth and dissemination.

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3.10 TABLES

Table 3.1: Outputs of Generalized Linear Mixed-Effects Models

For two-way comparisons, the comparison is explained in parentheses next to the variable name

a) Outcome: Parasite Peak Burden on Individual Patches (111 Degrees of Freedom)

	Estimate (±SE)	z-value	p-value
Group Size (isolated vs. grouped)	-30.8 (±12.9)	-2.392	0.015
Sex (male vs. female)	-4.8 (±1.7)	-2.754	0.006
Kn [1]	-1.8 (±1.1)	-1.536	0.124
*Group Size*Sex*	34.7 (±19.3)	1.798	0.072
*Sex ** Kn	3.7 (±1.8)	2.076	0.038
*Group Size ** Kn	29.4 (±12.2)	2.411	0.016
*Sex*Group Size**Kn	-33.0 (±18.2)	-1.809	0.070

b) Outcome: Parasite Peak Burden in Tanks (27 Degrees of Freedom)

	Estimate (±SE)	z-value	p-value
Group Size (isolated vs. grouped)	-2.3 (±0.5)	-4.717	<0.001
Sex (males vs. females)	0.6 (±0.5)	1.1	0.300
Sex (females vs. mixed)	-0.1 (±0.5)	-0.175	0.861
Sex (males vs. mixed)	0.5 (±0.5)	0.905	0.366
*Group Size * Sex*	1.2 (±0.7)	1.855	0.064

c) Outcome: Time to Peak Burden on Individual Patches (110 Degrees of Freedom)

	Estimate (±SE)	**t-value**	**p-value**
Sex (males vs. females)	-2.8 (±0.9)	-3.241	0.002

d) Outcome: Parasite Persistence on Individual Patches (111 degrees of Freedom)

	Estimate (±SE)	**t-value**	**p-value**
Sex (males vs. females)	25.6 (±11.9)	2.151	0.033
Kn	22.4 (±7.8)	2.881	0.048
Sex * Kn	-26.7 (±15.5)	-1.722	0.089

e) Outcome: Parasite Persistence in Tanks (27 Degrees of Freedom)

	Estimate(±SE)	**t-value**	**p-value**
Group Size (isolated vs. grouped)	-7.5 (±2.1)	-3.574	0.001

[1] Kn is the relative condition index based on weight (W) and standard length (SL) of each fish relative to all other fish of the same sex in the experiment. See methods section for calculation.

3.11 FIGURES

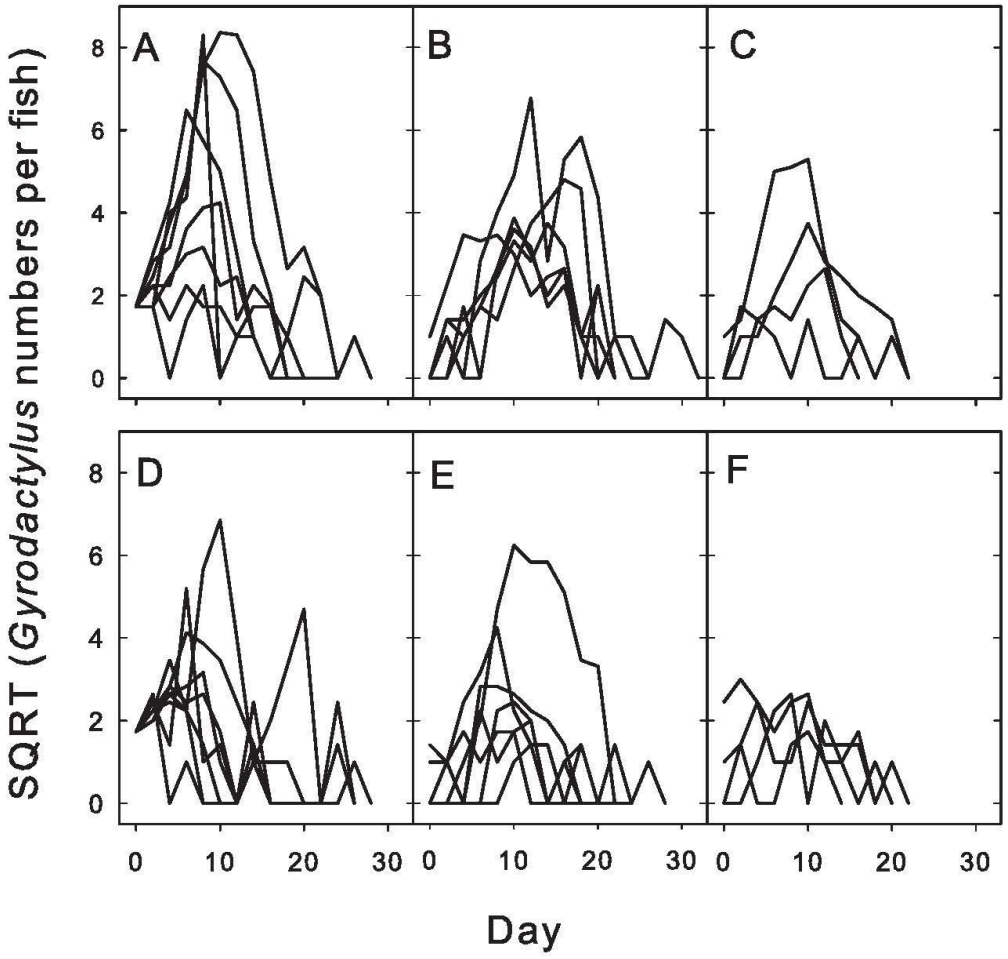

Figure 3.1: Parasite Population Dynamics for all Treatments

(a) individual isolated females, (b) individual females in a sample all-female tank, (c) individual females in a sample mixed-sex tank, (d) individual isolated males, (e) individual males in a sample all-male tank, (f) and individual males in a sample mixed-sex tank. Data are square-root transformed for graphing purposes but were not transformed for analysis. For all panels, "Day 0" indicates the day on which at least 3 parasites were first found in the tank.

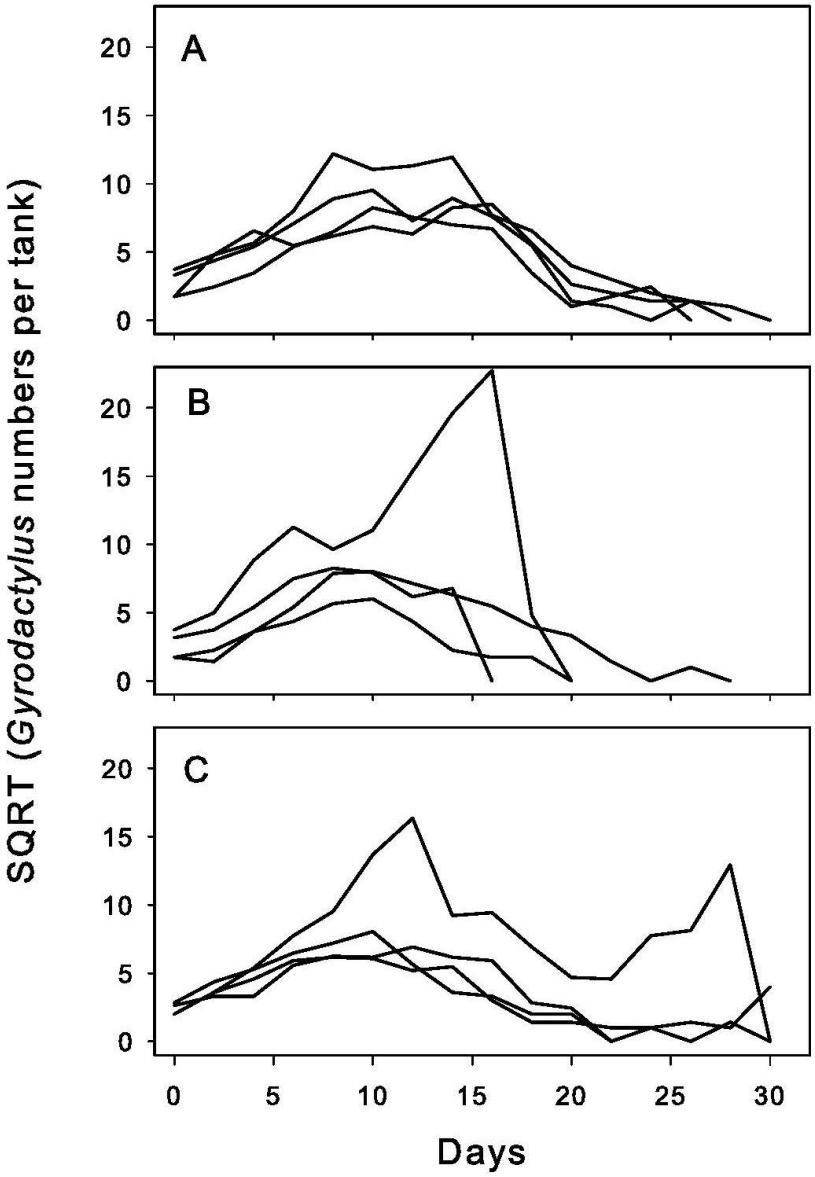

Figure 3.2: Total Parasite Population Numbers over the Course of the Experiment in Groups

(a) male groups, (b) female groups (c) mixed-sex groups. Data are square-root transformed for graphing purposes but were not transformed for analysis. For all panels, "Day 0" indicates the day on which at least 3 parasites were first found in the tank.

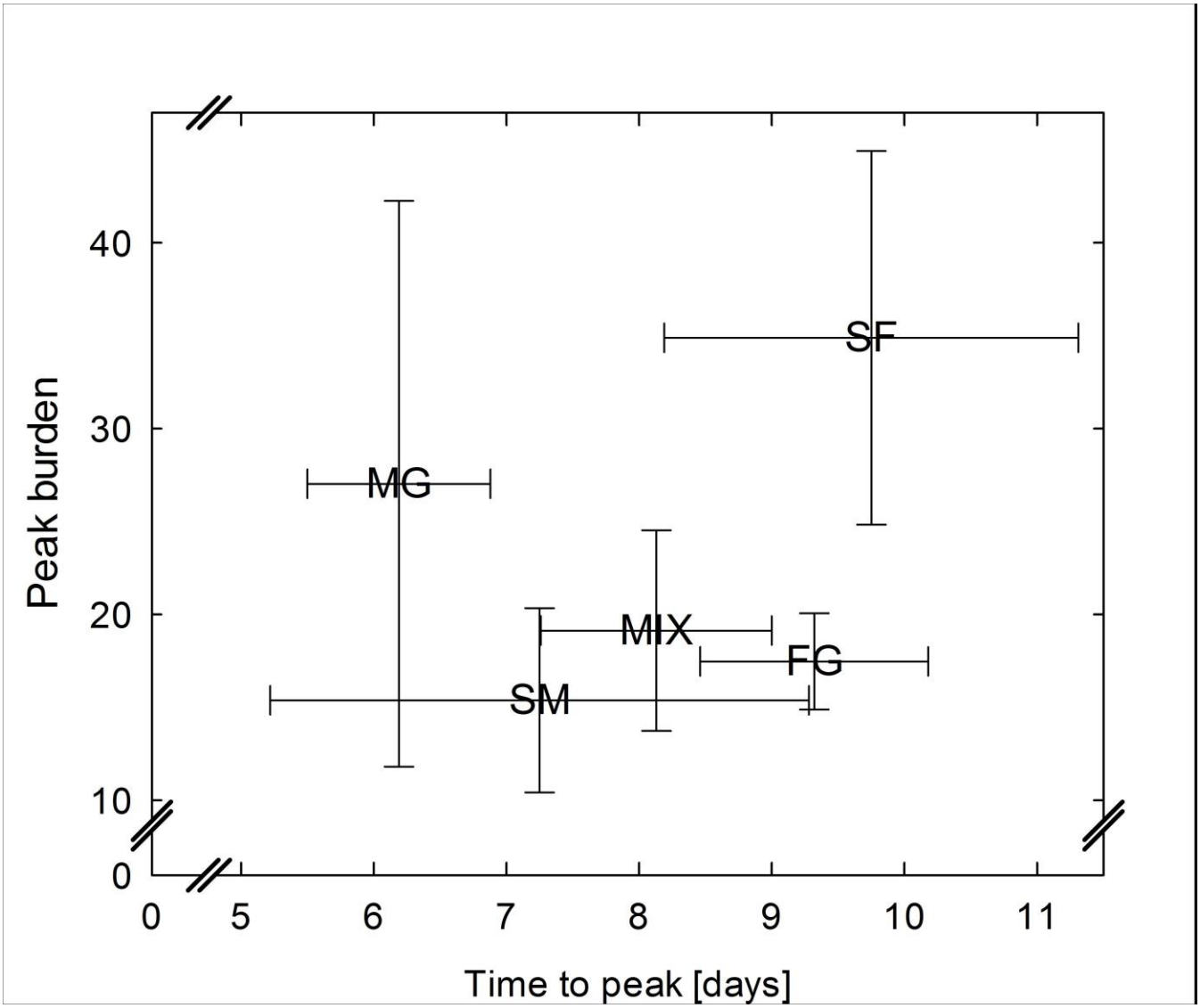

Figure 3.3: Mean Peak Burden (±SE) vs. Mean Time to Peak (±SE) for Individuals

SF: single (isolated) females, SM: single (isolated) males, FG: female groups, MG: male groups, MIX: mixed groups.

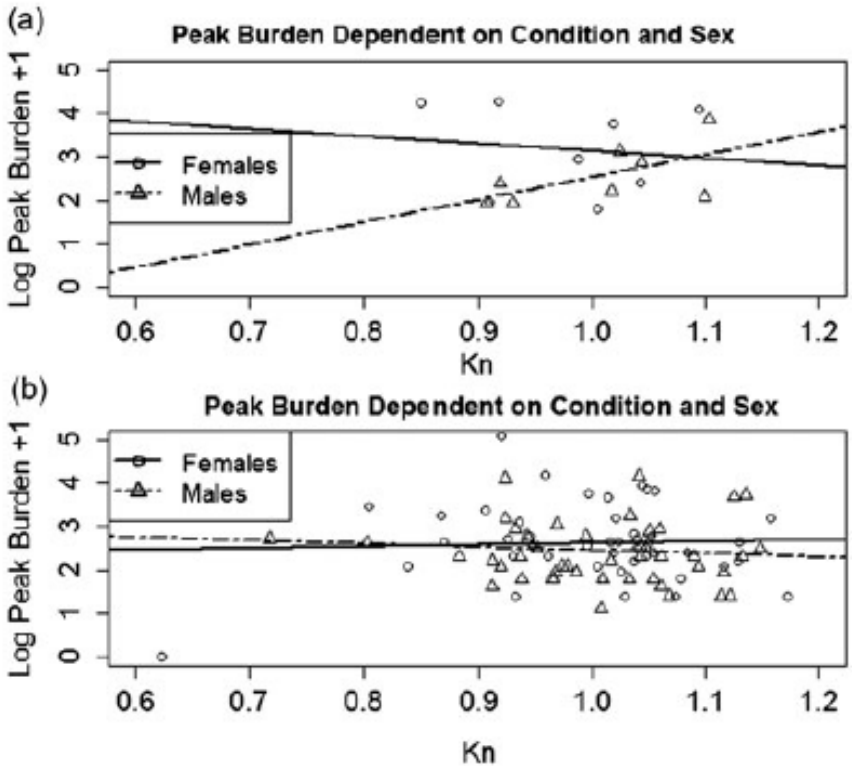

Figure 3.4: Interaction of Kn and Sex on a) isolated fish and b) grouped fish

CONNECTING STATEMENT 2

In Chapter 4, I return to data collected from my Chapter 2 experiment, this time moving a further level down in scale from Chapter 3 to investigate impacts of individual-level host characteristics on host metapopulation-level dynamics. Rather than looking at the general impacts of connectivity among subpopulations on long-term metapopulation dynamics, I here explore the impact that the specific individual who migrates and its parasites have on the host-parasite population that it enters, and which individual host characteristics are of primary importance to this impact. I also investigate the impacts of individual-level heterogeneity rather than metapopulation-level heterogeneity on metapopulation dynamics due to an individual's competence as a host.

Host competence, an individual's propensity to transmit parasites to new hosts, is crucial to the spread of disease. Most work investigating host competence focuses on the super-spreader hypothesis, which posits that few individuals are often responsible for a large proportion of transmission. In this chapter I explore how an individual's resistance and tolerance to parasites may translate to its competence as a host, and how variation in host competence may influence metapopulation dynamics.

4 CHAPTER 4: PARASITE SPREAD IN EXPERIMENTAL METAPOPULATIONS: RESISTANCE, TOLERANCE AND HOST COMPETENCE

Tadiri, C.P., Scott, M.E., Fussmann, G.F. 2019. "Parasite spread in experimental metapopulations: A role for super-spreaders?" In review: *Journal of Animal Ecology*

4.1 ABSTRACT

1. Host competence, an individual's propensity to transmit infection, is one of the most important aspects of heterogeneity among host individuals that may impact host-parasite dynamics in a metapopulation, yet it is still underexplored experimentally.
2. This study used data from experimental epidemics of the ectoparasite *Gyrodactylus turnbulli* in metapopulations of guppies to identify the characteristics of the more competent hosts and determine the degree to which they influence epidemic dynamics.
3. We characterized fish as having either intense infections, prolonged infections, both or neither to explore how resistance and tolerance relate to degree of host competence.
4. Fish with both intense and prolonged infections were larger than fish with neither, indicating that an individual's size may influence its tolerance (the ability to withstand infection). Fish with prolonged infections had more contacts and were responsible for more transmission than other fish, regardless of infection intensity. We found a positive association between the number of fish with prolonged infections and parasite metapopulation persistence, and a positive interactive effect of the number of fish with both prolonged and intense infections on metapopulation parasite load, indicating tolerant fish contribute the most to metapopulation loads.
5. These findings highlight the importance of disentangling different facets of host competence, particularly the underrecognized mechanism of tolerance in host competence.

Keywords: epidemic dynamics, guppies, *Gyrodactylus*, host competence, metapopulations

4.2 INTRODUCTION

The importance of understanding and therefore being able to anticipate infectious disease dynamics is one of the most fundamental concerns for human health, and only increases as networks of contacts and species ranges expand with anthropogenic change (1, 2). Within a metapopulation, connectivity among subpopulations is known to facilitate the spread of infectious disease by providing an avenue whereby parasites can be introduced into new, susceptible populations, and by allowing an influx of naïve hosts to populations in which the parasite is endemic (3, 4). Early models of microparasite transmission focussed on populations of susceptible, infected and resistant hosts (5, 6), however more recent work has acknowledged that heterogeneity among individuals can often be an important factor in epidemic dynamics, particularly in the context of migration within metapopulations (7-9). A large body of theoretical work has been developed to better understand disease transmission in metapopulations and incorporate host heterogeneity (10-15), but there is a need to merge it with experimental data that specifically tests these concepts.

One aspect of individual heterogeneity that influences parasite dynamics is host competence, an individual's propensity to transmit infection to new hosts (16), most commonly exemplified by the superspreader hypothesis (17-19), which posits that often a few individuals are responsible for a large proportion of subsequent infections in a population. Thus, identifying superspreaders and tracing their networks of contacts has become an important focus of epidemiology, with

connectivity to other individuals being one of the main features of superspreaders (17, 18). However, it is often difficult to trace networks of contacts, particularly with microparasitic infections such as viruses and bacteria which aren't clearly visible, and other factors can be important to increasing host competence, for example infectiousness and infectious period (20).

In addition to connectivity, an individual's parasite burden (and therefore its potential to shed parasites to others) and duration of infection (and therefore the length of time over which it can infect others) will contribute to its competence as an agent of transmission. Variability in these two factors may be due to a host's resistance or tolerance to infection. A resistant individual will limit parasite population growth and will therefore have a lower infection burden and period of infectiousness, and therefore be a less competent host than a tolerant one, that can withstand higher burdens for longer periods of time (20). However, the extent to which tolerant individuals influence transmission is underexplored (21), and disentangling the components of host competence and their influence on epidemic dynamics even more so. For example, individuals with high infectiousness or long periods of infection may contribute not only to high transmission rates, but also to the cumulative parasite burden in the population, or parasite persistence. We set out to explore these concepts in an experimental setting, using data from experimental metapopulation epidemics in the guppy (*Poecilia reticulata*)-*Gyrodactylus* model system.

The guppy-*Gyrodactylus* model system is used to investigate host-parasite epidemic dynamics (22-25), evolution (26-29) and behavioural ecology (30-34). Guppies are small, live-bearing

teleost fish that are relatively easy to rear in a laboratory. Their ectoparasite, *Gyrodactylus turnbulli,* reproduces rapidly on the fish skin; it is transmitted via direct contact (35) and hosts remain infectious for the duration of their infection. *Gyrodactylus* can cause high mortality rates in laboratory settings (27, 36) and induces a temporary refractory period (37, 38), thereby causing epidemics characteristic to microparasites, but parasite numbers are easily quantifiable without destructive sampling, making this system ideal for studying epidemic dynamics particularly in the context of individual host competence.

Importantly, much is already known about heterogeneity in resistance to *G. turnbulli* and the influence of many individual characteristics on parasite load (with higher loads indicating lower resistance). Post-infection, recovered fish exhibit a waning acquired resistance to *Gyrodactylus* (37, 38). It has also been found that innate resistance to infection can vary among populations, indicating evolutionary pressures selecting for resistance to parasites (27, 36), and studies of the major histocompatibility complex (MHC) have demonstrated evidence for a genetic basis for resistance (39). In terms of heterogeneity in parasite loads, females often have more parasites than males, (33, 40, 41), larger fish often harbour more parasites than smaller ones (42, 43) and bolder fish are more likely to be infected than shyer ones (44-46). Some previous work in this system has explored the evolution of tolerance, demonstrating that the removal of parasites selects for increased resistance, but not for decreased tolerance (27), that individuals under high predation regimes may evolve more tolerance, in accord with the pace-of-life hypothesis (47) and that selection for larger body size in females may also select for greater tolerance (48).

However, host tolerance, arguably a key characteristic facilitating the spread of parasites, as a contributor to host competence has been underexplored experimentally in this system. Additionally, some work has already been done investigating the role of individual heterogeneity and infection dynamics on the probability of transmission. For example, it was found that higher infection load (lower resistance) increased the rate of transmission in isolated guppy pairs (49). Yet, how these effects play out at the population or metapopulation level was not experimentally investigated. Similarly, heterogeneity at the subpopulation level and its impacts on metapopulation dynamics have been studied, finding no influence of the way parasites were initially distributed among subpopulations on metapopulation parasite persistence or intensity (50). However, the impact of individuals on metapopulation dynamics has not been examined in this metapopulation context.

We set out to determine the degree to which individuals drive metapopulation epidemic dynamics by identifying the more competent hosts. We hypothesized that fish with high infection intensity and duration would be more competent due to their greater potential for transmitting parasites, and that they would be larger and have a larger network of contacts than other individuals, and therefore be responsible for more transmission, and be associated with more intense and longer epidemic durations in their metapopulations.

4.3 METHODS

4.3.1 SOURCE OF DATA

Individual and metapopulation-level data were obtained from experimental metapopulations (50)

of adult guppies (age unknown) infected with *G. turnbulli.* A full description of the experiment including a diagram of the experimental design can be found in Tadiri et al. 2018 (48). In brief, fish were weighed, measured, individually marked and assembled into eight metapopulations, each consisting of four sub-populations (6L tanks) of eight male guppies. Metapopulations were experimentally infected with eight *G. turnbulli* and individual infection loads were monitored every other day by counting the number of parasites on each individual anaesthetized fish using a dissecting microscope for 120 days or until parasites died out in the metapopulation. Epidemics in metapopulations were initiated either by infecting one fish in each of the four tanks with two parasites, or by infecting four fish in only one tank with two parasites each. In all metapopulations, migration among tanks occurred every ten days by randomly selecting one fish from each tank and moving it to the subsequent tank in a unidirectional loop; note was made of the identity of each moved fish and its new tank. All fish deaths were recorded. These raw data were used to calculate individual- and metapopulation-level explanatory and outcome variables.

For each individual fish, the cumulative parasite burden and total number of days infected over the course of the experiment were calculated. For each fish that was moved from one tank to another, we estimated both fish connectivity and incidence. Fish connectivity was calculated for every fish in each metapopulation; connectivity was defined as the total number of distinct individual fish with which each fish shared a tank at some point during the course of the experiment. The contribution of each fish that was transferred from one tank to another to transmission was estimated as the incidence, calculated as the number of previously uninfected

fish that became infected within 2 days of introducing the fish into the tank. For fish that were moved more than once, both the total and average incidence were recorded.

At the metapopulation level, we calculated the peak prevalence (percent of fish infected), peak parasite burden, the duration of infection, and mortality (percent of fish that died).

All analyses were performed in R Language and Environment for Statistical Computing v. 3.5.3. For all analyses the significance level α was set at 0.05.

4.3.2 Defining and Identifying Potentially More-Competent Hosts

To determine if specific groups of individuals were more responsible for transmission than others, we focussed on two infection-level variables that we hypothesized would increase host competence: individual parasite load and infection duration. Fish were categorized based on whether or not they had intense infections (cumulative burden of the individual fish throughout the experiment greater than mean cumulative burden of all fish across all metapopulations) and prolonged infections (total number of total days infected throughout the experiment greater than the mean number of days infected across all metapopulations). From these variables, we defined four categories: both intense and prolonged infections, intense infection only, prolonged infection only, and neither intense nor prolonged infection.

4.3.3 Shared Characteristics

To determine whether fish within each infection category were characterized by specific traits, we tested whether standard length (SL) and weight (W) differed among infection categories

using ANOVA (function *aov*) with the Tukey-Kramer post-hoc test (function *TukeyHSD*). We also tested whether fish connectivity differed by infection category using a Kruskal-Wallace (function *Kruskal.test*) and Dunn post-hoc test (package *dunn.test*, function *dunn.test*) because the number of contacts per fish was not normally distributed.

4.3.4 Epidemic Outcomes

In order to determine if infection category was associated with epidemic outcomes at the metapopulation level, we compared total and average incidence among infection categories using a Kruskal-Wallis and Dunn post-hoc test because incidence was not normally distributed. We also tested whether parasite persistence, peak parasite load, peak prevalence and metapopulation mortality were influenced by the number of fish with intense infections or the number of fish with prolonged infections using generalized linear models (GLM) with different error distributions depending on the nature of the data. For parasite persistence and peak parasite load, we used a GLM with a negative binomial distribution (package *stats,* function *glm.nb*) and for peak prevalence and mortality, we used a GLM with a normal distribution (package *stats*, function *glm*). Models were simplified first by removing the interaction term, then each variable (number of fish with intense infections or number fish with prolonged infections) individually, comparing AICs with the full model at each step to find the model with the lowest AIC.

4.4 Results

Results of statistical analyses for post-hoc tests (Table 4.1) and GLMs (Table 4.2) can be found in supplemental materials.

4.4.1 Basic Dynamics

A total of 256 fish were used in this experiment. They had an average cumulative burden of 319.2 (range: 0 to 7608) parasites and an average infection duration of 21.8 (range: 0-110) days. Eighteen fish never became infected. Over the 120 days of the experiment, 133 fish died, and the average lifespan of the fish that died was 39.2 (range: 1-120) days. A total of 144 fish were moved at least once.

4.4.2 Identifying Potentially More-Competent Hosts

Of the 256 fish, a total of 83 fish were characterized as having intense infections, 109 fish had prolonged infections, 30 shared both characteristics, and 94 fish had neither characteristic.

4.4.3 Shared Characteristics

Fish categorized as having both prolonged and intense infections were significantly larger both in terms of weight ($p=0.003$) and length ($p=0.014$) than fish with neither characteristic (Figure 4.1). With regard to connectivity, fish categorized as having prolonged infections were more connected than those without prolonged infection ($p<0.001$), but infection intensity had no effect on number of contacts (Figure 4.2).

4.4.4 Epidemic Outcomes

After introduction to a new tank, fish categorized as having prolonged infections over the course of the experiment were responsible for more transmission (higher total and average incidence) than those without prolonged infections ($p <0.001$ for both, Figure 4.3), but infection intensity had no effect on the total or average number of individuals infected. Similarly, the number of

fish with prolonged infections in the metapopulation was positively associated with metapopulation parasite persistence ($p<0.001$, Figure 4.4), with no impact of the number of fish with intense infections on persistence. In contrast, peak burden was negatively associated with the number of fish with intense infections ($p=0.01$) and the significant positive interaction term ($p=0.05$) reflects an additive effect of the occurrence of fish with both intense and prolonged infections. Neither peak prevalence nor mortality were significantly affected by the number of fish with intense infection or prolonged infection (data not shown).

4.5 DISCUSSION

We identified four separate categories of fish based on their presumed degree of competence as hosts: those having intense infections, prolonged infections, both or neither. The range for infection duration was large, and the range for infection intensity larger, thus these categories capture extreme ends of the spectrum. Fish with both intense and prolonged infections were larger than fish with neither. Fish with prolonged infections had more contacts than other fish and were responsible for higher incidence upon introduction to a new tank than fish without prolonged infections, regardless of infection intensity. Parasite persistence in the metapopulation was longer when there were more fish with prolonged infections. Peak burden in metapopulations was higher when there were more fish with both intense and prolonged infections. These findings highlight that prolonged and intense infections contribute to maintenance of infection in the metapopulation.

We expect cumulative parasite intensity to be inversely related to a host's resistance, as it

indicates an inability of the host to limit parasite growth (26, 27). Therefore, fish without intense infections would be considered the most resistant. Tolerance, on the other hand, represents an individual's ability to withstand infection and limit damage due to infection (51). Here, fish that were able to survive for a longer period of time with infection could be considered the most tolerant. We found a significant difference in size between fish with both intense and prolonged infection compared with fish with neither, the two opposite extremes of this spectrum, indicating that an individual's size may play a role in its ability to provide a resource for parasites. This finding is in agreement with numerous other studies on this system that found that larger fish often have more parasites and potentially have a greater capacity to tolerate higher loads (43) and may also increase parasite transmission to other fish (42). It also could imply that fish resistance to infection plays a large role in competence, since parasite load is commonly used as a proxy for host resistance in this system, with high loads indicating lower resistance (26, 36, 52). In fact, more recent work has shown that an individual's low resistance may increase its probability of transmitting parasites to a single other host (49). Our experiment takes this concept one step further, by demonstrating the interplay among individual resistance and tolerance, connectivity to multiple contacts, and transmission dynamics.

The defining characteristic of a competent host is its propensity to transmit parasites to other hosts, an ability most expressed when the host is highly connected to other individuals (17, 18). We found that individuals with prolonged infections, regardless of their parasite load, had a higher number of contacts than those infected for a shorter period of time. In the experimental

design for this study fish that were able to survive with infection had a higher chance of contacting many other individuals than those that succumbed to infection early, due to movement and the introduction of a new contact occurring every 10 days. Furthermore, movement, which would expose an individual to even more new contacts was also highly dependent on fish survival, because the probability of being selected for movement was higher when there were fewer fish in the tank. Although connectivity is one of the most important characteristics of a competent host, precisely because our experimental design removed any link between connectivity and social characteristics, we were able to isolate the infection-level mechanisms of intensity and duration to show that fish with long infection periods were the most connected and the most likely to infect other individuals.

Not only did fish with prolonged infections have the opportunity to act as agents of transmission, they actually did, as we found that fish with prolonged infections were responsible for more transmission than other fish, indicating that duration of infection, rather than intensity, was most important to spreading infection. The number of fish with prolonged infections was also associated with prolonged parasite persistence in the metapopulation, regardless of whether or not individuals also had above average numbers of parasites, potentially due to not only their own infection durations, but their reproductive number, as they subsequently infected other fish in the metapopulation. That fish who were infected for the longest period of time were important to spreading and maintaining parasite populations in the system is in accord with other experiments and theoretical work (53, 54) and our hypothesis. However we had also

hypothesized that parasite burden on fish that moved among tanks would impact the spread of infection. That incidence was not higher if the fish that moved had above average intensity of infection highlights the parallel between our system and other microparasitic systems, in which an individual's viremia, bacteremia or parasitemia is usually assumed to be irrelevant to its infectiousness (5, 6) and the importance of disentangling different types of host competence and their influence on overall dynamics (21).

The interaction of the number of fish with prolonged infections and intense infections impacted metapopulation peak parasite load, with fish with both intense and prolonged infections positively associated with peak parasite load, whereas the occurrence of individuals with intense infections only was, to our surprise, negatively associated with peak parasite load, indicating that tolerance may play a large role in maintaining high levels of infection in a population. We also found that the number of individuals in the metapopulation with prolonged infections was positively associated with parasite metapopulation persistence. These results could demonstrate that those fish more prone to high parasite burdens were more responsible for supporting parasite population growth, while those more able to endure infection were responsible for spreading and prolonging parasite persistence, indicating that both resistance and tolerance are important to parasite dynamics overall (55). Untangling these two variables and their causes is important to understanding the dissemination of infectious disease. The results of this analysis support the hypothesis that heterogeneity among individuals in their resistance or tolerance to infection may drive parasite dynamics in metapopulations (16, 20).

We recognize a few key limitations of this project. First, we estimated the number of contacts as the total number of fish with which an individual ever shared a tank, but we do not have any behavioural data to confirm that fish actually came into physical contact with all other fish in a tank during the ten days they were together. However, guppies are highly social and shoal tightly together (45), and moreover our tanks were kept at slightly higher densities than observed in the wild (56), so it is a fair assumption to make in this case, especially since contact does not need to be long or intense for *Gyrodactylus* spp. transmission to occur (*pers. obs*). Second, we only estimated the number of fish that an individual infected within two days of being moved to a new tank because *G. turnbulli* are indistinguishable from each other and it is impossible to determine whether the source of a new infection was from the recently moved fish or another infected fish already in the tank, however, it is our best estimate based on the limitations of this system and experimental design. Nevertheless, the number of newly infected fish is most important for determining infection spread, thus strengthening our argument for estimating incidence. Thirdly, movement of fish within the system was imposed by the experimenter's haphazard selection, and therefore was not a reflection of an individual fish's natural sociability or inclination to migrate. Behaviour of infected fish and avoidance behaviour of uninfected fish may play an important role in transmission dynamics (45, 46), and further work is necessary to disentangle behavioural factors in host competence. Therefore, our results cannot indicate a causal link between connectivity and any of our other individual characteristics. Finally, this experiment was cut off at 120 days, artificially truncating the potential parasite persistence in metapopulations, and possibly the effect of infectious periods of individuals. Nevertheless, we were still able to detect

an influence of infectious period on both incidence and parasite persistence within a metapopulation, indicating a robustness of this relationship.

This analysis used pre-existing experimental metapopulation data to determine which characteristics of individual hosts had the greatest impact on metapopulation parasite dynamics, finding that cumulative duration of infection had the greatest impact on transmission and parasite persistence in the metapopulation. Rather than finding a small group of highly competent individuals, we found that many individuals contributed to the spread and persistence of infection, highlighting the importance of recognizing different forms of host competence, and particularly the role that tolerance may play in the spread of disease. Future work should incorporate genetic analysis, such as MHC expression (39, 57) to determine if the relationship of particular genetic profiles to resistance and/or tolerance influences metapopulation parasite dynamics, and if genetic profiles align with the infection profiles that we characterized. This study provides experimental evidence that individual tolerance and resistance to parasites can broadly influence host competence and therefore higher-level host-parasite dynamics.

4.6 Acknowledgments

We thank Stanley King for identifying the species of *Gyrodactylus*, and Mark Romer and Claire Cooney for assisting with administration and maintenance of our facilities.

4.7 Authors' Contributions

All authors contributed to the design of the study. CPT collected and analysed data. CPT wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

4.8 FUNDING

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4.9 DATA ACCESSIBILITY

The primary data set supporting this manuscript has been submitted as supplementary material and will be uploaded to an external repository upon review of this article.

4.10 ETHICS STATEMENT

Approval for animal care and research was obtained from the McGill University Animal Care Committee (AUP 2014-7547) in compliance with the Canadian Council on Animal Care.

4.11 COMPETING INTERESTS

We have no competing interests.

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4.13 FIGURES

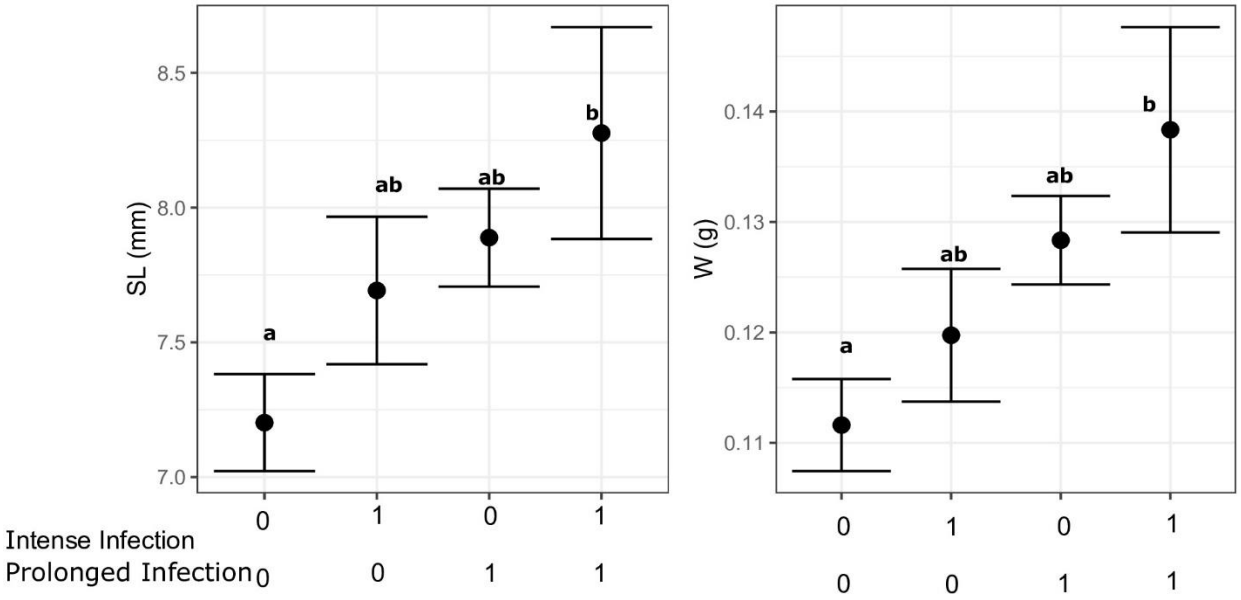

Figure 4.1: Guppy size in terms of standard length (SL) (left) and weight (W) (right) as a function of whether the fish was categorized as having intense and/or prolonged infection ("1": yes; "0": no) throughout the course of the experiment.

Error bars represent mean +/- standard error. Identical letters above error bars designate treatments that are not significantly different from one another.

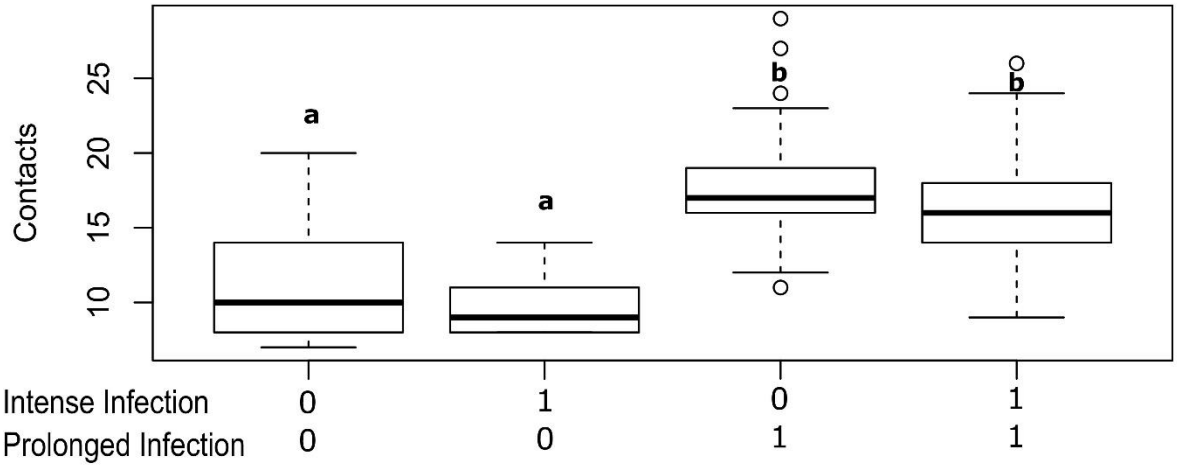

Figure 4.2: Box and whisker plots of connectivity, estimated as the total number of other fish in contact with the fish, as a function of whether the fish was categorized as having intense and/or prolonged infection ("1": yes; "0": no) throughout the experiment.

Data pooled from all 8 metapopulations and all migration days (which occurred at 10-day intervals). Identical letters above error bars designate treatments that are not significantly different from one another.

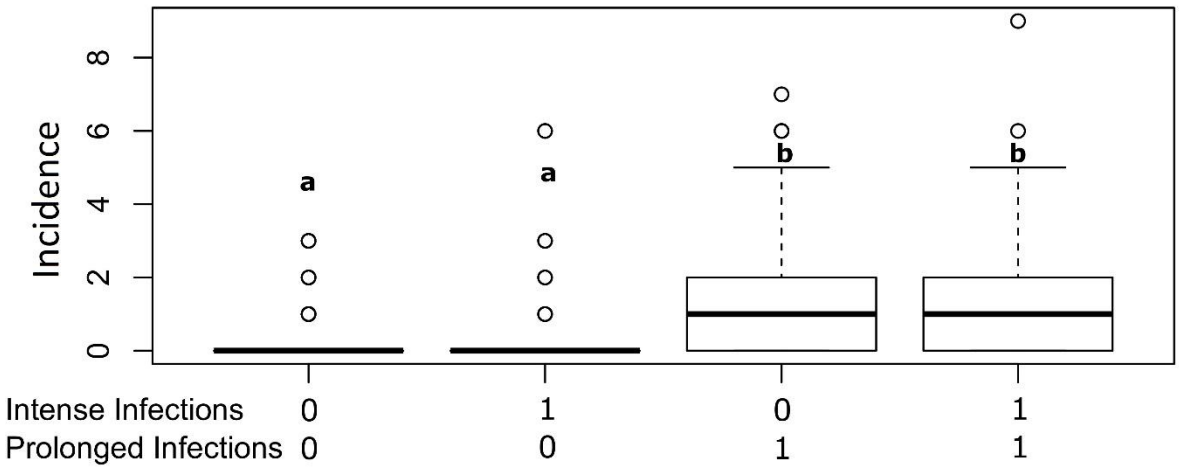

Figure 4.3: Box and whisker plots of our estimate of incidence of new infections within 2 days of arrival of a fish from another tank, as a function of whether the new fish in the tank was categorized as a fish with intense infection and/or prolonged infection ("1": yes; "0": no) throughout the experiment.

Data pooled from all 8 metapopulations and all migration days (which occurred at 10-day intervals). For fish that migrated more than once, we show the total number of infections they caused over all migrations. Identical letters above error bars designate treatments that are not significantly different from one another.

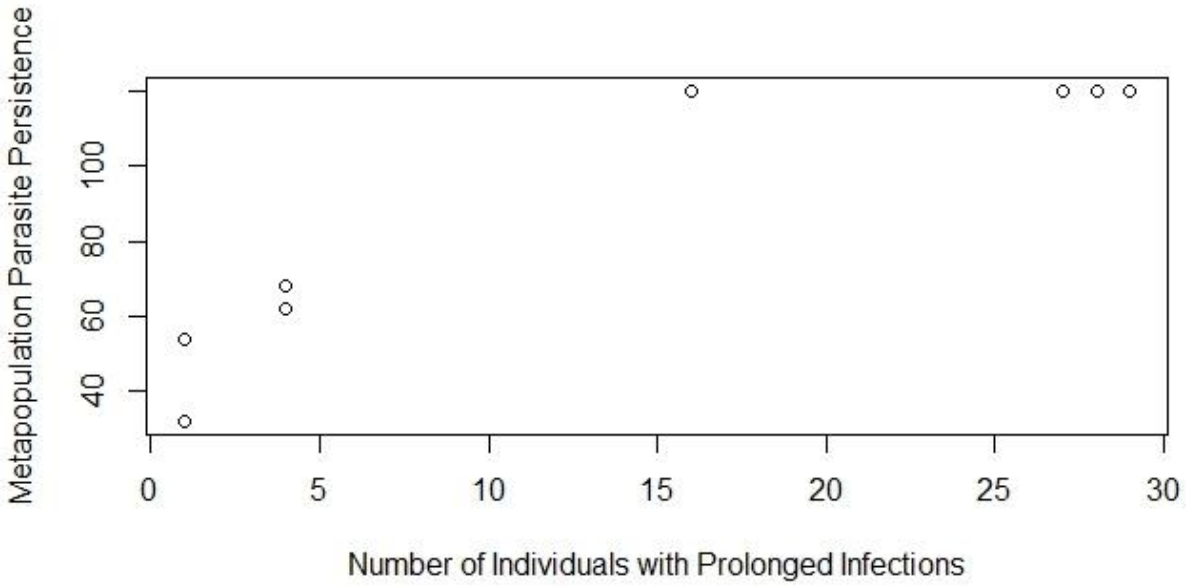

Figure 4.4: Parasite persistence in the metapopulation (days) as a function of the number of individuals in the metapopulation with prolonged infections.

Note that the experiment was terminated at 120 days, so we do not have information on how the presence of those individuals might prolong metapopulation parasite persistence beyond that endpoint.

4.14 SUPPLEMENTAL MATERIALS

Table 4.1: Post-hoc pairwise comparisons of infection categories for outcome variables.

ANOVA or Kruskal-Wallis results are listed in heading of each section for each outcome variable. Significant p-values are noted with an asterisk

Tukey Test Standard Length by Infection Category (ANOVA df=3, p<0.001)

	Diff.	Lower	Upper	p
Neither-Intense	0.49	-0.31	1.29	0.39
Neither-Prolonged	0.19	-0.63	1.02	0.92
Neither-Both	1.07	0.09	2.05	0.02*
Prolonged-Intense	0.19	-0.63	1.02	0.92
Prolonged-Both	0.38	-0.61	1.39	0.74
Intense-Both	0.58	-0.48	1.65	0.49

Tukey Test Weight by Infection Category (ANOVA df=3, p=0.005)

	Diff.	Lower	Upper	p
Neither-Intense	0.00	-0.01	0.02	0.65
Neither-Prolonged	0.01	0.00	0.03	0.04*
Neither-Both	0.02	0.00	0.04	0.01*
Prolonged-Intense	0.00	-0.01	0.02	0.64
Prolonged-Both	0.01	-0.01	0.03	0.66
Intense-Both	0.01	-0.00	0.04	0.19

Dunn test Number of Contacts by Infection Category (Kruskal-Wallis: chi-squared=141.76 df=3, p<0.001)

	z	p

Neither-Intense	1.72	0.04
Neither-Prolonged	-9.63	<0.0001*
Neither-Both	-5.18	<0.0001*
Prolonged-Intense	-9.95	<0.0001*
Prolonged-Both	1.78	0.03
Intense-Both	-6.05	<0.001*

Total Incidence by Infection Category (Kruskal-Wallis: chi-squared=37.59, df=3, p<0.001)

	z	**p**
Neither-Intense	0.12	.4
Neither-Prolonged	-4.58	<0.0001*
Neither-Both	-4.3	<0.0001*
Prolonged-Intense	-4.06	<0.0001*
Prolonged-Both	-0.96	0.16
Intense-Both	-4.06	<0.0001*

Average Incidence by Infection Category (Kruskal-Wallis: chi-squared=29.92, df=3, p<0.0001)

	z	**p**
Neither-Intense	0.17	0.4
Neither-Prolonged	-3.87	<0.0001*
Neither-Both	-4.06	<0.0001*
Prolonged-Intense	-3.49	0.0002*
Prolonged-Both	-1.21	0.11
Intense-Both	-3.85	0.0001*

Table 4.2: Analysis of occurrence of individuals with prolonged and intense infections and their interactions on metapopulation outcomes of peak parasite load and parasite persistence.

Models were simplified by removing non-significant interactions and then each variable if not significant. For each analysis, the model with the lowest AIC was chosen as the final result. Mortality and peak prevalence are not shown, as no results were significant for those models.

GLM negative binomial distribution Metapopulation Peak Burden

	Estimate (+/- standard error)	z	p
Number of individuals with Intense Infections	-0.06 (0.04)	-1.58	0.11
Number of Individuals with Prolonged infections	-0.08 (0.03)	-2.43	0.01*
Interaction	0.01 (0.003)	2.74	0.005*

GLM Negative Binomial Distribution Metapopulation Parasite Persistence

	Estimate (+/- standard error)	z	p
Number of Individuals with Prolonged infections	0.03 (0.005)	5.52	<0.0001*

CONNECTING STATEMENT 3

In my final Chapter, I use data obtained from experiments in Chapters 2 and 3 to inform a mathematical model to better describe dynamics of the guppy-*Gyrodactylus* system. We use the vast amount of data obtained to train and test a mathematical model that improves upon early work with this system when little was known about the dynamics of this system. We then conduct a sensitivity analysis and determine which parameters have the greatest influence on outbreak dynamics. By predicting the factors for which heterogeneity may have the largest impact on the system, we can prioritize factors that warrant further investigation and incorporation into future models.

I see this final chapter as the theoretical connection between the experimental work and findings of my thesis with future directions for further work. My experiments explore heterogeneity and connectivity at different scales and their impact on host-parasite dynamics. This model assumes homogeneity at the individual-level and accurately describes the general dynamics of the system, but the results of the sensitivity analysis imply that individual heterogeneity in resistance can have large impacts on epidemic dynamics, highlighting the need for a more individual-based model that I hope to develop in the future.

5 CHAPTER 5: A DATA-VALIDATED HOST-PARASITE MODEL FOR INFECTIOUS DISEASE OUTBREAKS

Christina P. Tadiri[*], Jude D. Kong[*], Gregor F. Fussmann, Marilyn E. Scott, and Hao Wang. (2019) A data-validated mathematical model for guppy-*Gyrodactylus* interactions. *Frontiers in Ecology and Evolution* 7, 307.

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5.1 ABSTRACT

The use of model experimental systems and mathematical models is important to further understanding of infectious disease dynamics and strategize disease mitigation. Gyrodactylids are helminth ectoparasites of teleost fish which have many dynamical characteristics of microparasites but offer the advantage that they can be quantified and tracked over time, allowing further insight into within-host and epidemic dynamics. In this paper, we design a model to describe host-parasite dynamics of the well-studied guppy-*Gyrodactylus turnbulli* system, using experimental data to estimate parameters and validate it. We estimate the basic reproduction number ($\mathcal{R}_0$), for this system. Sensitivity analysis reveals that parasite growth rate, and the rate at which the guppy mounts an immune response have the greatest impact on outbreak peak and timing both for initial outbreaks and on longer time scales. These findings highlight guppy population average resistance and parasite growth rate as key factors in disease control, and future work should focus on incorporating heterogeneity in host resistance into disease models and extrapolating to other host-parasite systems.

Keywords: Epidemic dynamics, mathematical model, guppy, *Gyrodactylus,* host-parasite interactions

5.2 INTRODUCTION

The guppy-*Gyrodactylus* system is a well-known model host-parasite system, used in numerous experimental and field studies (1-3). Guppies, *Poecilia reticulata,* are a common ovoviviparous tropical teleost fish whose abundance and ability to survive a broad range of environmental

variables and availability in pet stores worldwide have made them ideal subjects for research in various disciplines. *Gyrodactylus* spp. (Monogenea) are ectoparasites which feed on the epithelial cells and mucus of many marine and freshwater teleost fish species (4). They attach to the epidermis of their host via specialized hooks and are directly transmitted primarily by jumping to a new host during contact (5, 6). They also reproduce directly on the host, with the developing embryo containing within itself a second developing embryo, which allows for rapid population growth of the parasite directly on an infected host (4, 6). Upon infection, hosts mount an immune response, including mucus secretion (7), as well as a non-specific complement which kills gyrodactylids (4, 8-10). Gyrodactylid infection can result in high rates of mortality (11), and induce a temporary refractory period in surviving hosts (1, 12).

In general, parasites are typically divided into two categories: microparasites (such as viruses and bacteria) which are microscopic and tend to proliferate and transmit rapidly, often leading to high morbidity and mortality and inducing acquired resistance in surviving hosts and therefore causing periodic epidemics, while macroparasites (such as worms and insects) often have more complex life cycles and persist in populations, often with overdispersed distributions among hosts and rarely causing severe morbidity and mortality or acquired resistance (13, 14). Due to their rapid growth rate and infection-induced refractory period, gyrodactylids cause periodic epidemic outbreaks, making their population dynamics typical of microparasites like viruses and bacteria (13) despite being helminths which traditionally fall into the macroparasite category. However, they have a key distinction from other typical microparasites in that parasite

population size or burden is a central factor in determining host-parasite dynamics, as it directly influences transmission, mortality and several other parameters. Intrinsic population dynamics of *Gyrodactylus* sp. on isolated fish have been identified under standardized environmental conditions (1, 15). Both short- and long-term dynamics of *Gyrodactylus* sp. within laboratory populations of guppies have also been observed (2, 16-18). However, the need for a comprehensive model that can describe and make predictions for this system and others like it still exists.

Traditional microparasite Susceptible, Infected, Recovered (SIR) models can effectively describe epidemic dynamics of infectious diseases for which the parasite population size is unknown or less relevant than host category of infection (13, 19, 20). Yet, SIR models are less applicable to parasites such as *Gyrodactylus* spp., where parasite burden plays a crucial role in host-parasite population dynamics. Although macroparasite models directly consider parasite number, they also often overlook dynamics in parasite numbers within individual hosts (13, 21, 22). As gyrodactylids and many other parasites do not fit neatly into the micro-/ macro-parasite dichotomy, there is a clear need for a unifying framework which considers both host and parasite populations (23). Previous efforts to mathematically describe this system using various types of models have captured basic initial epidemic dynamics, but failed to effectively describe longer-term fluctuations due to gradual loss of immunity over time (5, 24). Similarly, infection dynamics on individual fish have been simulated, but the broader scale transmission and population dynamics were not incorporated (24). The objective for this paper is to establish a

mathematical model that effectively describes experimental data on guppy-*Gyrodactylus* dynamics, particularly with regards to host immunity waning and longer-term dynamics and to estimate the sensitivity to various parameters that we have not been able to effectively test in the laboratory. Ideally, this model can be applied to other directly transmitted, directly reproducing parasites for which parasite burden impacts host-parasite relations, with a waning immunity post-infection.

5.3 METHODS

The guppy-*Gyrodactylus* system shares some key characteristics with common directly transmitted infectious disease dynamics (e.g., infection-induced host mortality, refractory period, infection by host-to-host contact). Therefore, we design an SIR-type model with distributed delay (which captures the varying immunity period of the guppy) to describe the dynamics of guppies and *Gyrodactylus*. Since the guppy immune response plays a crucial role in eliminating *Gyrodactylus*, we explicitly integrate the dynamics of the immune response into the model. Thereafter, the distributed delay model is converted to an equivalent system of ordinary differential equations using the linear chain approach. Next, we ensure that non-negative initial values do not give rise to a negative solution. To determine the *Gyrodactylus* basic reproduction number ($\mathcal{R}_0$), the stability analysis of the *Gyrodactylus*-free equilibrium point was performed. This threshold is particularly of use because it allows us to determine the maximum potential number of *Gyrodactylus* that will be produced due to the introduction of one *Gyrodactylus* in a *Gyrodactylus*-free population of guppies, which can help inform control measures. Model

parameters unavailable in the literature were estimated by data fitting using previously published experimental data. Next, the model was validated by comparison to measurements from independent but analogous laboratory experiments. Finally, using the estimated parameters, together with the parameters from the literature, the sensitivity of the outbreak peak magnitude and the time to outbreak peak to the parameters of the model was determined. This sensitivity could be useful in determining the most influential parameters for designing control measures.

5.3.1 DERIVATION OF THE MODEL

In this section we derive a guppy-*Gyrodactylus* interaction model with distributed delay. Figure 5.1 provides a conceptual flowchart for the system. The model consists of four coupled equations tracing the rates of change of guppy population (G), guppy immune response (Y) and *Gyrodactylu*s population (X). The guppy total population is divided into three sub-groups: susceptible (S), infected (I) and recovered (R) guppies. The change in number of susceptible guppies could be due to (1) birth by any guppy (we assume all guppies are born susceptible), (2) loss of immunity by a recovered guppy, (3) death of a susceptible guppy or (4) becoming infected due to contact with an infected guppy. We assume the guppy population to be homogenous and the natural birth rate of the guppy is assumed to be constant, α. The birth rate of infected individuals is diminished by a function that is linearly proportional to the number of parasite it harbours, which we assumed to be $\eta\left(\frac{X}{I}\right) = e^{-\xi\frac{X}{I}}$, where ξ is the steepness of parasite-induced fecundity reduction. Although it is unclear whether guppy fecundity is reduced by *Gyrodactylus* infection, this is the case for many infectious diseases, including those of fish (25)

so we allow for it in our model, while defaulting the parameters to 0 in our simulations because in our experimental populations no birth was observed. Instead of the exponential growth assumed in typical SIR-type models, we consider a logistic growth for the guppy population because uninfected guppies exhibit density-dependent population growth up to a carrying capacity K (26). Thus, the growth rate of the guppy is $\alpha\left(S+\eta\left(\frac{X}{I}\right)I+R\right)\left(1-\frac{S+I+R}{K}\right)$. Transmission ($\beta$), the rate at which susceptible fish become infected, is described by the function $\beta\left(\frac{X}{I}\right)$:

$$\begin{cases} \beta = 0, & if\ \frac{X}{I} \leq 1 \\ \beta\left(\frac{X}{I}\right) = b\frac{X}{I}, & if\ \frac{X}{I} > 1 \end{cases}$$

where b is a constant. The natural death rate of guppies is assumed to be a constant d. The parasite mean intensity is represented as $\frac{X}{I}$. The population of infected guppies can increase when an infected guppy contacts a susceptible guppy, resulting in transmission, and decreases when any one of them dies or recovers. In addition to the natural death rate of guppies, infected guppies may also be killed by *Gyrodactylus* at a rate described by the function $\delta\left(\frac{X}{I}\right) = \mathcal{E}\frac{X}{I}$ where $\mathcal{E}$ is a constant. The recovery rate function is assumed to be directly proportional to the average immune response Y (i.e. recovery function $\propto Y$) and inversely proportional to the parasite intensity $\frac{X}{I}$ (i.e. recovery function $\propto \frac{1}{X/I}$), implying that the recovery rate function is proportional to $\frac{Y}{X/I}$ (i.e., the recovery rate function is $\lambda\frac{Y}{X/I}$ where λ is the proportionality

constant). Immunity is assumed to affect both the rate of parasite population growth and the rate at which infected fish become recovered and recovered fish regain susceptibility. Every recovered guppy is assumed to acquire an immunity that wanes with time following initial infection. To model immunity waning, we assume that the immunity period of every fish varies from 0 to ∞ in order to capture the wide variability in the period of acquired resistance observed among many guppy populations (1-3, 12). We let $g(\psi)$ denote the probability density function that a fish takes exactly ψ time units to lose its immunity after recovering from infection, which implies that the probability that a fish's immunity is lost τ time units after recovering from infection, is $\int_0^{\tau} g(\psi)d\psi$. Thus the probability that the fish's immunity is not lost τ time units after recovering from infection is $\int_{\tau}^{\infty} g(\psi)d\psi$. We assume that at a time $t-\tau$, $\lambda\frac{Y(t-\tau)I(t-\tau)}{X(t-\tau)}I(t-\tau)$ fish left the infected population compartment and joined the recovered population. The probability that these fish are still alive τ time units after leaving the infected compartment is $e^{-d\,\tau}$. Hence the total number of recovered fish at time t, is:

$$R(t) = \int_0^{\infty} \lambda\frac{Y(t-\tau)I(t-\tau)}{X(t-\tau)}I(t-\tau)\,e^{-d\,\tau}\int_{\tau}^{\infty} g(\psi)d\psi d\tau.$$

$$R(t) = \int_{-\infty}^{t} \lambda\frac{Y(\tau)I(\tau)}{X(\tau)}I(\tau)\,e^{-d\,(t-\tau)}\int_{t-\tau}^{\infty} g(\psi)d\psi d\tau. \qquad (1)$$

$$\Rightarrow \frac{dR}{dt} = \lambda \frac{Y}{X} I^2 - \int_0^{\infty} \lambda \frac{Y(t-\tau)I(t-\tau)}{X(t-\tau)} I(t-\tau)\, e^{-d\,\tau} g(\tau) d\tau - dR$$

We consider $g(\tau)$ to be the density function for a gamma distribution $g(\tau) \coloneqq \frac{c^n \tau^{n-1} e^{-c\tau}}{(n-1)!}$, $n = 1,2,3, \ldots, c > 0$, and $\frac{n}{c}$ to be the average duration of the immune memory. As the number of parasites increases, the guppy immune system gradually builds a defence against the parasite at a rate $f\left(\frac{X}{I}\right) = \frac{\frac{\theta X}{I}}{\kappa + \frac{X}{I}}$, proportional to the density of non-specific immune complement responsible for killing *Gyrodactylus*, where θ is the maximum rate of increase of immunity. This defence gradually reduces the guppy parasite carrying capacity. The per capita growth rate of the parasite follows a logistic growth: $\left(1 - \frac{X}{P(Y)I}\right)$, where $p(Y)$ is the average parasite carrying capacity of an infected guppy. $p(Y)$ is assumed to be an exponentially decreasing function of the average immune response of the guppy $p(Y) = re^{-\gamma Y}$, where r and γ are constants. The natural parasite death rate is assumed to be a constant, ω. We assume that when a guppy dies, all the parasites on it die, since dead guppies are more likely to be predated or washed downstream (27). In our experiments, dead fish were removed from tanks less than one day after death in order to minimize transmission from dead fish, but it's possible some could have occurred. Thus, the total per capita parasite death rate is $\omega + d + \delta\left(\frac{X}{I}\right)$. Thus, we have the following system of delay differential equations:

$$\frac{dS}{dt} = \alpha\left(S + \eta\left(\frac{X}{I}\right)I + R\right)\left(1 - \frac{S+I+R}{K}\right) - \frac{\beta\left(\frac{X}{I}\right)SI}{S+I+R} - dS$$
$$+ \int_0^\infty \lambda \frac{Y(t-\tau)I(t-\tau)}{X(t-\tau)} I(t-\tau)\, e^{-d\,\tau} g(\tau) d\tau$$
$$\frac{dI}{dt} = \frac{\beta\left(\frac{X}{I}\right)SI}{S+I+R} - \left(d + \delta\left(\frac{X}{I}\right)\right)I - \frac{\lambda YI}{X} I$$
$$\frac{dR}{dt} = \frac{\lambda YI}{X} I - \int_0^\infty \lambda \frac{Y(t-\tau)I(t-\tau)}{X(t-\tau)} I(t-\tau)\, e^{-d\,\tau} g(\tau) d\tau - dR \qquad (2)$$
$$\frac{dX}{dt} = \mu X\left(1 - \frac{X}{p(Y)I}\right) - \left(d + \delta\left(\frac{X}{I}\right)\right)X - \omega X$$
$$\frac{dY}{dt} = Y\left(\frac{\frac{\theta X}{I}}{\kappa + \frac{X}{I}} I - \nu\right)$$
$$\big(S(s), I(s), R(s), X(s), Y(s)\big) = \big(\phi_1(s), \phi_2(s), \phi_3(s), \phi_4(s), \phi_5(s)\big), s \in (-\infty, 0]$$

where $\phi_i, i = 1, 2, 3, 4\ and\ 5$ are bounded continuous functions, μ is the maximum per capita parasite growth rate. We assume that

$$X(I < 1) = 0$$

5.3.2 Reduction to an Ordinary Differential Equation Model

We assume that $g(\tau) = ce^{-c\tau}$ $(i.e. n = 1)$ and apply the chain trick method (28) to convert System (2) to a system of ordinary differential equations: Let

$$\bar{S} = \int_0^\infty \frac{Y(t-\tau)I(t-\tau)}{X(t-\tau)} I(t-\tau)\, e^{-d\,\tau} g(\tau) d\tau$$

$$= \int_0^{\infty} \frac{Y(t-\tau)I(t-\tau)}{X(t-\tau)} I(t-\tau)\, e^{-d\,\tau} c e^{-c\tau} d\tau$$

$$= \int_{-\infty}^{0} \frac{Y(u)I(u)}{X(u)} I(u)\, e^{-d\,(t-u)} c e^{-c(t-u)} du$$

$$= c e^{-(c+d)t} \int_{-\infty}^{0} \frac{Y(u)I(u)}{X(u)} I(u)\, e^{-(d+c)u} du$$

$$\Rightarrow \frac{d\bar{S}}{dt} = c\big(-(c+d)\big) e^{-(c+d)t} \int_{-\infty}^{0} \frac{Y(u)I(u)}{X(u)} I(u)\, e^{-(d+c)u} du + c e^{-(c+d)t} e^{(c+d)t} \frac{Y(t)I(t)}{X(t)} I(t)$$

$$= (c+d)\bar{S} + c\frac{Y(t)I(t)}{X(t)} I(t)$$

Substituting this in System (2) reduces it to the following system of ODE:

$$\begin{aligned}
\frac{dS}{dt} &= \alpha\left(S + \eta\left(\frac{X}{I}\right)I + R\right)\left(1 - \frac{S+I+R}{K}\right) - \frac{\beta\left(\frac{X}{I}\right)SI}{S+I+R} - dS + \lambda\bar{S} \\
\frac{dI}{dt} &= \frac{\beta\left(\frac{X}{I}\right)SI}{S+I+R} - \left(d + \delta\left(\frac{X}{I}\right)\right)I - \frac{\lambda Y I}{X} I \\
\frac{dR}{dt} &= \frac{\lambda Y I}{X} I - \lambda\bar{S} - dR \\
\frac{dX}{dt} &= \mu\, S\left(1 - \frac{X}{p(Y)I}\right) - \left(d + \delta\left(\frac{X}{I}\right)\right)X - \omega X \\
\frac{dY}{dt} &= Y\left(\frac{\frac{\theta X}{I}}{\kappa + \frac{X}{I}} I - \nu\right) \\
\frac{d\bar{S}}{dt} &= (c+d)\bar{S} + c\frac{Y(t)I(t)}{X(t)} I(t) \\
\big(S(0), I(0), R(0), X(0), Y(0)\big) &= \big(\phi_1(0), \phi_2(0), \phi_3(0), \phi_4(0), \phi_5(0)\big)
\end{aligned} \tag{3}$$

$$\bar{S}(0) = \int_0^{\infty} \frac{\phi_4(-\tau)\phi_2(-\tau)}{\phi_3(-\tau)} \phi_2(-\tau)\, ce^{-(c+d)\tau} d\tau$$

5.3.3 POSITIVITY AND BASIC REPRODUCTION NUMBER

In this section, we show that nonnegative initial data give rise to nonnegative solutions, establish conditions for the existence and stability of the *Gyrodactylus*-free equilibrium point of the system and determine the basic reproduction number.

5.3.3.1 Positivity

Positivity and boundedness of a model guarantee that the model is biologically well behaved. For positivity of the System (3), we have the following theorem:

Theorem 1. *All solutions of System (3) are positive for all t in* $(0, \infty)$

Proof. We need to show that $S(t) \geq 0,\ \ I(t) \geq 0,\ R(t) \geq 0,\ Y(t) \geq 0,\ X(t) \geq 0,\ \bar{S}(t) \geq 0$ for $S(0) \geq 0, I(0) \geq 0, R(0) \geq 0, Y(0) \geq 0, X(0) \geq 0$ *and* $\bar{S}(0) \geq 0$. Note that $\frac{X}{I} = 0, \frac{I}{X} = 0$ and $\frac{Y}{X} = 0$ for $X < 1$ or $I < 1$. We start by proving that if $I(0) \geq 0, \Rightarrow I(t) \geq 0$ for all $t > 0$. From the second equation of Systems (3), we see that $\dot{I}(I = 0) = 0$. Thus $I(t) \geq 0$ for $t \geq 0$. Also, from the third equation, we have that $\dot{X}(X = 0) = 0$ for $X(0) \geq 0$. Hence $X(t) \geq 0$ for $t \geq 0$. From the last equation, we have that $\dot{\bar{S}}(\bar{S} = 0) = \frac{cY}{X} I^2$. Since $I(t) \geq 0$ and $\frac{X}{Y} \geq 0$ for $t \geq 0, I(0) \geq 0$ and $\frac{X(0)}{Y(0)} \geq 0$, we have that $\dot{\bar{S}}(\bar{S} = 0) \geq 0$, $t \geq 0$ and $\bar{S}(0) \geq 0$. The non-negativity of $R(t)$ for $t \geq 0$ follows from the integral

representation (1) and non-negativity of $\frac{Y(t)}{X(t)}$. From the first equation, we have that

$$\dot{S}(S=0) = \alpha\left(\eta\left(\frac{X}{I}\right)I + R\right)\left(1 - \frac{I+R}{K}\right) + \lambda\bar{S}.$$

From the non-negativity of $\frac{X}{I}, I, R$, we have that if $S(0) \geq 0$ implies that $S(t) \geq 0$ for $t \geq 0$.

5.3.3.2 *Gyrodactylus*-free Equilibrium Point (GFE) and $\mathcal{R}_0$

For the GFE we have that $X = 0$, implying that I, R, Y and $\bar{S}$ are null. Plugging this in System (3), we have that $S = \frac{k(\alpha-d)}{\alpha}$. Since $S > 0$, we have that the GFE exists iff $\alpha > d$ and is given by $\left(\frac{k(\alpha-d)}{\alpha}, 0, 0, 0, 0, 0\right)$. The linearized system corresponding to this equilibrium point is:

$$\begin{pmatrix} \dot{S} \\ \dot{I} \\ \dot{R} \\ \dot{X} \\ \dot{Y} \\ \dot{\bar{S}} \end{pmatrix} = \begin{pmatrix} d-\alpha & 2d-\alpha & 2d-\alpha & d\eta - b & 0 & \lambda \\ 0 & -d & 0 & b-\varepsilon & 0 & 0 \\ 0 & 0 & -d & 0 & 0 & -\lambda \\ 0 & 0 & 0 & \mu - d - \omega & 0 & 0 \\ 0 & 0 & 0 & 0 & -\nu & 0 \\ 0 & 0 & 0 & 0 & 0 & -(c+d) \end{pmatrix} \begin{pmatrix} S \\ I \\ R \\ X \\ Y \\ \bar{S} \end{pmatrix}$$

The corresponding eigenvalues are:

$$\lambda_1 = d - \alpha,\ \lambda_2 = -d,\ \lambda_3 = -d, \lambda_4 = \mu - d - \omega, \lambda_5 = -(c + d).$$

$\lambda_1, \lambda_2, \lambda_3\ and\ \lambda_5$ are all less than zero and λ_4 is less than zero iff $\mu < d + \omega$. This leads to the definition of the *Gyrodactylus* basic reproduction number, $\mathcal{R}_0 := \frac{\mu}{d+\omega}$. Observe that GFE is locally asymptotically stable iff $\mu < d + \omega$, i.e. GFE is locally asymptotically stable iff $\mathcal{R}_0 < 1$ and unstable if $\mathcal{R}_0 > 1$. Therefore, if $\mathcal{R}_0 < 1$, the parasite dies out and if $\mathcal{R}_0 > 1$, the parasite

will invade the guppy population. $\mathcal{R}_0 = 1$, is a threshold below which the *Gyrodactylus* dies out and above which there is an outbreak. $\mathcal{R}_0$ has an intuitive biological interpretation: it is the average number of *Gyrodactylus* resulting from the introduction of a single *Gyrodactylus* into an otherwise *Gyrodactylus*-free population over the course of its life span.

5.3.4 Parameter Estimation and Model Validation Using Independent Measurements

We used data obtained from separate laboratory previously published experiments to respectively estimate the model parameters not available in the literature and to test the fit of the model. To estimate the model parameters we used experimental data averaged from four groups of eight male fish where one fish per group was infected with two parasites and the infection was allowed to spread naturally throughout the tank (29). These fish were bred in the lab from pet-store "feeder" guppies. To test the model, we used experimental data averaged from four groups of eight fish (four males and four females) where parasites were introduced to each group via a donor juvenile fish infected with three parasites that was removed once at least three parasites had naturally transferred to the experimental fish (17). These fish were third-generation lab reared fish bred from 33 original family lines originally obtained from wild populations in Trinidad but mixed haphazardly in experimental tanks. In both cases, 2-3 parasites were introduced in order to keep the introduction as close to one as possible while minimizing the probability of accidental parasite death or that an old or male parasite would be introduced preventing reproduction. No difference in host-parasite dynamics was found among all-male, all-female and mixed sex groups of eight fish (Tadiri et al 2016).

In both experiments, each fish was individually marked, and the number of parasites on each fish was counted every other day to obtain SIR numbers and total parasite population size. In all our experimental groups, no birth was observed within the 42 days. Hence, we ignore vital dynamics for guppies in the model.

To estimate the parameter values of System (3), we use the nonlinear regression function nlinfit(.) in MATLAB. The function nlinfit(.) uses the Levenberg-Marquardt algorithm (30) to fit the solution of the biodegradation module to the data. Some of parameters used in solving System (3) namely ω, d, and K, were taken from the literature (5, 26): the units, values and source of these parameters are provided in Table 5.1.

The validity of our model in predicting *Gyrodactylus* outbreak was evaluated by using the estimated parameters in the model to generate S, I, R *Gyrodactylus* and immune response data then comparing the predicted data to measured data using the goodnessOfFit(.) function in MATLAB.

5.3.5 SENSITIVITY ANALYSIS

The objective of this subsection is to discuss the sensitivity of the magnitude of the initial *Gyrodactylus* outbreak peak, and time to the initial outbreak peak to the parameters of the system. For this analysis, we use the normalized forward sensitivity index (31):

$$\textit{sensitivity index (S.I.)} = \left(\frac{\partial F^*}{\partial(parameter)}\right)\left(\frac{parameter}{F^*}\right) \qquad (4)$$

where F^* is the quantity being considered.

Since we do not have the explicit formula for the initial outbreak peak, or time to peak, we use central difference approximation to estimate them:

$$\frac{\partial F^*}{\partial parameter} = \frac{F^*(parameter + h) - F^*(parameter - h)}{2h} + O(h^2).$$

Letting $h = 1\%$ of the parameter value (P), Equation (4) becomes:

$$S.I. = \frac{F^*(1.01P) - F^*(0.99P)}{0.02(F^*(P))} \qquad (5)$$

5.3.6 Longer-term Dynamics and Generic Sensitivity Analysis

In this section, we simulate the longer-term parasite dynamics in a system that allows for guppy vital dynamics. We use an average birth rate estimated from literature of 0.4/fish/day (26) with no infection-induced reduction in fecundity. We equally assess the sensitivity of the generic outbreak peaks and periods to the parameters of the system from the long-term simulation.

5.4 Results

5.4.1 System Basic Reproduction Number and Gyrodactylus-Free Equilibrium Point

Using the parameters in Table 5.1 we have that $\mathcal{R}_0 = 2.63$. Since $\mathcal{R}_0 > 1$ for the estimated parameters values, the GFE is not asymptotically stable, meaning that an introduction of one

Gyrodactylus into a naïve guppy population would result in an outbreak. Next, we illustrate the dynamics of the system for $\mathcal{R}_0 < 1$ and for $\mathcal{R}_0 > 1$ using values very close to one. Rearranging the fourth equation of System (2), we have:

$$\begin{aligned}\frac{dX}{dt} &= \mu X\left(1 - \frac{X}{p(Y)I}\right) - \left(d + \delta\left(\frac{X}{I}\right)\right)X - \omega X \\ &= (d+\omega)(\Re_0 - 1)X - X\left(\frac{\mu X}{p(Y)I} + \delta\left(\frac{X}{I}\right)\right) \qquad (6)\end{aligned}$$

Figure 5.2 shows the long-term behaviour of the *Gyrodactylus* population for $\mathcal{R}_0 = 0.9(< 1)$ (A), $\mathcal{R}_0 = 1.1$ and $\mathcal{R}_0 = 2.63(> 1)$ (C) respectively. When $\mathcal{R}_0 < 1$, the system will stabilize to its *Gyrodactylus* free equilibrium $\left(\frac{k(\alpha-d)}{\alpha}, 0, 0, 0, 0, 0\right)$ (Panel A). The number of *Gyrodactylus*, the number of infected guppies, the number of recovered guppies and the guppy immune compliment density tend to zero as t increases. When $\mathcal{R}_0 > 1$, there will be a *Gyrodactylus* outbreak (Panel B and C). These outcomes are robust for large sets of initial values and parameter values.

5.4.2 FITTING THE MODEL TO DATA

Table 5.1 contains the value of the parameters obtained from fitting System (2) to the experimental data described above. Figure 5.3 shows the simulated susceptible, infected, recovered guppies and *Gyrodactylus* dynamics along with measured data. We obtained a goodness-of-fit statistic (NMSE) value of 0.99. This statistic indicates that the model is able to predict the training data accurately.

5.4.3 Model Evaluation Using NMSE

Using the parameter values in Table 5.1, with the procedure described above, we assess the validity of our model in predicting guppy-*Gyrodactylus* dynamics data. Figure 5.4 shows a comparison between our simulated and measured data. The goodness-of-fit statistics suggests that System (2) with the given parameter values is a good fit for guppy-*Gyrodactylus* dynamics data (NMSE = 0.70).

5.4.4 Sensitivity of the Magnitude of the Initial Outbreak Peak

The sensitivity indices of the magnitude of the peak of the initial outbreak measure how the magnitude of the peak of the initial outbreak depends on different parameters. Table 5.2 contains the sensitivity indices of the amplitude of the first outbreak peak obtained using Equation (5). The two parameters with the greatest independent influence on the system were parasite increase rate (μ), and maximum rate of increase of immunity (θ).

5.4.5 Sensitivity of the Time to Initial Outbreak Peak

Sensitivity indices of the time to initial outbreak peak measure how the first epidemic outbreak time depends on different parameters as seen in the Table 5.3. Similar to the sensitivity of the magnitude of the peak of the first outbreak, the parasite increase rate (μ) and the maximum rate of the increase of immunity (θ) were the most influential parameters.

5.4.6 Longer-Term Dynamics

By allowing for natural guppy birth in our systems, we are able to simulate steady state oscillating dynamics such as those expected of epidemic infectious diseases with generic

peaks and periods (Figure 5.5).

5.4.7 Sensitivity of the Generic Outbreak Peak Magnitude

Sensitivity indices of the generic outbreak peak measure how the magnitude of subsequent outbreak peaks under steady state oscillating dynamics depend on different parameters (Table 5.4). The guppy immune related parameters, θ (maximum rate of increase of immunity and v (rate of decay of immunity) have the strongest relationship to the outbreak peak. The negative value of the sensitivity of the outbreak to θ, indicates that a low value of θ will lead to a more severe parasite outbreak. The positive value of the sensitivity of the outbreak peak to v, on the other hand, tells us that if the guppy immunity wanes faster, there will be a severe outbreak.

5.4.8 Sensitivity of the Generic Outbreak Period

Sensitivity indices of the generic outbreak period measure how the time to subsequent outbreak peaks under steady state oscillating dynamics depend on different parameters (Table 5.5). The parasite increase rate (μ) and the maximum rate of increase of immunity (θ) have the strongest relationship to the outbreak period.

5.5 Discussion

In this paper, we define a mathematical model that effectively describes guppy-*Gyrodactylus* dynamics in small populations. In estimating parameters based on both literature and our own experimental data we determined our model to accurately describe the dynamics of this system. Additionally, we validated our model using a neutral data set

from a separate experiment and found that it fit reasonably well. We also find the model to be mathematically and biologically sound through our analysis of $\mathcal{R}_0$, which indicates that an outbreak will occur when the $\mathcal{R}_0$ is greater than one and that the system will stabilize to a *Gyrodactylus*-free equilibrium when $\mathcal{R}_0$ is less than one. With our parameters, $\mathcal{R}_0$ was greater than one, indicating that an outbreak will occur in our system with the introduction of one parasite. This model builds off previous efforts to model this system, (5) but incorporates a more realistic representation of immunity. Firstly, and most importantly, we describe fish immune response to infection in its own equation, rather than assuming a linear constant to represent immunity. We specifically also describe the waning of immunity post-infection using a distributed delay function, which allows for repeated, dampening cycles of outbreaks without constant immigration of naive hosts. We also allow for host population growth rather than fixed immigration and consider a parasite-induced reduction in fecundity (32), which had not been investigated at the time of previous models. Longer-term simulations using population growth estimates from literature with our other parameter estimates from experiments demonstrate that our model is capable of describing oscillating parasite dynamics typical of those observed in the wild (27). These developments are important to more accurately explaining guppy-*Gyrodactylus* dynamics and could have a broader applicability to other systems as well.

Gyrodactylus are a large genus of over 400 ectoparasites infecting at least 20 orders of teleost fish (33), and our model can most directly be applied to other species of this genus.

Gyrodactylids have had significant economic impact, causing epizootics in many resource fish such as carp, trout and African catfish (9) and, most notably Atlantic salmon fisheries, particularly in Norway in the 1960's and '70's which saw large declines due to *G. salaris* (4, 34), and efforts to recover these populations and prevent disease spread to other watersheds are still ongoing (35). Since the basic life cycle of gyrodactylids and their relationships with their hosts are similar (4, 33) this model would only need reparameterization to be applied to a range of other aquaculture species. Beyond other gyrodactylids, many infectious diseases also confer immunity that decays over time. Our methods of applying a distributed delay to describe waning guppy immunity to *Gyrodactylus* are novel to this system, and can also be used for other infectious diseases with declining immunity, most notably being comparable to the waning of vaccine-induced immunity which has observed in many human diseases (36) such as measles (37), pertussis (38), malaria (39) and varicella (40) and modelled using different methods. Given the broader applicability of our methods, our results have important implications for disease management, as we identify the most impactful parameters on disease outbreaks, and thus crucial intervention points.

Our sensitivity analysis found that the most influential parameters on both initial outbreak amplitude and time to initial outbreak in our system were parasite increase rate (μ) and maximum increase rate of guppy immunity (θ). These results indicate parasite growth rate and host resistance play the strongest role in the severity and speed of an outbreak, which makes logical sense. The higher parasite growth rate, or lower the immune response, the

greater the parasite abundance will be. Given the small population sizes of our experiments, it is possible that host density may have affected the relative importance of these variables compared to the transmission rate or population size. The density of fish in our experiments was higher than wild populations (41), but lower than in commercial guppy populations (42), therefore an average approximation of the different conditions in which *Gyrodactylus* outbreaks may occur. Given the relatively short timescale of our experiments, we did not observe any impacts of longer-term parameters such as guppy birthrate or natural guppy mortality. However, sensitivity analysis of our generic outbreak magnitudes and periods consistently demonstrate that parasite virulence and parameters relating to guppy immunity have the strongest impact on our system and therefore this result was not an artefact of our experimental design. Both guppy resistance (11, 43), and parasite virulence (3) are known to evolve rapidly and vary widely among populations due to different selective pressures and our findings indicate that understanding this heterogeneity is of significance to predicting and controlling disease outbreaks.

One limitation of this model is that it was based on laboratory, rather than field data and large differences in both host mortality and parasite burdens have been observed between the lab and field settings. Gyrodactylids persist in the wild and are observed at typically low burdens, however mark-recapture experiments have suggested that infection can cause severe mortality (27) typical of epidemics and in aquaculture (34, 44), and laboratory (5, 11) settings, where outbreaks are known to cause severe disease and mortality. Also, in the wild,

guppies inhabit streams which are punctuated by “pools” separated by waterfalls, thus creating a network of populations among which unidirectional migration of hosts (and potentially parasites) downstream is possible (27, 45), however in our current model we focus only on populations in isolation. Furthermore, as previously mentioned, the population sizes were much smaller than those in a natural setting, and as such, the timescales used to estimate some of the longer-term parameters of our model, such as host birth and immunity waning to full susceptibility may not fully reflect dynamics in the wild (27). Nevertheless, our estimates obtained from both literature and short-term data show a good fit for our data and could potentially be directly applied to aquaculture settings with only reparameterization specific to the species of interest. Moreover, longer-term simulations with our model show its ability to predict longer-term fluctuating dynamics such as those observed in the wild, indicating the predictive value of this model to more natural settings.

Another limitation is our assumption of homogeneity of hosts, which is not accurate in this system. Guppies are known to exhibit a broad range in both life history traits (46, 47) and innate resistance to parasites (48-50), both within and among populations. Additionally, individual guppies may vary in their susceptibility to parasites due to individual characteristics such as size (3, 18), carotenoid colouration (51, 52) and sex (17, 43, 53, 54). Our parameters don’t capture the wide variability that occurs in nature, or how this heterogeneity may influence host-parasite dynamics in the population but were instead based on average values obtained from literature and our own laboratory observations.

Moreover, significant variability even in average population-level resistance has been observed among wild populations and domestic fish to various strains of gyrodactylids (3, 11, 50, 55) and it's possible that our estimated parameters may not fit some extreme cases of particularly low- or high-resistance populations. However, despite not accounting for such complexities, our model fit data from two experiments, one which used fish from various wild populations from Trinidad and one which used domestic fish, therefore we find these average values to be a decent approximation.

In conclusion, we were able to develop and validate a mathematical model that more effectively describes the guppy-*Gyrodactylus* system, thus contributing to a further understanding of disease dynamics. Through sensitivity analysis, we were able to identify key factors affecting outbreaks to strategize control measures for parasites which increase in numbers due to reproduction directly on the host (in the absence of transmission) and are directly transmitted via host contact, particularly those relating to parasite growth rate and host resistance. Our findings have implications for a broader range of systems, with our model being most directly applicable to other gyrodactylids such as *G. salaris*, which is known to cause severe mortality and morbidity in Atlantic salmon fisheries (34, 56), but these methods could be also applicable to many other infections for which immunity decays over time, such as that observed for some vaccines.

5.6 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or

financial relationships that could be construed as a potential conflict of interest.

5.7 Author Contributions

The mathematical model was developed at a meeting between CPT, JDK, GFF and HW. CPT collected the data (either experimentally or from published literature) used for parameter estimates and model fitting, while JDK validated the model and conducted the sensitivity analysis. CPT wrote the initial draft of the manuscript, with editing input from all co-authors.

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5.10 Data Availability Statement

The experimental data used to estimate and validate parameters for this experiment are included as supplemental materials.

5.11 Ethics Statement

Approval for animal care and research was obtained from the McGill University Animal Care Committee (AUP 2014-7547) in compliance with the Canadian Council on Animal Care.

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5.13 TABLES AND FIGURES

Table 5.1: Estimated parameter values used to train and test the model and initial values from experiments.

Volume of experimental tanks was 6L. As no birth was observed in our experiments, we estimate α to be 0 and neglect the parasite induced fecundity reduction.

Parameter	*Sym.*	*Estimate*	*Unit*	*source*
Initial number of susceptible guppies	S (0)	7	-	this study
Initial number of infected guppies	I (0)	1	-	this study
Initial number of recovered guppies	R (0)	0	-	this study
Initial number of parasites	X (0)	2	-	this study
Initial number of immune cells	$Y(0)$	5.1440	-	this study
transmission rate	β	0.0468	/day/host/parasite	this study
recovery rate	λ	0.0080	/day/host	this study
half-saturation constant of per-capita parasite killing rate	r	176.7035	-	this study
maximum parasite killing rate	γ	0.1084	/day/no immune cells	this study
parasite increase rate	μ	0.6395	/day	this study
maximum rate of immunity increase	θ	0.0562	/day	this study
half-saturation constant of immunity increase	κ	7.1411	-	this study

rate of decay of immunity in the absence of parasites	ν	0.0321	/day	this study
guppy carrying capacity	K	9.600	/litre	(1)
steepness of distribution kernel	c	0.0100	/day	this study
parasite-induced mortality rate	ε	0.0012	/day	this study
guppy birth rate	α	0.0	/fish/day	this study
natural guppy mortality rate	d	0.0049	/day	(2, 3)
natural parasite mortality rate	w	0.24	/day	(2)

Table 5.2: The sensitivity of the magnitude of the peak of the first outbreak to the parameters

Parameter	*Definition*	*Sensitivity index*
β	transmission rate	0.0130
λ	recovery rate	-0.0516
μ	parasite increase rate	1.5662
r	half-saturation constant of per-capita parasite killing rate	0.4707
γ	maximum parasite killing rate	-0.6369
θ	maximum rate of increase of immunity	-0.8186
κ	half-saturation constant of increase of immunity	0.3367
v	rate of decay of immunity in the absence of parasites	0.2253
α	birth rate	0.0216
d	natural guppy mortality	-0.0178
K	half-saturation constant for guppy growth	0.2541
c	$1/c$ is the average duration of immune memory	-0.0016
ε	parasite-induced mortality rate	-0.0568
ω	natural parasite mortality	-0.6862

Table 5.3: The sensitivity of the first outbreak peak time to the parameters

Parameter	*Definition*	*Sensitivity index*
β	transmission rate	-0.7344
λ	recovery rate	-0.1732
μ	parasite increase rate	-1.0673
r	half-saturation constant of per-capita parasite killing rate	-0.1847
γ	maximum parasite killing rate	0.1212
θ	maximum rate of increase of immunity	-1.1184
κ	half-saturation constant of increase of immunity	-0.0880
v	rate of decay of immunity in the absence of parasites	-0.1329
α	birth rate	0.0593
d	natural guppy mortality	0.0088
K	half-saturation constant for guppy growth	-0.8757
c	$1/c$ is the average duration of immune memory	-0.0055
ε	parasite-induced mortality rate	0.0057
ω	natural parasite mortality	-0.2868

Table 5.4: The sensitivity of the magnitude of the peak of a generic outbreak to the parameters

Parameter	*Definition*	*Sensitivity index*
β	transmission rate	-0.8688
λ	recovery rate	0.1385
μ	parasite increase rate	0.5096
r	half-saturation constant of per-capita parasite killing rate	0.1465
γ	maximum parasite killing rate	-0.4790
θ	maximum rate of increase of immunity	-1.5751
κ	half-saturation constant of increase of immunity	0.1726
ν	rate of decay of immunity in the absence of parasites	1.9784
α	birth rate	-0.0344
d	natural guppy mortality	-0.4309
K	half-saturation constant for guppy growth	-0.5502
c	$1/c$ is the average duration of immune memory	-0.3137
ε	parasite-induced mortality rate	-0.0754
ω	natural parasite mortality	-0.4031

Table 5.5: The sensitivity of a generic outbreak period to the parameters

Parameter	*Definition*	*Sensitivity index*
β	transmission rate	-0.2360
λ	recovery rate	-0.2712
μ	parasite increase rate	-0.5972
r	half-saturation constant of per-capita parasite killing rate	0.1035
γ	maximum parasite killing rate	-0.1115
θ	maximum rate of increase of immunity	0.5502
κ	half-saturation constant of increase of immunity	0.1037
v	rate of decay of immunity in the absence of parasites	-0.3536
α	birth rate	-0.1824
d	natural guppy mortality	0.1708
K	half-saturation constant for guppy growth	0.2089
c	1/*c* is the average duration of immune memory	0.08776
ε	parasite-induced mortality rate	-0.1128
ω	natural parasite mortality	-0.0537

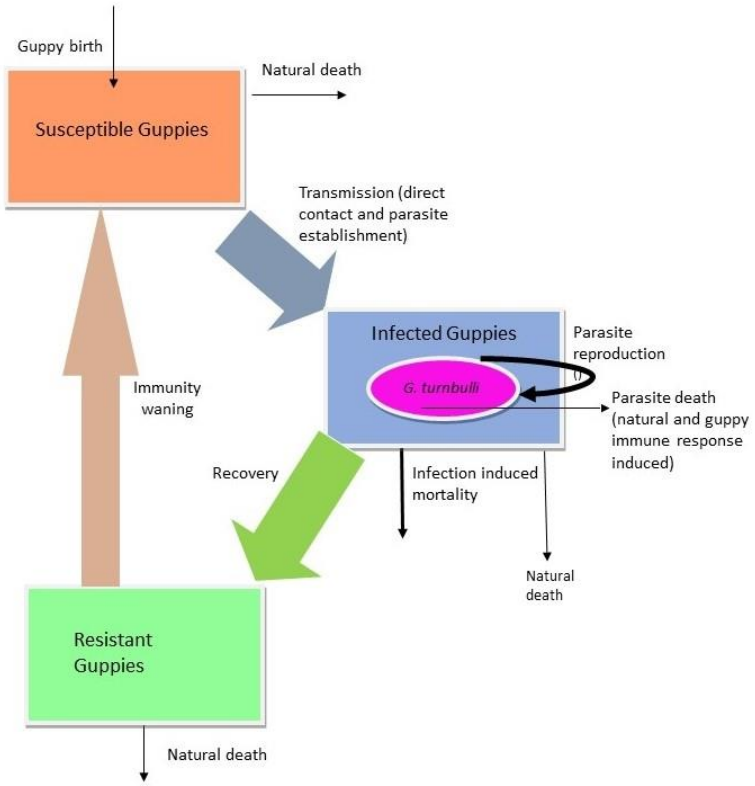

*Figure 5.1: Conceptual model of the Guppy-*Gyrodactylus *system*

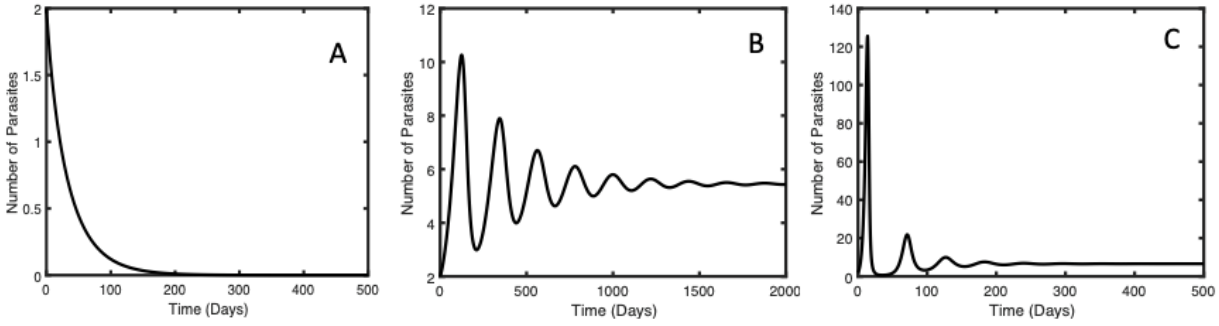

Figure 5.2: Gyrodactylus *dynamics with different basic reproduction numbers ($\mathcal{R}_0$). Panel A: $\mathcal{R}_0$=0.90 and panel B: $\mathcal{R}_0$=1.1 and panel C: $\mathcal{R}_0 = 2.59$, demonstrating that parasites will die out with an $\mathcal{R}_0$ of less than one, and persist if $\mathcal{R}_0$ is greater than one.*

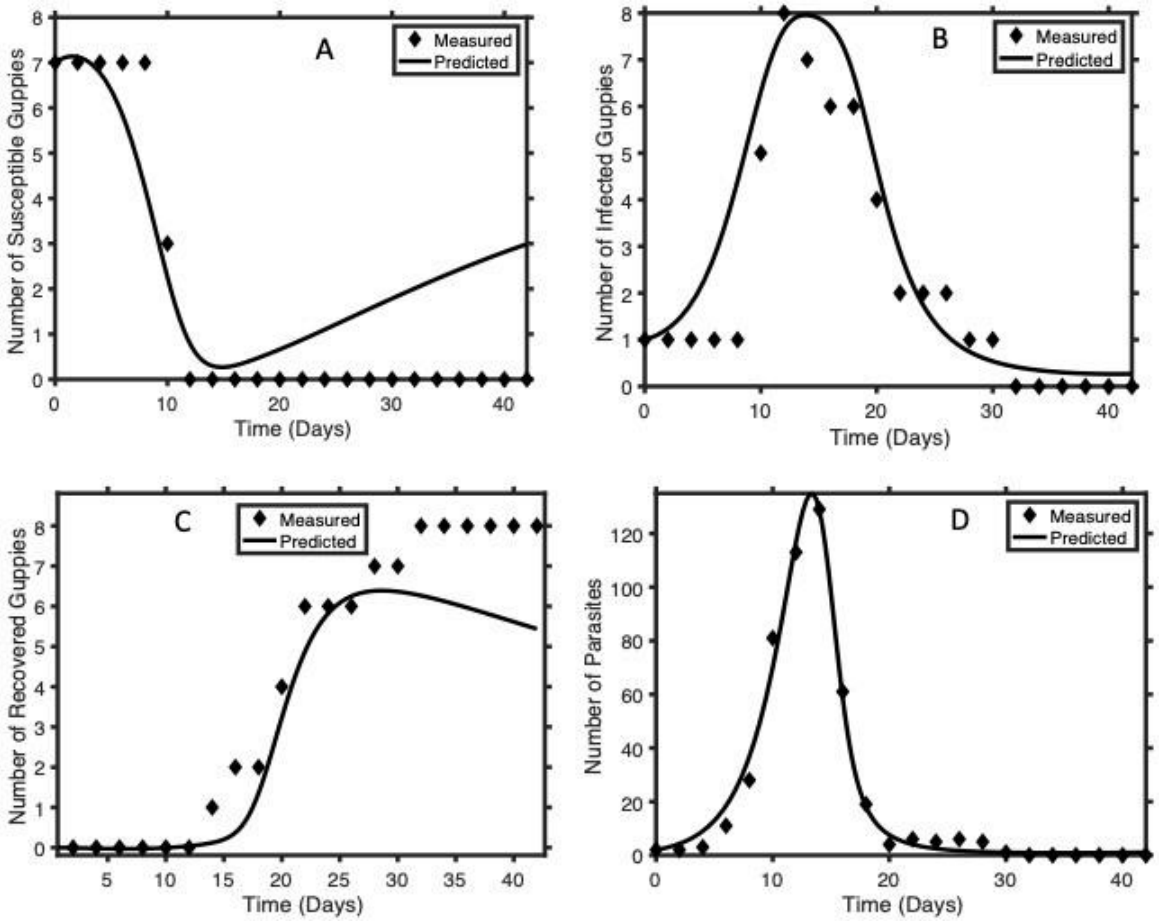

*Figure 5.3: Comparison of model predictions (solid line) of Guppy-*Gyrodactylus *dynamics using the parameters in Table 5.1 with measured values from averages of laboratory results (diamonds).*

In panels A, B, C, and D we have the time course dynamics of the number of susceptible guppies, infected guppies, recovered guppies and Gyrodactylus *per tank, respectively.*

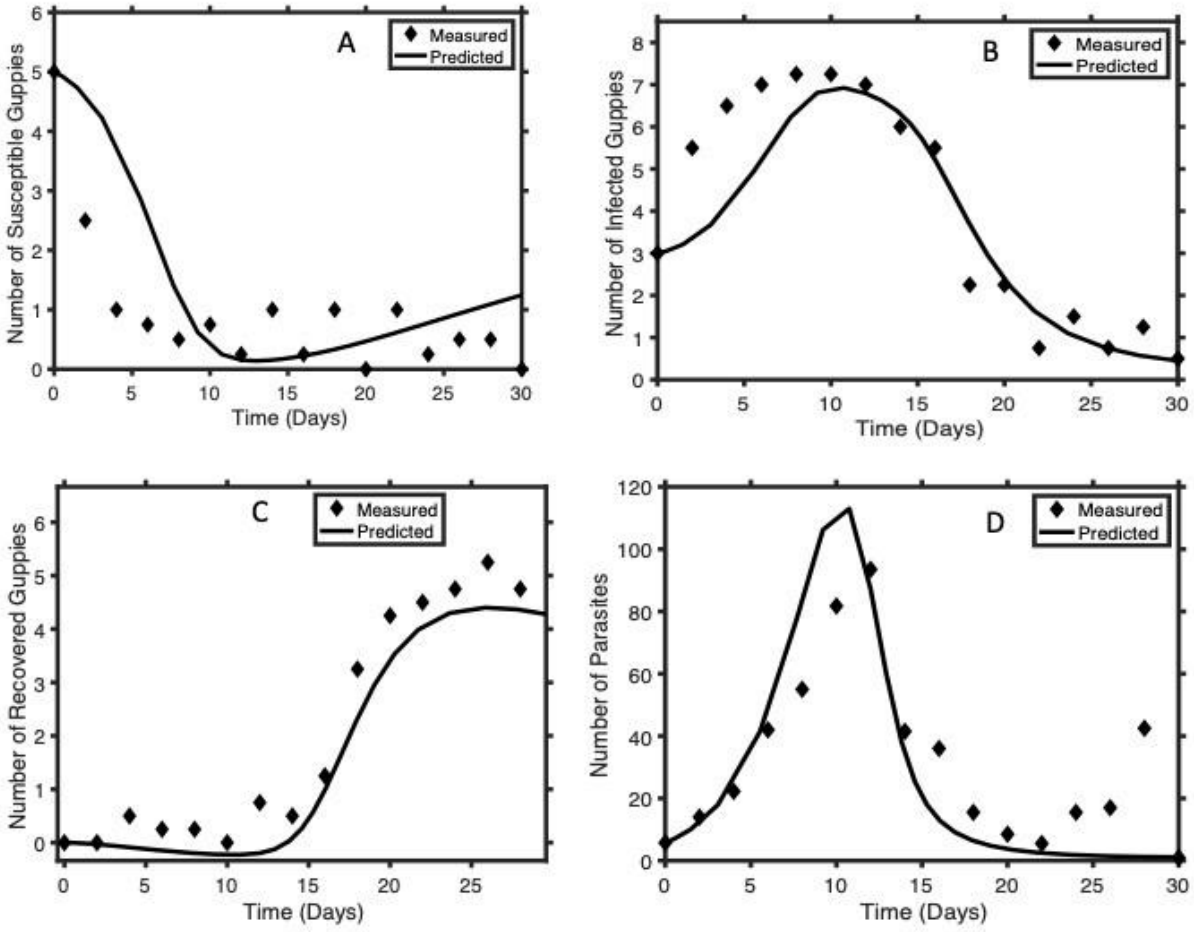

*Figure 5.4: Comparison of model predictions of Guppy-*Gyrodactylus *dynamics (solid lines) with averages from laboratory data (diamonds) independent from the data used to estimate the model parameters (Table 5.1).*

In panels A, B, C, and D we have the time course dynamics of the number of susceptible guppies, infected guppies, recovered guppies and Gyrodactylus *per tank, respectively.*

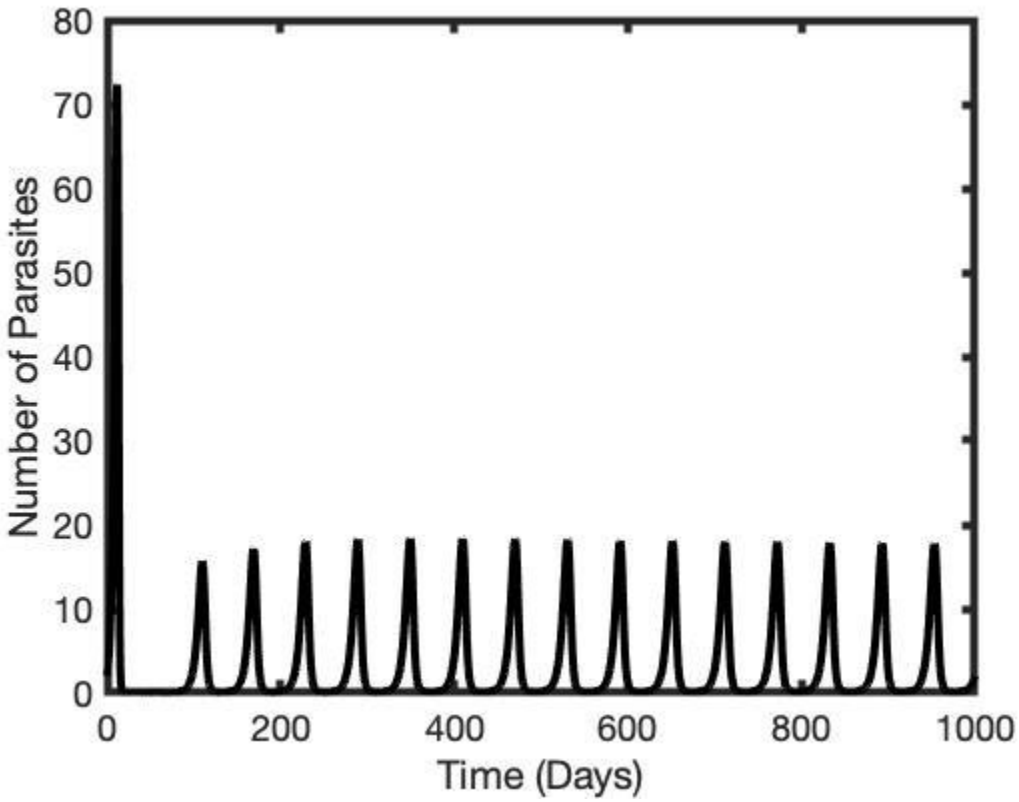

Figure 5.5: Long-term Gyrodactylus *dynamics when guppy birth is included in the system. The Figure was generated using the parameter values in Table 5.1 with α= 0.4/fish/day.*

6 CHAPTER 6: GENERAL DISCUSSION

6.1 OVERVIEW

In this thesis I have investigated the impacts of heterogeneity and connectivity on host-parasite metapopulation dynamics at various levels of analysis and types of heterogeneity using both experimental and theoretical approaches. I began by exploring host metapopulation heterogeneity in terms of parasite introductory distribution (concentrated or dispersed) and connectivity among patches. I then moved down one level to study within-population heterogeneity in the form of sex and connectivity and explored the concept of each host acting as a patch for the parasite metapopulation. I then moved down one level further to explore individual host-level heterogeneity and determine the characteristics of more competent hosts, and the influence they have on metapopulation parasite loads, transmission and persistence. Finally, I developed a mathematical model to better describe this host-parasite system and conducted a sensitivity analysis to identify for which parameters variability could cause the largest impact on outbreak size and timing.

In Chapter 2 I found that there is an impact of host population connectivity on parasite persistence over the metapopulation, but no effect of spatial heterogeneity in terms of parasite introductory distribution on parasite persistence. I did find an important interaction between connectivity and initial parasite load influencing mean parasite burden for hosts, where those with high initial parasite introductions benefited from connectivity to other subpopulations by experiencing lower mean burdens than those in isolated populations. In Chapter 3 I again found an influence of connectivity on parasite persistence, with parasite populations persisting longer

on groups of fish than on isolated ones. I again found an interactive effect of connectivity with heterogeneity where females, the sex that experienced higher loads in isolation, benefitted from being in a group by experiencing lower mean burdens than when alone. I also found that males, the more resistant sex, experienced earlier parasite peaks than females regardless of whether isolated or grouped, indicating that they are better at clearing infection earlier. In Chapter 4 I examined the role of heterogeneity in resistance and tolerance at the individual level on metapopulation dynamics by identifying individuals with intense and/or prolonged infections and found that individuals with intense and prolonged infections were associated with high metapopulation parasite burdens, while individuals with prolonged infections were the most connected and more responsible for transmission and parasite persistence regardless of parasite burden, thus providing evidence that tolerance is important to parasite dynamics and highlighting the need to consider different facets of host heterogeneity. In Chapter 5 I used a theoretical model to describe host-parasite dynamics in this system and demonstrated through sensitivity analysis that heterogeneity in host resistance to infection may have a strong impact on the timing and magnitude of epidemic outbreaks. Together, these chapters highlight not only the importance of connectivity and heterogeneity in resistance to parasite dynamics, but also demonstrate that the level of analysis considered is highly important to detecting outcomes.

6.2 SPECIFIC IMPLICATIONS

The importance of connectivity runs thematically through my three experimental chapters, primarily by prolonging parasite persistence as in Chapters 2 and 3, where the inclusion of

multiple patches (either as hosts or sub-populations of hosts) provided additional resources for the parasite. In Chapter 4 I also found that the most competent hosts, the ones with prolonged infections, also had the greatest number of contacts (and were therefore the most connected) and were able to successfully transmit parasites to other hosts and that their occurrence in a metapopulation was associated with longer parasite persistence. That metapopulation persistence and disease transmission are driven by connectivity is not surprising, as it is one of the most important elements of metapopulation (4, 5) and epidemiological theory (6-8). However, this body of work provides experimental evidence supporting these concepts, which have previously been largely theoretical or observational, and also highlights the many parallels between these two fields (9, 10), and the importance of drawing from multiple pools of knowledge in an increasingly connected world (11-14). Together, these three chapters demonstrate experimentally that connectivity plays an important role in the spread and persistence of infectious disease at the individual, population and metapopulation level.

These studies also reflect the varied and important role that heterogeneity plays in parasite dynamics, at the individual, population and metapopulation level. Chapter 2 focused on spatial heterogeneity in terms of parasite introductory distribution and found no difference in metapopulation-level dynamics between metapopulations with concentrated and dispersed introductions. Spatial and temporal heterogeneity in metapopulations have been a large focus of theoretical work (13, 15, 16), but we were unable to experimentally establish sustained asynchrony among our subpopulations via introductory distribution of parasites, indicating that

variability in individual resistance may play a larger role contributing to asynchrony.

Using data from this same experiment as Chapter 2, I found in Chapter 4 that heterogeneity in individual resistance and tolerance may have overridden any of our imposed spatial heterogeneity in this system. Individuals with prolonged infections were responsible for more transmission and were associated with longer metapopulation persistence, while the occurrence of individuals with intense *and* prolonged infections influenced peak parasite load in the metapopulation, indicating that variability in individual host defence against parasites plays a large role in population- and metapopulation-level dynamics. This finding is also theoretically supported by our sensitivity analysis in Chapter 5, which demonstrated that slight changes to the parameters relating to resistance had the greatest influence on outbreak magnitude and time. In Chapter 3 I suggested that males were more resistant than females and found earlier outbreak peaks in males than females, indicating an influence of heterogeneity in resistance on outbreak timing again supporting the findings of our sensitivity analysis from Chapter 5, where time to first outbreak and general outbreak period were most sensitive to variables having to do with host resistance. The wide variation in resistance to parasites and the evolution thereof has been a large focus of study in the guppy-*Gyrodactylus* system (17-19). My studies show that heterogeneity in resistance may have a large impact on parasite population growth at the broader scale. I also find evidence for an influence of tolerance, an aspect understudied in this system and context (19, 20), indicating the importance of studying heterogeneity in various aspects of host defence against parasites.

In conducting a multilevel analysis of metapopulation dynamics, examining dynamics on the individual host, subpopulation and metapopulation levels, I found an importance of scale in detecting results. In both Chapters 2 and 3, I found a dampening effect of connectivity on the impacts of heterogeneity compared to in isolated patches. Heterogeneity in parasite distribution among subpopulations did not influence metapopulation-level parasite persistence or intensity in Chapter 2, however heterogeneity in individual-level host competence did have a strong impact on parasite dynamics in those same experimental metapopulations, as demonstrated in Chapter 4. In Chapter 3, I detected a difference in infection intensity between isolated males and females, but not at the grouped or mixed population level. I also considered host heterogeneity as a difference in "patch" quality for a metapopulation of parasites, highlighting again the parallels between ecology and epidemiology (9, 21-23) and the importance scale. My experimental chapters considered the metapopulation-, sub-population- and individual-host levels as the focus of each, and in Chapter 5, I considered the individual parasite level by incorporating an equation to specifically model the parasite population dynamics, an addition not typical to SIR models (24, 25), and showed that outbreaks are also highly sensitive to parasite virulence. Importantly, these findings imply that some relationships found at one scale may be unimportant or undetectable at another, or may have a strong influence on higher-level dynamics, and that it is important to consider all levels of analysis to fully understand host-parasite metacommunity dynamics.

6.3 General Implications and Future Work

Together, this body of work merges experimental evidence and theory to provide further insights into host-parasite dynamics. The spread and persistence of infectious disease is of major concern to our world, particularly as we become more connected. Therefore gaining a better understanding of the mechanisms behind it is crucial to fisheries and aquaculture management (26-28) resource and crop management (29-31), species conservation (32-37) and human health (38, 39). Our model system may most generally be analogous to other gyrodactylid species such as *G. salaris*, which has been devastating to the North Atlantic salmon fisheries and shares many features with *G. turnbulli* (40). However, given the microparasite-like behaviour of *Gyrodactylus* spp., it may serve as a model to offer further insight into epidemics of other infectious diseases, and may advance our understanding particularly of the impacts of connectivity and heterogeneity in resistance.

One of the main strategies in resource or species conservation is to establish corridors over a fragmented landscape to allow for metapopulation persistence, however as demonstrated in this thesis, it may also allow for the spread and persistence of infectious disease. In parallel, one of the major strategies for disease mitigation is containing it through quarantine, however my work has also shown that in some cases, infected hosts may benefit from connectivity. My work highlights the importance of considering scale and specifics of the system in question when applying best practice.

I also consistently demonstrate an importance of host resistance and heterogeneity thereof to

parasite dynamics at the individual, population and metapopulation level. Although the resistance focused on in our experiments was mainly ostensibly innate, with the help of modern medicine and technology human society is capable of augmenting resistance through treatment and vaccines, both for human diseases and those infecting other species of concern. Unfortunately, we are currently seeing an emergence of disease virulence due to over-use and improper use of treatments, particularly the broad applications of antibiotics when unnecessary (in humans and livestock) as well as patient failure to finish the full course (41, 42). Similarly, refusal of vaccines is currently causing a re-emergence of some human diseases in countries in which they were previously considered eradicated (43, 44). My work emphasizes the importance of treatments being properly used and vaccines being broadly applied in order to prevent spread of infectious disease by maintaining a high, homogenous level of resistance in a host population.

This body of work offers experimental and theoretical insights into host-parasite dynamics and focuses on the role of connectivity and heterogeneity (particularly in host resistance) on disease transmission and persistence, however there is room for more work to be done. Although laboratory experiments are important to isolating variables of study and theoretical work is important to forecasting longer-term effects, it is important to note that other environmental impacts for which I controlled may also play a role in the dissemination of disease, and therefore field studies could complement this work and offer further insight. This work also focused on a very simplified form of connectivity and expanding upon it to examine different patterns or rates of migration could be useful. Similarly, I focus largely on heterogeneity in resistance and

tolerance to parasites using small laboratory populations, but other host factors such as behaviour and sociability may be important in larger populations with less controlled environments. Also, although I was able to develop a modified SIR model that more accurately describes this system, it assumed homogeneity within the host population and since analysis showed that outbreaks were sensitive to differences in population-level average resistance, building a more individual-based model that allows for heterogeneity in resistance and connectivity could help to merge my experimental findings with real-world variability. Finally, this work largely focused on the importance of scale and heterogeneity in host resistance but used *G. turnbulli* from the same isogenic line—since identifying different species or strains of *Gyrodactylus* would require destructive sampling—thus eliminating any potential influence of heterogeneity at the lowest scale in this system: the individual parasite level. However, *Gyrodactylus* strains and species are also known to exhibit different degrees of virulence which also may potentially co-evolve with hosts (45, 46), and exploring heterogeneity in parasite virulence could be a difficult but interesting avenue for future work, as indicated by the results of the sensitivity analysis in Chapter 5. This thesis advances our understanding of disease dynamics in heterogenous metapopulations and highlights the need for merging ideas from different fields, as well as bridging theory and observation with experiments to address real-world problems.

6.4 REFERENCES

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