Natural history of host-parasite interactions in an invaded community

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Dedicado a mi madre, que me abrió los ojos a la belleza de la naturaleza,

and to my father, who taught me that nature is beautiful, but not always pretty.

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ABSTRACT

Biological invaders typically lose parasites and pathogens from their native range but can acquire parasites from their native range or from their introduced range. As a result, host-parasite communities in invaded habitats need to be re-assembled – and this assembly can echo aspects of host-parasite associations in the native range. In Panama, the invasive red-rimmed melania snail *Melanoides tuberculata* (Muller 1774) is commonly infected by trematode parasites with complex life cycles. In this research, I investigate the ecological factors driving the transmission of parasites in an invaded community by considering the interactions of trematodes with different host species throughout their life cycle. I specifically consider the ecological processes that affect the extent to which biological invasions facilitate the transmission of introduced parasites in expanded geographic ranges.

In the first chapter, I test the idea that "host diversity begets parasite diversity" in an introduced range by evaluating the influence of native bird hosts and habitat features on the abundance and species richness of trematode parasites infecting *M. tuberculata* in an invaded range. I show that the abundance and diversity of trematodes in *M. tuberculata* is strongly associated with the abundance and diversity of potential bird hosts in lake sites but found no evidence for this association from field surveys in stream sites. This system therefore shows how the assembly of host-parasite associations in new ranges is a complex mosaic of historical processes, novel associations and environmental effects. In the second chapter, I investigate how these trematodes described in the previous chapter interact with first intermediate snail hosts. Specifically, I consider the effect of parasitism by an invasive trematode, *Centrocestus*

formosanus (Nishigori 1924), on snail reproduction and investigate the extent to which infected hosts exhibit plasticity in life-history strategies in response to parasitic castration. Field surveys showed that infected snails are castrated and unable to produce new offspring. However, infected snails could harbor juvenile snails in their brood pouch and thus provide maternal investment in brood development post-infection. Snails infected in laboratory had depressed growth rates and produced larger juveniles compared to uninfected snails. These results support the idea that trematode infection induces a plastic response in the energetic budget allocated to reproduction to boost reproduction at the cost of growth. I discuss the implications of the ability of snails to "make the best, if not the most, of a bad situation " In the third chapter, I consider how *C. formosanus*, which is transmitted by snails to fish, interacts with a community of potential fish hosts with which they do not share a common evolutionary history. Field surveys across three field sites in Gatun Lake, Panama, revealed that the invasive peacock bass, *Cichla monoculus*, was more commonly infected by *C. formosanus* than were three other common cichlid fishes. Laboratory infection experiments were conducted to determine if parasitism might be driven by differential encounter to parasites or by differential infection susceptibility/preference across different host species. In all cases, the peacock bass exhibited higher infection rates relative to other potential fish hosts. These data provide support that an introduced 'generalist' parasite shows specialization on a novel host, which could be the product of rapid local adaptation post-invasion.

As a whole, this research illustrates the ecological complexity associated with the establishment of parasites with complex life cycles in new environments, creating a mosaic of interactions with different host species that are driven by different ecological and evolutionary processes.

RÉSUMÉ

Dans la plupart des cas, les espèces biologiques envahissantes ne transportent pas de parasites ou d'agents pathogènes natifs à leurs aires de répartition originelles. Ces espèces peuvent tout de même aquérir des parasites dans leurs habitats originelles ou envahies. Ceci a pour conséquence un réassemblement des communautées hôtes-parasites dans les habitats envahis – et ce réassemblement peut présenter des aspects qui reflètent ceux des associations hôtes-parasites présents dans leurs aires de répartition naturelles. Dans la République du Panama, l'espèce envahissante Mélanie tropicale *Melanoides tuberculata* (Muller 1774) est couramment infectée par des parasites trétamodes (*Tretamoda*). Ces parasites ont des cycles de vie relativement complexes. Dans cette thèse, j'ai examiné les facteurs écologiques conduisant à la transmission de parasites dans une communauté précédemment envahie, en prenant en considération les intéractions des trétamodes avec leur differentes espèces-hôtes au cours de leur cycle de vie. J'ai plus spécifiquement pris en considération les processus écologiques qui affectent l'étendue avec laquelle les invasions biologiques facilitent la transmission de parasites introduits dans des aires géographiques étendues.

Dans mon premier chapitre, je teste l'idée que "la diversité des hôtes engendre la diversité des parasites" dans une aire de répartition importée en évaluant l'influence des oiseaux-hôtes (natifs de l'aire de répartition) et les particularités de l'habitat sur l'abondance et la richesse des parasites trématodes infectant *M. tuberculata* dans une aire de répartition envahie. Je démontre que l'abondance et la diversité des trématodes de *M. tuberculata* sont fortement associées avec l'abondance et la diversité des potentiels oiseaux-hôtes dans des sites lacustres. Je n'ai cependant trouvé aucune évidence de cette association dans les ruisseaux étudiés. Ce

système montre donc comment le réseau formé par les association hôtes-parasites dans de nouvelles aires de répartition est une mosaïque complexe de processus historiques, de nouvelles associations, et d'effects environnementaux. Dans mon second chapitre, j'examine comment les trématodes décrits dans le chapitre précédent intéragissent avec leurs premiers hôtes intermédiaires (escargots aquatiques). Plus spécifiquement, je considère l'effect du parasitisme par un trématode invassif, *Centrocestus formosanus* (Nishigori 1924), sur la reproduction des escargots aquatiques, et j'examine l'étendue avec laquelle les hôtes infectés montrent une certaine plasticité dans leur stratégie d'histoires de vie en réponse à leur castration parasitique. Les études de terrain ont montré que les escargots aquatiques infectés sont castrés et donc incapables de se reproduire. Ces escargots peuvent cependant porter des jeunes individus dans leur poche pour couvain and peuvent donc fournir un investissement maternel dans le dévelopement des couvets post-infections. En laboratoire, comparés aux escargots non-infectés, les escargots infectés montrent des taux de croissance inférieurs et produisent des juvéniles plus larges. Ces résultats supportent l'idée que l'infection par les trématodes induit une réponse plastique dans le budget énergétique alloué à la reproduction, et ce dans le but de booster la reproduction. Cette réponse se fait cependant au détrimant de la croissance. Je discutons les implications de l'abilité des escargots aquatiques à "tirer le meilleur parti d'une mauvaise situation". Dans le troisième chapitre, j'examine comment *C. formosanus,* qui est transmis aux poissons par les escargots, intéragit avec une communautée potentielle de poissons-hôtes, avec qui ils ne partagent aucune histoire évolutive. Des études de terrain ont été réalisées à travers trois sites situés dans le lac Gatun, Panama. Elles ont montré que l'espèce invasive tucunaré – ou peacock bass – *Cichla monoculus*, était moins souvent infectée par *C. formosanus* que les trois autres espèces communes de poissonss Cichlidae. Des expériences d'infection réalisés en

laboratoire ont été réalisées pour déterminer si le parasitisme pouvait être conduit par des rencontres différentielles aux parasites, ou par une susceptibilité/préference envers les infections différentielles à travers differentes espèces-hôtes. Dans tous les cas, le tucunaré montre des taux d'infection plus élevés par rapport aux autres espèces-hôtes potentielles. Ces données soutiennent qu'un parasite "généraliste" introduit montre une certaine spécialisation pour son nouvel hôte, ce qui pourrait être le produit d'une rapide adaptation locale post-invasion.

Dans son ensemble, cette recherche illustre la complexité écologique complexe associée avec l'établissement des parasites avec des cycles de vie complexes même dans un nouvel envireonnement. Ceci permet la création d'une mozaïque illustrant les intéractions entre différences espèces-hôtes qui sont poussés par différents processus écologiques et évolutifs.

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PREFACE

"I shall always feel respect for every one who has written a [dissertation], let it be what it may, for I had no idea of the trouble which trying to write common English could cost one."

CHARLES R. DARWIN ,
Letter to W. D. Fox, 1837

"All that we can do, is to keep steadily in mind that each organic being is striving to increase in a geometrical ratio; that each at some period of its life, during some season of the year, during each generation or at intervals, has to struggle for life and to suffer great destruction. When we reflect on this struggle, we may console ourselves with the full belief, that the war of nature is not incessant, that no fear is felt, that death is generally prompt, and that the vigorous, the healthy, and the happy survive and multiply."

CHARLES R. DARWIN
The Origin of Species (1859)
Chapter III: The Struggle for Existence

Statement of original scholarship

The Panama Canal is the crossroads of oceans and continents. Due to the globalization of the world's economies, human activities have facilitated the geographic expansion of species beyond their natural distributions. A major concern with the spread of these non-native species is their potential to facilitate the spread of parasites that can cause disease in humans, wildlife and commercially valuable species. In the Isthmus of Panama, I have developed a research program that investigates how trematode parasites with complex life-cycles have established in the Isthmus of Panama. This research program was first developed with the discovery of a unique diversity of trematode parasites infecting *Melanoides tuberculata* (Muller 1774), the ecologically dominant invertebrate in the Panama Canal. Trematode parasites have established in various lake and stream sites in the Isthmus of Panama where their snail host has invaded. I discovered that trematode assemblages occur in different abundances and diversities in their snail host across sites. This natural variation in parasite abundance and diversity provided the foundation to investigate what ecological and evolutionary factors are driving the transmission of these parasites in an introduced range. As these parasites have a complex life-cycle and interact with various host species in different stages of their life-cycles, I divide my thesis into three chapters that explore basic processes in the ecology of host-parasite associations; community ecology in chapter 1, phenotypic plasticity in chapter 2 and encounter/compatibility in chapter 3.

Chapter 1:

Parasites contribute substantially the diversity of life on Earth, yet we know little about what factors influence parasite distributions and abundances in natural systems and even less in the expanded geographic ranges of host-parasite associations. One idea that has received a lot of

empirical support is that host communities structure parasite communities; since parasites live within their hosts and require hosts for transmission, parasite abundance and diversity should correlate with the abundance and diversity of hosts. Can we expect the same patterns for trematode parasites infecting snail hosts in an introduced region and across broad spatial scales encompassing different habitat types? In this study, I put the "host diversity begets parasite diversity" hypothesis to the test in an introduced habitat. This is a valuable contribution to parasite ecology theory as it tests the generality of this hypothesis in an introduced range and among host-parasite associations in a different ecological context where host and parasites do not share a common evolutionary history.

Observations from a field survey of lake and stream sites in the Isthmus of Panama show natural variation in bird and trematodes across habitat types and led us to evaluate the relationship between bird abundance and diversity on that of trematodes across all sites and within lake and stream sites. My study is the first to my knowledge that demonstrates that a community of native host species predicts the abundance and diversity of parasites infecting an invasive host in its introduced habitat. Specifically, a greater abundance and diversity of definitive bird hosts was associated with a greater abundance and diversity of trematode parasites in invasive snail hosts. In addition, my study appears to be the first to consider how habitat type influences the observed association between host abundance/diversity and parasite abundance/diversity in an invasive species context.

In the second part of the study, I employ a mark-recapture field experiment exposing uninfected "sentinel" snails to infection by trematodes across lake and stream sites and ask if the rate of trematode infection over a one-year period differs across different habitat types. By measuring the success of recapture of these "sentinel" snails, I am able to estimate if the

persistence of snails is consistent among in lake and stream habitats, and allow us to make inferences regarding the influence of habitat characteristics on the observed associations between native bird hosts and trematodes infecting the invasive *M. tuberculata* in the Isthmus of Panama. By integrating how biotic and abiotic factors affecting both parasites and hosts, I uncover important factors structuring invasive host-parasite communities. Thus, my work on this system has revealed a complex and dynamic re-assembly of host-parasite interactions in a novel location through a set of complementary associations at different levels of the parasite life cycle.

Chapter 2:

Phenotypic plasticity can provide the means to respond to both extrinsic and intrinsic factors that affect fitness, including aboiotic stress and natural enemies. Plastic responses in life-history strategies reflect a re-allocation of the energetic budget allocated to reproduction to maximize fitness and can be expressed as changes in the number and size of offspring, the size and age at maturity, and the timing in which these changes are expressed from one year to another or the lifespan of an individual. However, these shifts in life-history strategies can be expressed "pre-infection" due to genetic effects or "post-infection" as a consequence of phenotypic plasticity. For this reason, I investigate the extent to which trematode infection affects snail reproduction in field surveys and evaluate through a lab experiment if infected hosts display plastic responses in life history strategies. By jointly considering the results of field surveys and the lab experiment, I consider the broader implications that residual reproduction

after infection and associated changes in life-history traits have for the persistence of an invasive snail and the parasites that infect them in an introduced range.

Parasitic castrators, such as trematodes of snail hosts, can severely restrict or completely eliminate the reproductive potential of their hosts. Exposure to infective stages of trematodes can drive shifts in life-history strategies in snail hosts to increase growth and rates of egg production in response to risk of parasitism, but it is often unclear to what extent observed shifts in life-history traits are the product of genetic canalization versus phenotypic plasticity. In order to control for genetic effects and phenotypic plasticity, reciprocal transplants have been employed, but this is, to my knowledge, the first time that an experimental infection in controlled laboratory experiments specifically tests for the effect of trematode infection in driving plastic shifts in life history traits associated with reproduction.

In this study, I provide original research for the first time in the Isthmus of Panama that an invasive snail, *M. tuberculata*, is frequently infected by a trematode parasite, *Centrocestus formosanus* (Nishigori 1924). To my knowledge, this is the first investigation into the impacts of parasitism by *C. formosanus* on host reproduction in both natural field surveys and experimental infections, confirming that this trematode is a parasitic castrator of *M. tuberculata.* I also find, for the first time, that infected snails provide residual reproduction after infection and shift the size of juveniles they release to the environment, a change in life-history strategies that could enhance the survivorship of *M. tuberculata* to desiccation to "make the best (if not the most) of such a bad situation."

Chapter 3:

Host specificity is a central feature of the ecology and evolution of host-parasite interactions and an important determinant of the spread of parasitic diseases, yet surprisingly little is known about the factors underpinning a parasite's host range in natural settings. Host-parasite associations in nature are determined both by the encounter rate and compatibility of a parasite with potential host species. For this reason, generalist species are predicted to be more successful than specialists at colonizing new environments with novel host species. Here, I ask to what extent a generalist parasite demonstrates specialization to a novel host species in an expanded geographic range. To address this question, I evaluate the distribution of an invasive 'generalist' trematode parasite, *Centrocestus formosanus*, across four cichlid species in an invaded range. Natural patterns of parasitism across four sympatric fish species in Gatun Lake, Panama, demonstrate striking limitations in their distribution across sympatric fish hosts, such that the parasites in nature were commonly found in the peacock bass, and rarely in other host species. I then conducted two laboratory experiment, the first comparing infection compatibility in single species trails and the second host preference in mixed species trials, in order to control for the contribution of potential differences in encounter to parasitism in nature.

My study is unique in two ways. First, tests that attempt to elucidate ecological and evolutionary drivers of host specificity typically focus on native species and long-established host-parasite associations. By contrast, my use of non-native parasites and novel hosts is more informative for inferring interactions during the early stages of host-parasite community assembly. Second, laboratory experiments testing for patterns of host specificity typically focus on parasites thought to be highly host-specific, and often document infection compatibility with an unexpectedly broad range of novel host species typically not encountered in nature.

Conversely, my experiments consider whether a parasite putatively considered a generalist (from its native range) shows unexpectedly high host-specificity in its introduced range among a set of host species with which the parasite does not share a common evolutionary history. Overall, my combined survey-experimental approach for introduced parasites on novel hosts should provide theoretical and practical insights concerning the ecological and evolutionary processes underpinning host-parasite associations in expanded geographical ranges.

Author contributions (by chapter)

CHAPTER 1

Title:

SUPPORT FOR THE 'HOST BEGETS PARASITE' HYPOTHESIS IN AN INVADED COMMUNITY: NATIVE BIRD HOSTS DRIVE TREMATODE ABUNDANCE AND DIVERSITY IN AN INTRODUCED HOST.

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CHAPTER 2

Title:

PHENOTYPIC PLASTICITY IN LIFE-HISTORY STRATEGIES IN RESPONSE TO PARASITIC CASTRATION

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CHAPTER 3

Title:

HOST PREFERENCE OF A "GENERALIST" PARASITE FOR A NON-NATIVE HOST

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GENERAL INTRODUCTION

When people travel internationally, they are forced to go through a customs checkpoint. In customs forms in many countries, travelers are asked a series of probing questions regarding what they are bringing in to the destination country. The Unites States Customs and Border Protection - Customs Declaration Form, for example, asks if travelers are bringing "a) fruits, vegetables, plants, seeds, food, insects, b) meats, animals, animal/wildlife products, c) disease agents, cell cultures, snails." On the latter question, which is associated with attempts to prevent the spread of disease, customs agents want to know if passengers are bringing snails because they are obligate vector hosts for trematode parasites that can cause disease in humans, wildlife and commercially valuable species. In this thesis, I investigate the ecological and evolutionary factors that drive host-parasite interactions of introduced trematode parasites with multiple host species in their life cycle.

Human activities have radically transformed the natural environment, ushering a new geologic era, the Anthropocene (Crutzen 2002, Zalasiewicz et al. 2010, Waters et al 2016) that has had dramatic impacts on patterns of biological diversity around the world (Myers and Worm 2003, Barnosky et al. 2012, Dirzo et al 2014). One of the most apparent and irreversible features of the Anthropocene is the rapid geographic expansion of species ranges to new environments (D'Antonoi and Vitousek 1992, Vitousek et al. 1996, Simberloff 2009). This rapid geographic expansion of species distributions is a phenomenon that is an unprecedented aspect of global change in Earth's history (Ricciardi 2007) and is likely facilitated by other stresses on the natural environment (Hobbs 1989, Hobbs and Huenneke 1992, Dukes 1999, Lake 2004). When species are introduced to new environments, they can become widespread and have pronounced effects

on ecological processes (Mack and D'Antonio 1998) and local patterns of biodiversity (Lövei 1997, Sax and Gaines 2008, McGeoch et al 2010). A major concern related to the spread of biological invasions in their potential to spread parasites that can cause disease in humans, wildlife and commercially valuable species (Crowl et al 2008) and can themselves be considered invasive.

Many invaders lose parasites from their native range when they are introduced to a new environment (Enemy Release – Darwin 1859, Keane and Crawley 2002) due to founder effects or incompatibilities of parasites with the abiotic and biotic environment in an expanded geographic range (Torchin et al. 2003). While there is vigorous debate on the extent to which the loss of natural enemies predicts demographic success in new environments (Colautti et al 2004, Liu and Stiling 2006), the loss of parasites and pathogens has been observed in many natural systems where invasive species have become widespread in a novel environment (Mitchell and Power 2003, Torchin et al 2005, Torchin et al. 2006), invaders can also acquire new natural enemies, including parasites and pathogens from their introduced range or can re-acquire parasites from their native range (Levigne et al. 2004, Torchin and Mitchell 2004, Parker and Gilbert 2004). As a result, host-parasite communities in invaded habitats need to be re-assembled – and this assembly can echo aspects of their previous associations in the native range. The central question motivating this research is 'What affects the transmission of an introduced parasite in a novel range?'

Parasites with complex life-cycles should not easily establish in new environments because of the ecological complexity involved in transmission to multiple host species with which the parasite might not share a common evolutionary history (Torchin et al 2003). However, many parasites can and do invade a new environment. In the Isthmus of Panama,

which is the crossroad of two oceans and two continents, I have discovered a diverse and abundant community of trematode parasites infecting an invasive snail host, *Melanoides tuberculata* (Muller 1774). These snails have invaded freshwater lakes and streams around the world (Pointier et al 1992, Facon et al 2005, Weir 2012), often bringing their parasites to new environments (Salgado-Maldonado 2000, Font 2003, 2007), including lakes and streams in the Isthmus of Panama (Frankel et al. 2015). Aquatic habitats in the Isthmus of Panama, including the Panama Canal, have also invaded by predatory fish hosts that have had dramatic and lasting effects on the fish communities and top-down and bottom-up effects on other trophic levels (Zaret and Paine 1973, Sharpe et al. 2016). These changes in the community ecology of these aquatic ecosystems likely affect the transmission pathways of trematode parasites that require snails, fish and bird hosts to complete their life-cycle. The central question driving this research program asks 'How are introduced parasites establishing their life-cycle in a new environment?'

In this thesis, I investigate the ecological and evolutionary factors driving the transmission of trematode parasites in an invaded community and test broad ecological hypotheses previously applied to predict the success of free-living invasive species to the success of an invasive parasite species. I explore three ecological and evolutionary processes that are driving parasites transmission. In the first study, I evaluate the influence of native bird hosts and habitat features on the abundance and species richness of trematode parasites infecting this snail in an invaded range, testing the hypothesis that "hosts begets parasites." By considering both the biotic and abiotic conditions that drive patterns of abundance and diversity of introduced parasites in an introduced host range, I am able to draw conclusions about the extent to which ecosystem-based features are predictive of the success of introduced parasite assemblages. In the next chapter, I investigate how one of these trematodes, *Centrocestus formosanus* (Nishigori

1924), which transmitted by bird hosts as described in the previous chapter, interact with first intermediate snail hosts. Specifically, I consider the effect of parasitism on host reproduction and investigate the extent to which snail hosts exhibit plasticity in life-history strategies in response to parasitic castration. In the final chapter, I consider how the parasite *C. formosanus*, which is the most common of these trematodes infecting *M. tuberculata* in Panama, interacts with a community of second intermediate fish hosts with which they do not share a common evolutionary history. In this final chapter, I consider the extent to which being a "generalist" facilitates transmission or if an introduced parasite demonstrates specialization and host preference to a non-native host, which could be the product of rapid local-adaptation in an invaded range.

As a whole, this research illustrates the complexity and answers important questions associated with the establishment of parasites with complex life cycles in new environments, creating a mosaic of ecological interactions at different stages of the life cycle of parasites that contribute to their success in an introduced range. On a functional level, I use parasite invasions to test predictions that explain the success of biological invaders, which are in themselves tests for more basic ecological questions determining the abundance and distribution of species in natural populations. On a practical level, I hope to provide insight on the factors that promote the spread of parasites that can cause disease in wildlife, commercially valuable species and human populations.

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LITERATURE REVIEW

ON THE ANTHROPOCENE, INVASIONS AND THE GLOBAL SPREAD OF DISEASE

ABSTRACT:

Biological invasions are an irreversible component of the impacts that human activities have had on the natural environment, with the capacity to affect ecological and evolutionary processes and facilitate the spread of parasites. In this literature review, I will ground biological invasions in the broader context of human impacts on the natural environment, and the significance of these activities on geologic and ecological processes, with a special emphasis of the role of human activities in shaping global patters of biological diversity. I will then consider how the spread of biological invaders is another major component of human impacts on biological diversity. I review an extensive body of literature considering the factors that contribute to the success of biological invaders in novel habitats and focus on differentiating species-based, ecosystem-based and pathway-based approaches to understanding and predicting the success of biological invasions. Distinctions between trait-based, ecosystem-based and pathway-based approaches to understanding the success of biological invaders provide a theoretical framework for me to develop hypotheses on the success of tremtode parasites in a novel range.

The Anthropocene is a term that has been proposed as a formal subdivision of geologic time to mark the current interval in which human activities have persistently and significantly impacted geological and ecological processes on Earth (Crutzen 2002, Zalasiewicz et al. 2008, Steffen et al. 2011, Lewis and Maslin 2015). While there is vigorous debate as to if and when this new geologic epoch officially began, and how it should be defined, there are various lines of evidence that the impact of human activities is significant and has already altered geologic and biological processes. The modern biosphere differs significantly from previous geologic epochs in such dramatic ways, marked by changes in the geologic and biological record in both terrestrial and marine environments, that the Anthropocene warrants characterization not only as an epoch within a period within an era, but as a new geologic era unto itself (Williams et al. 2015).

The first line of evidence comes from the stratigraphic record, which signals major distinctions in the global system from the Holocene to the present. The stratigraphic record provides evidence of the impact of human activities in transforming the Earth but also suggests that these changes are accelerating, as the most dramatic changes in the stratigraphic record have occurred since the mid 20th century (Ellis 2011, Zalasiewicz et al 2011, Lewis and Maslin 2015). These changes in the last 100 years mark radical changes in the cycling of phosphorus, nitrogen and carbon and include fallout from fossil-fuel combustion particulates and manufactured materials including concrete, plastics and aluminum. Human activities such as deforestation, mining, transport and urbanization also affected geomorphology, affecting rates of sedimentation and erosion by an order of magnitude in many parts of the world (Hook 2000, Wilkins 2005, Syvitski et al 2005, Syvitski and Kettner 2011).

The impact of agriculture on terrestrial systems is also significant, as an estimated 10^9 hectares of undeveloped habitat could undergo agricultural conversion in order to meet the demands of a growing human population. Indeed, more than one half of the earth's surface has already been transformed by human activities for agriculture and resource exploitation and more than 50% of surface fresh water is diverted for human use (Vitousek et al 1997). This dramatic increase in agriculture would not only affect the terrestrial ecosystem, but would also affect freshwater and near-shore marine environments due to phosphorus and nitrogen-driven eutrophication and the increasing use of pesticides (Tilman et al. 2001, Steffen et al. 2004). Human impacts on the climate system also help to distinguish the Anthropocene from the Holocene and are likely to have profound effects on both terrestrial and marine environments, which have clear implications for global patterns of biological diversity.

In addition to the evidence from the geological stratigraphic record and changes to the atmospheric, oceanographic and terrestrial environment, human impacts on the natural environment in the Anthropocene are also characterized by significant changes in global patterns of biological diversity (Myers and Worm 2003, Barnosky et al. 2012, Dirzo et al 2014). Indeed, the permanent effects of human domination of the planet's ecosystems on biological diversity is evident in new biostratigraphic zones (Barnosky et al. 2014). These changes in biological diversity are marked by accelerated rates of extinction in the last 500 years and notably since the 19^{th} century (Thomas et al 2004, Ceballos et al 2015). Habitat destruction ((Tilman et al 1994) and human population density (Kerr and Currie 1995) are considered significant drivers of species loss but it is likely that a time-lag of several generations will exist between the loss or fragmentation of habitat and when species extinctions occur (Tilman et al 1994). It is also likely that climate change will exacerbate rates of extinction (McLaughlin et al 2002). Estimates of

extinction levels due to climate change under mid-range warming scenarios suggest that 15% - 37% of species are "committed to extinction" by 2050 (Tilman et al. 2004). Indeed, in the next few centuries, 75% of species could go extinct if current trends in habitat loss, over-exploitation of natural and biological resources continue unabated (Barnosky et al. 2012).

Coupled with biodiversity loss in what is the Earth's sixth mass extinction, global patterns of biological diversity have also been characterized by changes in the assemblages of species and their relative abundances in terrestrial and marine ecosystems around the world (Williams et al 2015). These changes have been the product of both intentional resource exploitation and unintended consequences of the industrial revolution and the globalization of the world's economies. On the one hand, industrialization and mechanization of food production by fishing and trawling in aquatic systems (McConnaughey et al 2000, Myers and Worm 2003, Sheppard 2006) has affected the species abundances of marine ecosystems while agriculture and hunting has affected terrestrial systems (Ellis et al 2010).

Another important aspect of human influences on biological diversity on a planetary scale is the unprecedented rate of trans-global movement of introduced species that can become widespread and invade[1] new environments (Vitousek et al. 1996, Simberloff 2009). Changes in

[1] There are divergent opinions on how to describe biological invasions and the criteria for defining what is an invasive species (Davis and Thomson 2000, Valery et al 2008). The term "invasive" typically applies to non-indigenous / non-native species that are introduced to a new environment and have adverse effects on the ecological and/or economic systems in the habitat or bioregion where they establish. The term invasive can also refer to species that have recently become globally widespread but do not necessarily have adverse affects on their natural environment (Colautti and MacIsaac 2004)

the assemblage and relative abundance of species are the product of natural ecological and evolutionary processes, but human impacts on the environment have accelerated these changes at a phenomenal rate that is unprecedented in Earth's history (Ricciardi 2007). These rapid changes in species assemblages have already had profound impacts on fundamental ecological processes (D'Antonio and Vitousek 1992, Fritts and Rodda 1998, Brooks et al. 2004), ecosystem services (Perrings et al 2002), economic systems and human welfare (Pimentel et al. 2000, 2005, 2011) due to the rapid demographic expansion of introduced species that can invade new environments.

In one sense, the success of invasive species is a paradox for ecological and evolutionary theory (Sax and Brown 2000) as evolutionary theory predicts that success in a given environment is the product of long-term adaptation to that environment (Lande 1988). So why is it that invasive species can displace natives that have adapted to their environment? It is important to recognize that most introductions attempts fail (Veltman et al 1996). Indeed, most introduced species don't invade; most introductions don't establish viable populations. This is the case even for introduced species such as the European red deer, *Cervus elaphus* (Clarke 1971) and the European Starling, *Sturnus vulgaris* (Lever 1985) that eventually invade but only do so after multiple colonization attempts.

Demographic stochasticity could contribute to failed colonization attempts, as Allee effects and extinction rates are high in small, isolated populations (Gilpin and Soule 1986, Lande 1988, Simberloff 1988). Environmental stochasticity such as severe droughts, floods and biotic interactions could also contribute to local extinctions of founding populations of eventual invaders (Simberloff 1988, Lande 1988, Mack 1995). Of those introduced species that successfully establish in a new environment, most become naturalized in their new environment

but are un-noticed (Colautti and MacIsaac 2004). Thus, while many introduction events fail to establish and most that establish have negligible ecological impacts, many introductions lead to invasions where the organism becomes so widespread and abundant that it can dominate local habitats, displacing or depleting native species. An important question is why?

There has been a great emphasis in quantitatively predicting the likelihood of invasion based on factors that are thought to contribute to the demographic success of introduced species (Elton 1958, Williamson 1996, Kolar and Lodge 2001). Factors that can contribute to the success of invasive species are varied but can be classified under 1) species-based processes, 2) ecosystem-based processes and 3) dispersal pathway-based processes (Kot et al 1996, Ruiz et al. 2000, Kowarik 2003). Species-based approaches draw on case histories of invasive species and consider the role of phenotypic traits on the success of invasion in order to identify characteristics that predispose a species to become invasive. These trait-based approaches have received considerable attention in the last 20 years and have allowed some generalizations to be made about general traits of invasive species. These trait-based approaches have identified fecundity (which in turn is associated with mode and frequency of reproduction - vegetative and clonal, and the number and mass of seeds or eggs), body mass, taxon, mode and frequency of reproduction (vegetative and clonal), fast growth and broad dispersal ability, diet breadth (generalists) and the ability to tolerate a wide range of abiotic stressors (such as fire, temperature, drought, salinity and toxins) as key traits of invasive species (Williamson and Fitter 1996, Kolar and Lodge 2001), yet the varying success of invasive species in different habitats (Moyle and Light 1996, Williamson and Fitter 1996a, Williamson and Fitter 1996b, Hierro et al 2005) suggest that ecological context also contributes to what traits are predictive of invasion potential in different environments (Williamson 1996).

Ecosystem-based processes in novel environments underscore the importance of the ecological context in which species invade as a major factor determining the success of invasion (Levine 2000). Invaded environments are characterized by historical and geographic isolation (Elton 1958, Brown 1989) that have low diversity of native species that could provide biotic resistance to invasion (Elton 1958, Fox and Fox 1986, Case 1990). Invaded communities are often habitats that are disturbed by human activities (Burke and Grime 1996, Case 1996, Suarez 1998) and associated modifications of the environment, such as roads (edge effects), agricultural landscapes and urban areas (Elton, 1958, Hobbs 1989, Hobbs and Huenneke 1992, Dukes 1999, Lake 2004). These habitat characteristics are often also coupled with the loss of natural enemies such as predators, herbivores, pathogens and parasites in introduced habitats (Elton 1958, Ricklefs and Cox 1972, 1978, Newsome and Noble 1986, Keane and Crawley 2001).

The loss of parasites and pathogens in an introduced range is the product of founder effects, incompatibilities with the abiotic and biotic environment in an expanded geographic range (Torchin et al. 2003). The loss of parasites and pathogens has been observed in many natural systems where invasive species have become widespread in a novel environment (Mitchell and Power 2003, Torchin et al 2005, Torchin et al. 2006). Invaders can also acquire natural enemies, including parasites and pathogens, from their native range or new enemies from their introduced range (Levigne et al. 2004, Torchin and Mitchell 2004, Parker and Gilbert 2004). However, there is some debate on the extent to which the Enemy Release Hypothesis (ERH) is valuable in predicting the demographic success of invaders in new environments (Calautti et al 2004, Liu and Stiling 2006) and the extent to which this hypothesis is supported by empirical evidence.

In addition to the species-based and ecosystem-based approaches to predicting the success of invasion, invasion pathways and features of release events can also contribute to the likelihood of invasion. One aspect of pathway-based approaches is the association that the potential invader has with human activities. Many species that have become globally widespread are often associated with human activities such as agriculture and aquaculture, recreation and international trade and have become cosmopolitan following human settlement around the world (Elton 1958, Lever 1987). The links between human activities and invasion pathways are also quantitatively relevant to the success of founder populations, as the likelihood of invasion success invariably increases with the number of individuals released. (Kolar and Lodge 2001). Due to the close links between propagule pressure (both the volume and frequency of propagule movement) and the frequency of potential colonization events on the likelihood of invasion, it is likely that the number of successful species invasions will continue to increase as global trade routes and invasion pathways such as marine shipping also continue (Verling et al 2005).

There are multiple factors that contribute to the success of invasive species in novel environments. Species-based, ecosystem-based and pathway based approaches are not mutually exclusive, and likely contribute to the success of invaders in different transition points in the pathway for species establishment and invasion (Korand and Lodge 2001). For this reason, it is important to consider the entire life-history of invasive species and consider different factors that contribute to their successful establishment in introduced ranges.

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CHAPTER 1:

Support for the 'host begets parasite' hypothesis in an invaded community: Native bird hosts drive trematode abundance and diversity in an introduced host

ABSTRACT

Biological invaders typically lose parasites and pathogens from their native range. As a result, host-parasite communities in invaded habitats need to be re-assembled – and this assembly can echo aspects of their previous associations in the native range. In Panama, the invasive red-rimmed melania snail (*Melanoides tuberculata*) is commonly infected by trematode parasites with complex life cycles that typically use birds as definitive (final) hosts. In the present study, I evaluate the influence of native bird hosts and habitat features on the abundance and species richness of trematode parasites infecting this snail in an invaded range. A field survey of 13 lake and 13 stream sites shows that the abundance and diversity of trematodes in *M. tuberculata* is strongly associated with the abundance and diversity of potential bird hosts in lake sites but I found no evidence for this association from field surveys in stream sites. A mark-recapture field experiment exposing snails to infection at lake and stream sites found support for the role of birds in driving patterns of trematode infection across habitats, demonstrating that abiotic conditions in stream sites could affect the ability to detect the relationship between bird hosts and trematode parasites. This system therefore shows how the assembly of host-parasite networks in new ranges is a complex mosaic of historical and novel associations and effects.

INTRODUCTION

Parasites substantially contribute the world's biodiversity (Price 1980, Thompson 1994, Dobson et al 2008, Poulin and Morand 2014, Poulin 2014). Despite major advances in community ecology of free-living species in the last 100 years, we know little about what factors influence parasite distributions and abundances in natural systems (Johnson et al. 2016), and even less in the expanded geographic ranges of host species. One commonly observed phenomenon is that host communities structure parasite communities; because parasites live within their hosts and require hosts for transmission, parasite abundance and diversity should correlate with the abundance and diversity of hosts (Hechinger and Lafferty 2005, Kamiya et al. 2014, Thieltges et al. 2011, Poulin 2014). Parasites with complex life cycles are particularly useful for testing this idea as transmission to any particular host is dependent on the previous host in the lifecycle (Combes 2001, Poulin 2007). Indeed for trematode parasites with complex life cycles, the assertion that the distribution of final bird hosts shapes their abundance and diversity in snail hosts has received strong empirical support in the native range of trematodes and snail hosts (Smith 2001, Latham and Poulin 2003, Hechinger et al, 2005). Can we expect the same pattern that bird hosts drive trematode abundance and diversity for trematode parasites infecting snail hosts in an introduced region and across broad spatial scales encompassing different habitat types?

Little is known about the factors shaping the distribution and abundance of parasite assemblages in the introduced range of host species (Johson et al. 2016). This is because host-parasite associations in introduced ranges are decoupled from the ecological and evolutionary context in which they originally developed and need to be reassembled in an introduced range. Parasites are often lost when a host invades a new geographic area (Torchin et al. 2003) but

invaders can acquire parasites from both their native and introduced ranges when they invade (Torchin and Mitchell 2004, Mitchell et al. 2006). When biological invaders become widespread in a new environment, they can facilitate parasite transmission to native hosts (Torchin et al 2002). In cases where either parasites are introduced and hosts are native, or where hosts are introduced and parasites are native, parasite transmission requires the infection of novel host species with which the parasite does not share a common evolutionary history (Frankel et al 2015). As a result, those associations between parasites and hosts in novel environments might be quite different from that in their native range, especially if the parasites are poorly adapted for native hosts or native hosts are poorly adapted to invasive parasites (Poulin 2011). These potentially unique interactions between parasites and novel hosts should then influence the diversity and abundance of parasites in introduced host species. Despite these potential constraints to parasite transmission, invasive snails commonly facilitate the transmission of trematode parasites in their introduced ranges that have the potential to cause disease in humans, wildlife and commercially valuable species (Salgado-Maldonado 2000, Font 2003. Thus, for both theoretical and practical applications, it is important to consider the ecological drivers of parasite transmission in the introduced range of invasive snails.

The red-rimmed melania snail, *Melanoides tuberculata,* is a globally widespread invasive species (Facon 2003, Ben-Ami and Heller 2005) that is commonly infected by trematode parasites in both its native and introduced ranges (Font 2003, Genner 2005). These trematodes have complex life cycles involving 3 different hosts: a 1st intermediate snail host, an 2nd intermediate invertebrate or fish, and a final bird or mammalian host. Trematodes typically exhibit high host specificity to their 1st host but have increasingly broader host range in 2nd and final host (Poulin 2007). In several introduced regions, *M. tuberculata* is infected with

trematodes from its native range (Salgado-Maldonado 2000, Frankel 2015). In Panama, *M. tuberculata* is infected with several trematode species in lake and stream habitats that are now transmitted by native bird hosts.

Birds can disperse trematodes over broad distances (Miura et al. 2011, Torchin et al 2015) and have the potential to shape the distribution and abundances of trematode communities within snail populations (Smith 2001, Hechinger and Lafferty 2005), but little is known about the ability of birds to shape the distribution and abundances of trematode communities in expanded geographic ranges of snail hosts across broad spatial scales and across different habitat types. The invasion of *M. tuberculata* to freshwater lake and stream habitats in the Isthmus of Panama provides a great opportunity to test the hypothesis that bird host abundance and diversity "begets" that of trematode parasites in an expanded geographic range of snail hosts and further evaluate if this pattern is consistent in different ecological contexts.

Using observations from a field survey of 13 lake and 13 stream sites in the Isthmus of Panama, I first ask to what extent the abundance and diversity of birds and of tremtode parasites infecting *M. tuberculata* differ among habitat types (lakes versus streams). Using these field surveys, I also evaluate if patterns of trematode abundances and diversity in snail hosts are correlated with patterns of abundance and diversity of birds and if these associations are consistent across different habitat types (lakes versus streams). I predict that trematode assemblages in *M. tuberculata* will be higher in sites where potential native definitive bird hosts are more abundant and diverse. This prediction arises because the differences among bird species in diet and foraging will expose them to different trophically transmitted trematode species. I predict that bird abundance and diversity should be greater in lakes than in stream sites. Consequently, there should be differences in parasite abundance and diversity in different habitat

types where birds are more abundant.

In the second part of the study, I use a mark-recapture field experiment exposing uninfected "sentinel" snails to infection by trematodes across lake and stream sites and I ask if the rate of trematode infection over a one year period is different in different habitat types. By measuring the success of recapture of these "sentinel" snails, I am able to estimate if the persistence of snails is different across lake and stream habitats. This information informs conclusions regarding the influence of habitat characteristics on the observed associations between native bird hosts and trematodes infecting the invasive *M. tuberculata* in the Isthmus of Panama. By integrating how biotic and abiotic factors affecting both parasites and hosts, I uncover important factors structuring invasive host-parasite communities.

METHODS

Melanoides tuberculata is an introduced snail that has become established in tropical and sub-temperate fresh water throughout the world (Facon et al 2003, Facon et al 2005, Genner et al 2004, Weir & Salice 2012). This snail is parasitized by a diverse assemblage of trematode parasites in its native range of Southeast Asia, the Middle East and Africa (Ben-Ami & Heller 2005, Genner, Kalantan et al 1997, Michel & Todd 2008) and in many parts of its introduced range, including North and South America (Bogéa et al 2005, Salgado-Maldonado et al. 1995, Salgado-Maldonado 2000). Trematode parasites, typically exhibiting high host specificity to snail hosts, asexually reproduce within the snail, releasing free-swimming larval cercariae that either infect definitive hosts directly or encyst on second intermediate hosts. These hosts, such as fishes or crustaceans, infect definitive bird hosts through trophic transmission when they are consumed. Adult trematodes in the definitive hosts breed sexually and pass parasite eggs in their

feces into the water where susceptible snails are infected to restart the life-cycle.

Melanoides tuberculata became established in the Isthmus of Panama as recently as the turn of the 21st century, and it is now the dominant aquatic invertebrate (in terms of abundance, density and biomass) in freshwater lakes and streams in Central Panama (Garces and Garcia 2004). These two environment types vary in several important factors that could influence the population dynamics of snails and their trematode parasites. For instance, the distribution and abundance of *M. tuberculata* is limited to areas of relatively low flow and with no chance of complete desication (Pointier, Theron & Borel 1993, Facon et al. 2004, Giovanelli et al. 2005, Pointier 2001, Weir & Salice 2012). These same factors can influence the parasite: trematode eggs can be washed away by heavy flows and they are intolerant of desiccation (Pointier et al. 2001) In general, then, I might therefore expect numerically fewer snails and fewer parasites in high flow areas and at sites characterized by seasonal drying. Finally, different lake and stream sites vary in the abundance, species identity, and distribution of second intermediate fish hosts and definitive bird hosts (Sharpe et al. 2016).

Field surveys

I sampled snails and conducted bird surveys at 13 lake and 13 stream sites in central and eastern Panama. Of the lake sites, five were in Gatun Lake (*Lago Gatun*), four were in Madden Lake (*Lago Alajuela*), and four were in the western arm of Bayano Lake (*Lago Bayano*). All stream sites were along the northern Caribbean coast of Panama. All of the sites, even within a given lake, were separated from each other by at least 5 km, and I therefore consider them to be independent replicates.

Bird surveys were conducted at each lake and stream site every month for one year:

January 2011 to December 2011. For each monthly bird survey, I counted and identified birds along the same 20 by 50 m long shoreline transect every minute for one hour in the morning regardless of weather conditions. Counts were restricted to birds observed foraging in the water, or perching on trees along the shore where they their feces could end up in the water. Individual birds that were sighted multiple times were recounted. Counts thus excluded birds over-flying the site and birds not seen near the water. Due to these biologically relevant restrictions, most of the birds recorded were piscivorous, although non-piscivorous birds (e.g., grackles and vultures) were also recorded as they were often seen foraging at or near the water. Bird abundance was determined for each site by calculating the number of birds observed per square meter per hour (Hechinger and Lafferty 2005). Counts of birds in a given survey were summed for each species and for all species combined, and divided by 60 (the number of counts per hour) to obtain averages that included multiple birds of the same species occupying a site and multiple observations of the same individual bird at each site. These daily measures were then averaged across the monthly observations over the one-year period.

Snail surveys were conducted every other month in the same sites as the bird surveys. In each case, I established one 25 by 25 cm quadrat every five meters along the same 50 m belt transect used for the bird counts. All snails in each quadrat were collected at each site on each sampling day. *M. tuberculata* is an Old World species complex of snails with distinct morphological and genetic types that can be infected by different species of trematodes. Given that both African and Asian types of *M. tuberculata* are established in Panama (Frankel, unpublished data), some of the variation in our data could be due to variation in snail types. I therefore here limit our analysis to infection of Asian snails only, which are more abundant and more commonly infected than are African snails in Panama. To distinguish African and Asian, I

used diagnostic aspects of shell ornamentation, color, and shape (Genner et al 2004, Genner et al 2008).

A haphazard sample of 100 Asian snails from each lake and stream site was selected for parasitological analysis at each collection period (N = 15,600 across all sites and samples). These snails were measured for width to the nearest .01mm (length would be unreliable owing to broken shell tips) and averages were calculated across the 12 monthly samples per site. To assess if snails were infected, I used a shedding technique (protocol described by Lo and Lee 2001) and confirmed the efficacy of this technique to evaluate if snails were infected (that would be used in our mark-recapture experiment) by conducting a parasitological dissection of all 15,600 snails. Parasite identification (to the level of genus) was based on morphological features of cercariae (Martin 1958, Shell 1985).

Infection experiment using 'sentinel' snails

I quantified natural infection rates through a 'mark-recapture' field experiment exposing 'sentinel' snails to infection. Snails were collected from each site in January 2013 and brought back to the laboratory to test for infection (using the shedding protocol described by Lo and Lee 2002). For each site, 500 uninfected snails were then painted with non-toxic acrylic paint coated with superglue. The painted snails were then released into the same site from which they were originally collected. After a period of one year, the released snails were recaptured for parasitological analysis. Recapture efficiency was the number of snails recaptured per 500 released. I assumed that infection does not affect the probably of recapture, as infected snail can persist for long time periods (Frankel, personal observation), longer than the time-frame of our field exposure. Also, if there was mortality in snails, from parasitism or another factor (aside

from predation) during the exposure period, marked snail shells could still persist in the environment. Infection rate was the proportion of recaptured snails that were infected, as evaluated using the shedding method described above. All snails were dissected for parasitological analysis to confirm the efficacy of the shedding technique as was done with snails collected in the field surveys.

Abundance and diversity measures:

To measure bird abundance, I used raw measures of bird counts from field surveys. To measure parasite abundance, I used parasite prevalence, or the proportion of snails infected relative to the total examined, which is common in the parasite literature (Bush et al. 1997). I calculated parasite abundance (prevalence) as the mean number of infected snails per 100 snails at each site (Bush et al. 1997) across the year-long sampling period. Some previous studies (Kuris 1990, Lafferty et al. 1994) use "pre-interactive" trematode abundances to account for the loss of trematodes due to intra-guild predation. However, because multiple infections were rarely observed, and because the relative competitive dominance among trematodes infecting *Melanoides* is unknown, I used raw parasite abundances rather than 'pre-interactive' trematode abundances. I used rarified species richness as a measure of bird and trematode diversity. Species richness increases with sampling effort. I therefore rarefied estimates of species richness to control for sampling effort (Gotelli and Colwell, 2001). Due to the high variability of bird and trematode abundances in snail populations, I controlled for the sampling effort in snails for trematodes by rarifying bird and trematode species richness to a sample of 100 birds and infected snails, respectively, from each site (Aguirre de Carcer et al 2011, Warton 2015) using the Vegan Package in R (Legendre and Legendre, 2014).

Statistical analysis

I employed analysis of variance (ANOVA) and analysis of covariance (ANCOVA) to evaluate the effect of habitat (a categorical variable with two levels, stream and lake) on 1) bird abundances, 2) bird species richness, 3) parasite abundances and 4) parasite species richness while accounting for potential interactions with snail size (a continuous variable) and an interaction between habitat and snail size. I used linear models to evaluate the correlations between the abundance and species richness of parasites and birds. Throughout my analysis, I used Shapiro-Wilks tests to evaluate the error structure of my data and employed natural log transformations on trematode and bird abundance data to fit the data to a normal distribution.

RESULTS:

Field surveys

Field surveys across 13 lake and 13 stream sites in the Isthmus of Panama quantifying bird abundances (measured as the number of birds per 100 meter squared per hour) showed a lot of variation across sites, ranging from .3 $100m^{-2}\ h^{-1}$ to 41.1 $100m^{-2}\ h^{-1}$. I found differences in the abundance of birds in lake sites (N = 13, mean =13.68 $m^{-2}\ h^{-1}$, SD = 12.82) compared to stream sites (N = 13, mean = 3.68 $m^{-2}\ h^{-1}$, SD = 2.77), which shows that habitat has a strong influence on bird abundance ($F_{1,24} = 7.57$, $P = .01$). Parasite abundance (proportion of snails infected by any trematode) also varied across the 26 sites from 0% to 72.9%. Parasite abundance in lake sites (N = 13, mean = 24.29, SD = 23.94) was greater compared to stream sites (N = 13, mean = 7.62, SD = 4.00). Habitat had a significant effect on parasite abundance ($F_{1,22}$ value = 7.567, $P = .01$), but the effect of snail size was not significant ($F_{1,22}$ value = 1.130, $P = .29$). The interaction

between snail size and habitat had only a marginally significant effect on parasite abundance ($F_{1,22}$ value = 3.465 P = .07). I found significant positive correlations between parasite abundance and bird abundance ($F_{4,21}$ = 6.84, R^2 = 0.483, P < .01) but found no evidence that snail size, habitat nor snail size and habitat had a significant effect on parasite abundance. Considering these interactions, I recalculated the linear regressions for parasite abundance in lake and stream sites separately. Parasite abundance was positively correlated with bird abundance in lake sites ($F_{2,10}$ = 10.15, R^2 = 0.4985, P < 0.01) but I found no evidence for a correlation between parasite abundance and bird abundance in stream sites ($F_{2,10}$ = 0.868, R^2 = .0225, P = 0.21).

Similar to bird abundance data, I observed differences in the species richness of birds in lake sites (N = 13, mean =13.68, SD = 12.82) compared to stream sites (N = 13, mean = 3.68, SD = 2.77), demonstrating that habitat has a significant influence on the species richness of birds ($F_{1,24}$ = 10.07, P < .01). Parasite species richness (rarified) also varied across the 26 lake and stream sites from 0 to 3.20. Parasite richness in lake sites (N = 13, mean = 2.37, SD = 1.12) was greater compared to stream sites (N = 13, mean = 1.24, SD = 1.12). Habitat had a significant effect on parasite richness ($F_{1,22}$ value = 8.141, P < 0.01), but the effect of snail size was not significant ($F_{1,22}$ value = 0.110, P = .74), nor was the interaction between snail size and habitat ($F_{1,22}$ value = 1.141 P = 0.29). I found significant positive correlations between parasite richness and bird richness ($F_{4,21}$ = 5.647, R^2 = 0.4265, P < .01) but found no evidence that snail size, habitat nor snail size and habitat had a significant effect on parasite richness. I then recalculated the correlations for parasite richness in lake and stream sites separately. Parasite richness was positively correlated with bird abundance in lake sites ($F_{2,10}$ = 5.746, R^2 = 0.4417, P = 0.0105) but I found no evidence for a correlation between parasite abundance and bird abundance in

stream sites ($F_{2,10}$ = 0,3178, R^2 = 0.1283, P = 0.466).

Infection experiment using 'sentinel' snails

The recapture rate of sentinel snails after a one year period in lake sites (mean =.10, SD .05) was twice as high as that in stream sites (mean = .05, SD = .02), demonstrating that habitat had a significant effect on our ability to retrieve snails ($F_{1,24}$ = 10.84, P = .00307). I found no evidence that habitat had a significant effect on infection prevalence in sentinel snails ($F_{1,24}$ = 1.358, P = .255). A strong positive correlation was evident between parasite abundance in sentinel snails and abundance of birds across all sites ($F_{4, 21}$ = 7.219, R^2 = 0.4987, P < .01) as well as within lake sites ($F_{2, 10}$ 11.45, R^2 = 0.6352, P < .01) and stream sites ($F_{4, 21}$ = 4.096, R^2 = 0.3404, P = .04991. In a similar fashion, I found a strong positive correlation between parasite richness in "sentinel" snail and richness of birds across all sites ($F_{4, 21}$ = 4.702, R^2 = 0.372, P < .01) as well as in lake sites ($F_{2, 10}$ 11.45, R^2 = 0.6352, P < .01) and approached significance at stream sites ($F_{4, 21}$ = 2.541, R^2 = 0.2044, P = .0833). I found no significant effect of snail size, habitat or the interaction between snail size and habitat on the abundance or richness of parasites in either lake or stream sites.

DISCUSSION:

In our surveys of lake and stream sites in the Isthmus of Panama, birds are more abundant and diverse in lakes than in stream sites as are trematodes infecting the invasive snail, *M. tuberculata*. This natural variation in birds and trematodes across habitat types led us to evaluate the relationship between bird abundance and bird diversity on treamtode abundance and diversity across all sites and within lake and stream sites. My study is the first to my knowledge that

demonstrates that a community of native host species predicts the abundance and diversity of parasites infecting an invasive host in its introduced habitat. Specifically, a greater abundance and diversity of definitive bird hosts was associated with a greater abundance and diversity of trematode parasites in invasive snail hosts. In addition, my study appears to be the first to consider how habitat type influences the observed association between host abundance/diversity and parasite abundance/diversity in an invasive species context. Specifically, snails in lake sites had more parasites than did snails in stream sites and the above relationships between birds and parasites were present in lakes but not streams in field surveys. My field experiment exposing sentinel snails to infection across lake and stream sites demonstrated that lake and stream sites did not differ in the abundance and diversity of trematodes that infected them over a year-long exposure period. Lake and stream sites did differ significantly in the rate at which sentinel snails were recaptured, suggesting that the persistence of snails is greater in lakes than in stream sites, which could contribute to our inability to detect a relationship between bird abundance and diversity and that of trematodes in stream sites.

These results are consistent with several previous studies focusing on host-parasite dynamics in native ranges. For instance, Smith (2001) found that the abundance of native trematodes in native snail hosts was high in sites where bird abundance was high. In addition, Hechinger and Lafferty (2005) reported differences in bird abundance between habitat types (pans and channels) in a coastal salt marsh that led to differences in trematode abundances and diversity. My documentation of similar associations in invasive host-parasite systems suggests that these relationships are fundamental drivers of host-parasite community dynamics across broad spatial scales and in de novo communities. These findings increase confidence in my ability to use native-range host-parasite dynamics to predict - and possibly control –invasive

range host-parasite dynamics.

These results are surprising and exciting because invasive parasites with recently-expanded geographic ranges often do not share a long evolutionary history with potential intermediate and definitive hosts. As a result, parasites might either increase their diversity of hosts (because many of the potential novel hosts are not adapted to resist them) or decrease their diversity of hosts (because parasites are not adapted to many of the novel hosts). In either case, the long-term and moderately stable host-parasite community interactions that have evolved in native ranges might be overturned in the short-term and dynamic host-parasite communities in invasive ranges. Yet my study showed that some of the key drivers of host-parasite interactions are faithfully maintained even in these "non-analog" communities.

My work on this system has revealed a complex and dynamic re-assembly of host-parasite interactions in a novel location through a set of complementary associations at different levels of the parasite life cycle. First, trematodes infecting an invasive snail are likely benefiting from the broad geographic distribution and high abundances of these invasive hosts in order to establish in second intermediate and definitive hosts. In the invaded range of their snail hosts, trematodes show that they can be strongly influenced by the diversity of native bird species.

My study has unique implications when I consider the ecological context of these associations between native hosts and invasive host-parasite associations. It is important to note that the lakes where were sampled are man-made reservoirs, the product of human modifications to aquatic habitats (for the construction of the Panama Canal (Gatun Lake and Madden Lake) and hydroelectric power (Bayano Lake). The differences in birds and trematodes abundances and diversity in these modified habitats compared to undammed streams generates interesting questions regarding the implications of human modifications to the environment on the

transmission of parasites that can cause disease in humans, wildlife and commercially valuable species.

Opportunities

Biological invasions provide important tests for the generalizability of hypotheses that describe a broad range of ecological processes, including the ecological drivers of host-parasite associations and disease transmission. Moreover, the introduction of host species to new geographic ranges provides the unique opportunity to evaluate the drivers of host-parasite community assembly at early stages in the processes. It is also fundamentally important to recognize that the spread of snail hosts that are vectors for trematode parasites can cause emerging diseases in humans, wildlife, and commercially valuable species. Thus, the application of ecological tests describing the abundance and diversity of parasites in a recently expanded geographic range of parasites and their primary hosts also provides important practical insights into the ecological processes underpinning parasite transmission in new environments.

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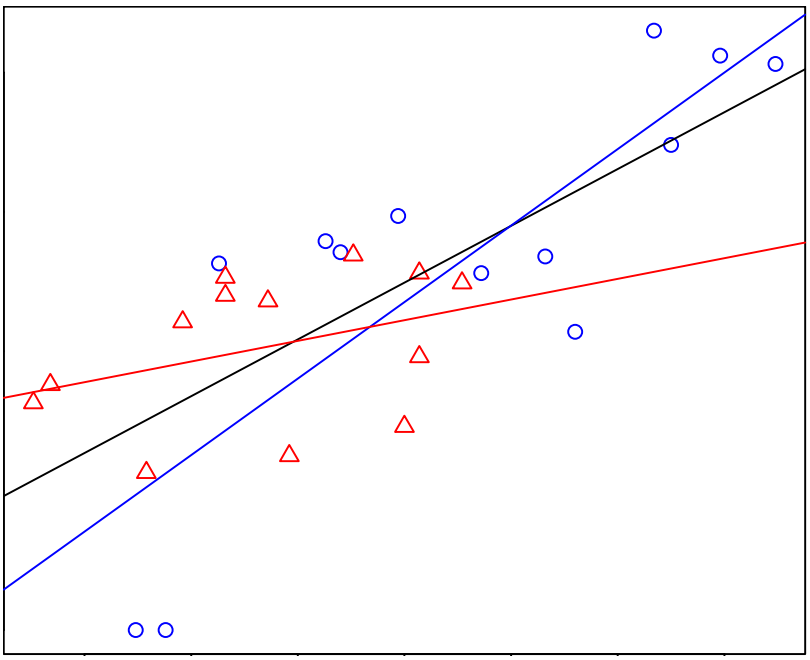

Figure 1: Relationship between bird abundance and parasite abundance in lake and stream sites in the Isthus of Panama. Positive correlations between parasite abundance (% of snails infected) and bird abundance (number m^{-2} h^{-1}) across 26 lake (circles) and stream (triangles) sites in the Isthmus of Panama ($F_{4,21} = 6.84$, $R^2 = 0.483$, $P < .01$). I did not find evidence that snail size, habitat or the interaction between snail size and habitat had a significant effect on parasite abundance. Recalculations of linear regressions for parasite abundance in lake and stream sites separately show that parasite abundance was positively correlated with bird abundance in lake sites ($F_{2,10} = 10.15$, $R^2 = 0.4985$, $P < 0.01$) but I found no evidence for a correlation between parasite abundance and bird abundance in stream sites ($F_{2,10} = 0.868$, $R^2 = -.0225$, $P = 0.2171$).

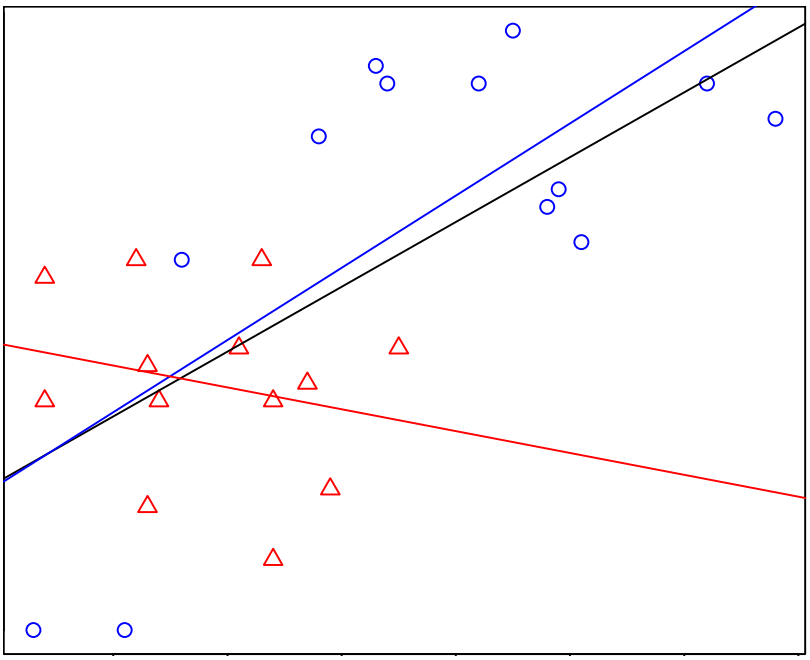

Figure 2: Relationship between rarified species richness of birds and trematode parasites in lake and stream sites in the Isthmus of Panama. Positive correlations between rarified parasite richness and rarified bird richness ($F_{4,21} = 5.647$, $R^2 = 0.4265$, $P < .01$) across 26 lake (circles) and stream (triangles) sites in the Isthmus of Panama. I found no evidence that snail size, habitat or the interaction between snail size and habitat had a significant effect on parasite richness. Recalculation of linear regressions for parasite richness in lake and stream sites separately show that parasite richness was positively correlated with bird abundance in lake sites ($F_{2,10} = 5.746$, $R^2 = 0.4417$, $P = 0.0105$) but I found no evidence for a correlation between parasite abundance and bird abundance in stream sites ($F_{2,10} = 0{,}3178$, $R^2 = -0.1283$, $P = 0.466$).

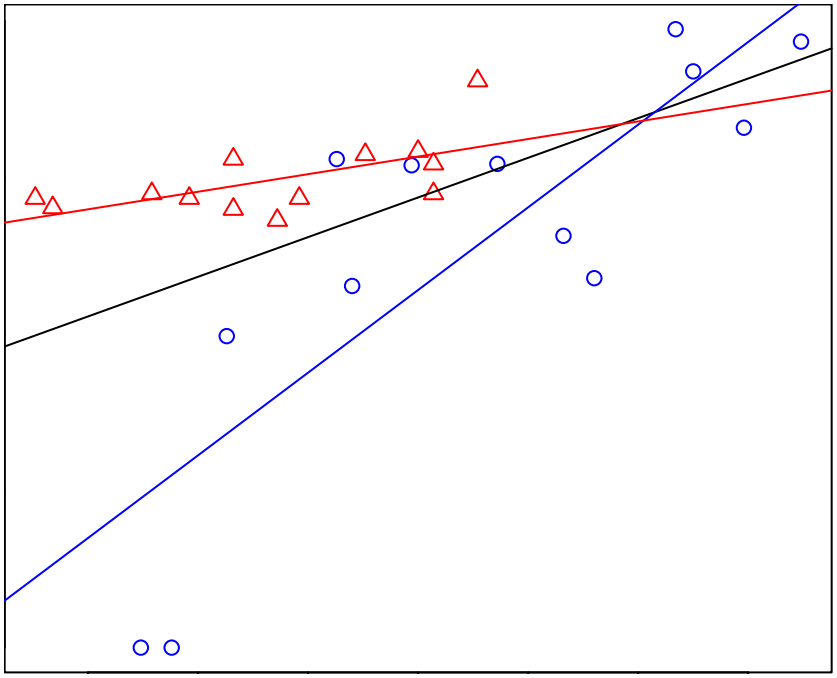

Figure 3: Relationship between bird abundance and trematode abundance in lake and stream sites in the Isthmus of Panama. Positive correlation between parasite abundance in sentinel snails and abundance of birds across all sites ($F_{4, 21} = 7.219$, $R^2 = 0.4987$, $P < .01$) as well as within lake (circles) sites ($F_{2, 10}$ 11.45, $R^2 = 0.6352$, $P < .01$) and stream (triangle) sites ($F_{4, 21} = 4.096$, $R^2 = 0.3404$, $P = .04991$).

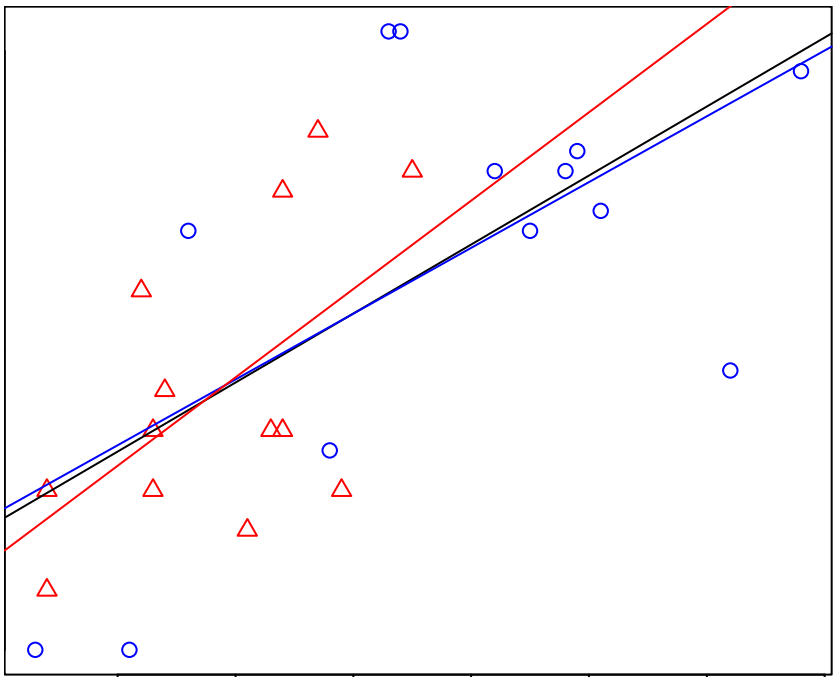

Figure 4: Relationship between rarified bird species richness and rarified trematode species richness in lake and stream sites in the Isthmus of Panama. Positive correlation between rarified parasite richness in sentinel snail and rarified richness of bird across all sites ($F_{4, 21} = 4.702$, $R^2 = 0.372$, $P < .01$) as well as in lake (circles) sites ($F_{2, 10}$ 11.45, $R^2 = 0.6352$, $P < .01$) and approached significance at stream (triangle) sites ($F_{4, 21} = 2.541$, $R^2 = 0.2044$, $P = .0833$).

CONNECTING STATEMENT (Chapter 1 → Chapter 2)

The general objective of this thesis is to consider the entire life-cycle of trematode parasites and the ecological processes that are driving their transmission in introduced ranges. In the first chapter, I evaluate the influence of native bird hosts and habitat features on the abundance and species richness of trematode parasites infecting *M. tuberculata* in an invaded range. I show that the abundance and diversity of trematodes in *M. tuberculata* is strongly associated with the abundance and diversity of potential bird hosts in lake sites but found no evidence for this association from field surveys in stream sites. This system therefore shows how the assembly of host-parasite networks in new ranges is a complex mosaic of historical and novel associations and environmental effects.

In the second chapter, I investigate how these trematodes transmitted by bird hosts, as described in the previous chapter, interact with first intermediate snail hosts. The second chapter logically flows from the first chapter in tracking the life cycle of the parasite from bird hosts to snail hosts, and elucidating the impact of parasitism on snail hosts at population level and at the individual level. Specifically, I consider the effect of parasitism by an invasive trematode, *Centrocestus formosanus*, on host reproduction and investigate the extent to which snail hosts exhibit plasticity in life-history strategies in response to parasitic castration.

CHAPTER 2
PHENOTYPIC PLASTICITY IN LIFE-HISTORY STRATEGIES IN RESPONSE TO PARASITC CASTRATION

ABSTRACT

Plasticity in life-history strategies is a common response in plants and animals to abiotic stress and natural enemies, including parasites. Parasitic castration, exhibited by some trematode parasites in snail hosts, is a form of parasitism that can cause the reproductive death of their hosts and could elicit a plastic response in life-history strategies to compensate for the fitness costs of infection. In this study, I evaluate if an invasive clonal snail, *Melanoides tuberculata*, demonstrates plasticity in life history strategies in response to infection by a globally widespread trematode, *Centrocestus formosanus*. Field surveys suggest that infection by *C. formosanus* eventually stops reproductive output in snail hosts, but some infected snails still harbored juveniles in their brood pouch and exhibited some brood development post-infection. Snails infected in laboratory experiments did not increase reproductive output (numbers of juveniles) but did produce larger juveniles compared to uninfected snails. Further, experimentally infected snails grew more slowly than uninfected snails during the experimental period. The increase in juvenile size, in conjunction with depressed growth in their mother snail, supports the idea that trematode infection induces a plastic response in energetic demands, boosting reproduction at the cost of growth.

INTRODUCTION

Parasites and pathogens can affect the viability of host populations through individual level effects on host reproduction and survival (Anderson and May 1978, Price 1980, Hamilton and Zuk 1982, Lehmann 1993), the primary determinants of individual fitness (Maynard-Smith 1989, Metz et al 1992). Host defenses against parasitism should first attempt to limit the frequency of encounter with parasites and decrease host susceptibility or compatibility of infection through immunological resistance or inducible defenses to parasitism (Poulin 20011). Parasitism can also select for shifts in life-history strategies in populations at high risk of parasitism to maximize reproductive potential before infection (Lafferty 1993, Gomariz-Zilber and Thomas-Orillar 1993). However, plasticity in life history strategies could also allow a host to compensate for the fitness effects of infection post-infection. Here, I evaluate the extent to which a parasitized host demonstrates phenotypic plasticity in life-history strategies in response to parasitism. Specifically, I examine how infected hosts initiate a reproductive shift to producing larger offspring after infection by a parasitic castrator.

Evolutionary theory predicts that selection should act on the way a given genotype alters its life history in response to change in its environment. Phenotypic plasticity, the capacity of a given genotype to generate distinct phenotypes in different environmental conditions (Schlichting and Pigliucci 1998, Debat and David 2001), can provide the means to respond to both extrinsic and intrinsic factors that affect fecundity and survival (Stearns 1989). Plastic responses in life-history strategies reflect a re-allocation of the energetic budget allocated to reproduction to maximize fitness in response to stress (Stearns 1989, Schaffer 2003). These modifications in life-history strategies can be expressed as changes in the number and size of

offspring, the size and age at maturity, and the timing in which these changes are expressed from one year to another or the lifespan of an individual (Steams 1976, Hutchings 2003). The effects of environmental factors such as temperature, habitat quality and food supply on plastic responses in life-history strategies have been well documented (Nylin and Gottard 1998, Sultan 2000). In addition to environmental factors, natural enemies such as herbivores and predators can also induce a plastic response in life history strategies (Crowl and Covich 1990, Reznick et al 1990, Stibor 1992, Martin 1995, Tollirian 1995, Boersma et al 1998, Sakwinska 1998, 2002, see Gotthard and Nylin 1995, Nylin and Gotthard 1998 and Lass and Spaak 2003 for reviews). Modifications in life history strategies of hosts have also been documented in response to parasitism (Vizoso and Ebert 2005). These include shifts to earlier reproduction (Chadwick and Little 2005), increase growth (Bize et al. 2003) and reproductive effort (Thornhill et al 1986, Polak & Starmer 1998, Krist 2001). However, it is often unclear to what extent phenotypic plasticity versus genetic differences contribute to these differences in life-history strategies (Michalakis and Hochberg 1994, Krist 2001). A better understanding of the ability of hosts to alter life-history strategies as a phenotypic plastic response to parasitism can provide valuable insights to the factors that promote the persistence of host populations and the transmission of the parasites that infect them.

Parasitic castration is a consumer strategy in whereby parasites acquire energy by eliminating host reproduction (Kuris 1974), effectively causing the "reproductive death" of a host (Minchella and Loverde 1981). Thus, there should be strong selection pressure in response to castration (Lafferty and Kuris 2009). Common parasitic castrators, such as barnacle and isopod parasites of crustaceans and larval trematode worms infecting snails, can severely restrict or completely eliminate the reproductive potential of their hosts directly by feeding on host

gonads or indirectly by producing hormones that affect host reproduction or energy allocation to gonad development (see Lafferty and Kuris 2009 for a review). Exposure to infective stages of trematodes, for example, drives shifts in life-history strategies in snail hosts; *Biomphalaria glabrata* increases growth and rates of egg production in response to schistosomes (Minchella and LoVerde 1981) and marine horn snails mature earlier in populations frequently parasitized by trematodes (Lafferty 1993). Observed shifts in life-history strategies of snails in response to trematode parasites could be linked to phenotypic plasticity in response to environmental factors such as maternal effects. Thus, I asked, to what extent can phenotypic plasticity allow a host to modify life history strategies associated with reproduction in response to parasitic castration?

One hypothesis is that parasites that have strong fitness impacts on their hosts should elicit a phenotypic response in an infected individual that reflects a terminal investment in reproduction to maximize fitness in the face of certain reproductive death (Williams 1966, Roitberg et al 1993, Engqvist and Saurer 2002, Daly and Wilson 2005). In some cases of parasitic castration, hosts are able to reproduce as the parasites are developing in the host in early stages of infection before host gonad tissue is completely absent (Fogelman et al. 2009). In other cases, snail hosts can respond to trematode parasites by boosting reproductive output in egg production in early stages of infection (Minchella and LoVerde 1981, Thornhill et al 1986) to compensate for future reductions in fecundity expected when tremaotodes develop within their snail hosts and castrate them (Keas and Esch 1997, Sorensen and Minchella 2001). Thus, if a host is able to produce offspring in early stages of infection by a parasitic castrator, it has the potential to reallocate energy away from growth to boost energetic expenditures in reproduction or maternal investment in juveniles, which could be reflected in increase in brood size or the size of juvenile offspring at birth.

To test this idea, I evaluate the effect of trematode infection on the reproduction of the snail, *Melnaoides tuberculata* (Muller 1774) by experimentally infecting snails to determine if hosts can modify life-history strategies in response to parasitism. *M. tuberculata* is a globally widespread clonal snail that is frequently infected by trematode parasites in native and introduced ranges. *M. tuberculata* snails are ovoviviparous and provide maternal investment for the development of juveniles; eggs are continuously produced and passed into a cephalic brood pouch where they develop inside the mother's shell until juveniles are released to the environment. Clonal lineages (also referred to as 'morphs') of *M. tuberculata* display phenotypic variance in fecundity, juvenile size, age at first reproduction and size at first reproduction (Facon et al 2003), yet variance in these reproductive traits is greater across different morphs than among morphs. Thus, differences in life history traits among clonal lineages are likely attributable to environmental factors (Facon et al 2003). This system thus provides an opportunity to test the effect of parasitism on life-history strategies by comparing brood size and juvenile size between infected and uninfected individuals within a single clonal lineage. Here, I investigate the extent to which trematode infection affects snail reproduction in field surveys and evaluate through a lab experiment if infected hosts display plastic responses in life history strategies. By jointly considering the results of field surveys and the lab experiment, I consider the broader implications that residual reproduction after infection and associated changes in life-history traits have for the persistence of *M. tuberculata* snails and the parasites that infect them in an introduced range.

METHODS:

I investigate the extent to which trematode infection affects snail reproduction in field surveys and use a lab experiment to test if infected hosts display plastic responses in life history strategies. I first surveyed wild populations of *M. tuberculata* naturally infected by parasitic trematodes in lake and stream sites in the Isthmus of Panama, quantifying 1) the prevalence of infection in different populations as a measure of "intensity" of infection at the population level and 2) the impact of infection on snail reproduction by measuring the brood size and number of new juveniles produced by infected snails compared to uninfected snails. I complement this field survey with a laboratory experiment where I infected *M. tuberculata* snails and quantified the number and size of juveniles produced over 16 week period post-infection. I first determined if infected snails fledge offspring after infection and whether infection changes the number or size of juvenile offspring produced after infection. I also measured snail growth in both infected and uninfected snails to consider the effect of infection on snail growth.

Field surveys

I conducted field surveys to examine parasitism in snail reproduction in wild populations. These field surveys allowed us to quantify 1) the frequency of trematode in different snail populations and 2) how infection affects brood size (or the number of juveniles found in the brood pouch of snail mothers, 3) the production of new offspring after infection and 4) whether infected snails can still harbor juveniles in early and late stages of infection, thus creating the conditions for residual maternal investment in juvenile development after infection. Field surveys of *M. tuberculata* snails took place at various lake and stream sites in the Isthmus of Panama (see Table 1) where snails are very abundant and commonly infected with trematodes. Snails collected in these field surveys were dissected by lightly crushing the shell, permitting the soft

tissue of the snail to be removed from the shell. Juvenile snails were teased away from cephalic brood pouch, sorted into groups based on different stages of development. Juveniles in the very early stages of development had a shell but no whorls, while juveniles in late development stages have 1 – 5 whorls. Trematodes infecting snails commonly occupy the gonad tissue of their hosts, developing asexually and increasing in biomass within their host as they consume snail tissue. Thus, I was able to classify whether infected snails were at different stages of infection (from recent to late stage infections) made by visually estimating the proportion of snail gonad tissue occupied by trematodes.

Lab experiment:

I experimentally infected *M. tuberculata* snails with a heterophyid trematode, *Centrocestus formosanus* (Nishigori 1924, Martin 1958), to evaluate the impact of parasite infection on snail reproduction and growth. I restricted my laboratory experiment to this parasite because it was the most common parasite in my surveys (Frankel et al. in prep) and its life-cycle has been extensively described in previous studies. Uninfected snails used in this experiment were collected in a near-shore freshwater habitat in Barro Colorado Island, an island in Gatun Lake, Panama. Immediately after collection, snails were transported to the Smithsonian Tropical Research Institute's Naos Marine Station in Panama City for the experiment. I was able to confirm that collected snails were uninfected by exposing snails to heat and light (see Lo and Lee 1996 for protocol) that would shed trematode larval cercariae from infected snail hosts and allow for visual determination of infection status. In order to control for the effects of snail size on reproduction, I restricted my experiment to snails that measured between 6.8 mm – 7.0 mm in length. After measuring snails, I randomly allocated snails into two treatment groups, 100 snails

in the infection group and and 100 uninfected snails as controls. All snails were housed at ambient temperature in the lab and kept in isolation from other snails. Throughout the duration of the experiment, all snails were fed twice each week with 1g of blended lettuce followed by a water change 24 hours after each feeding.

After a two-week period in which snails were acclimated to laboratory conditions, I began measurements of snail reproduction twice per week by collecting juvenile snails that were born (fledged) from their snail mother into the holding container. During this acclimation period, I was able to confirm that all snails were female as all snails produced juveniles. Juveniles were pipetted from the holding container and immediately euthanized by immersion in 95% ethanol. Reproduction was measured by quantifying the number of juveniles produced for each bi-weekly period. I also measured their size (length) by photographing the juveniles using a camera mounted on a stereo microscope and using the program ImageJ (Schneider et al. 2012) to measure length of juveniles. Measurements of brood number (output) and size were taken for four weeks prior to exposure to trematodes and 16 weeks after exposure.

Snails were experimentally infected with trematodes cultivated in the lab (see Chen 1942, 1958, Martin 1958 and Arizmendi 1992 for a detailed description of the parasite life cycle, protocols for lab cultivation and diagnostic morphological features in intermediate and definitive hosts). Parasite cercariae from *M. tuberculata* snail hosts were collected by "shedding" collected snails (see Lo and Lee 1996 for details on protocol). I used these carcariae to infect second intermediate fish hosts. Next, I exposed second intermediate fish hosts to 1000 parasite larvae shed from infected snails. I used the peacock bass (*Cichla monoculus*), a common fish host that is commonly infected by *C. fomrosanus* in Gatun Lake and can be successfully infected in laboratory experiments with this parasite (Frankel et al. 2015). After a three week infection

period allowing trematode parasites to encyst and fully develop in the fish host, I euthanized infected fish and extracted mature metacercariae from the gill filaments. Immediately after extraction, I fed approximately 500 metacercariae to chickens (*Gallus gallus domesticus*) that were raised in the laboratory (and therefore were previously uninfected). One week following the infection of the bird hosts, I confirmed the presence of *C. foromosanus* eggs in fecal samples (see MAFF 1986 for details on protocol for flotation in centrifuge) and exposed 100 snails to 1ml of fecal matter containing trematode eggs. After the 16 week infection period, in which many snails exposed to parasites were observed producing trematode cercariae eight to ten weeks after infection, I again measured snail size and dissected all snails to confirm parasite infection in the treatment group.

Statistical analysis:

I quantified snail reproduction by measuring the number and sizes of juveniles by dissecting snails in the field and by measuring juveniles released from experimental snails over a 22 week period, 4 weeks before infection and 16 weeks after infection twice per week. Of the 22 week infection period, I analyzed the last two-week measurement period of reproductive output and snail size and analyzed these data with analysis of variance (ANOVA) treating fecundity (brood production) as the response variable and infection type (uninfected vs infected) and infection level 0 (uninfected), 1 (early stage) to 5 (late stage) in separate analyses. I used a Tukey test on an ANOVA to compare pairwise differences in brood size in field captured snails for all five species of trematodes independently against each other and against uninfected snails. Finally, I used an ANOVA to compare differences in growth rates, calculated as growth in snail width over time timeframe of the experiment, of snails between the control, exposed and infected groups.

RESULTS

Field survey:

In total, 3,749 snails were collected in my field survey of five lake sites and four stream sites in the Isthmus of Panama. Of these snails, 462 (or 12.3%) were infected by trematodes. The most common of five species of trematode found infecting *M. tuberculata* was *Centrocestus formosanus*, with infection prevalence (or the percent of snails infected) ranging from 0% to 29.4% across sites but representing more than 70% of all trematode infections (see Table 1). Both infected and uninfected snails harbor juvenile snails in the brood pouch but brood size was smaller in infected snails (measured as the number of juveniles found in the cephalic brood pouch of female snails) when I compared all infected snails, irrespective of the parasite species, with uninfected snails (ANOVA: F (5, 3695) = 35.3, $P < .001$). Of the 5 trematode species infecting *M. tuberculata* only *C. formosanus* significantly reduced brood size (*C. formosanus*: N = 326, mean brood size = 7.86, St. dev = 16.93, uninfected: N = 3,287, mean brood size = 62.44, St. dev. = 76.23, ANOVA: F (5, 3559) = 41.56, $P < .001$) (see Figure 1, Table 2) (see Table 3). However, I found no difference in the number of juveniles found in early stage infections compared to late stage *C. formosanus* infections (see Table 4, Figure 2). I found a significant difference in the number of juveniles of the smallest size class produced by infected *M. tuberculata* snails (N = 326, mean= 0.21, St. dev. = 1.21) compared to uninfected snails (N = 3,287, mean = 16.83, St. dev. = 20.80, ANOVA: F (1, 3563) = 208, $P < .001$) (see Figure 3).

Laboratory experiment:

My laboratory experiment exposed 100 snails to trematode eggs and produced 43 infections, The 57 exposed but uninfected individuals were excluded from the analysis. The number of juveniles released by female *M. tuberculata* snails did not differ significantly between uninfected and infected snails (uninfected mean = 3.939, St. Dev = 1.975, infected mean = 3.396 St. Dev. = 1.390, ANOVA: F (1,141) = 2.705, P = 0.102) (see Figure 4). However infected snails produced significantly larger offspring (uninfected mean = 1.990mm, St. Dev = 1.114, infected mean = 2.534 St. Dev. = 0.51542, ANOVA: F (1,141) = 9.354, P = 0.003) (See Figure 5). I coupled the number and size of juveniles into a single measure of juvenile production, the product of the number of juveniles by the average size of the juveniles produced by infected and uninfected snails (Flemming and Gross 1990). I found a significant difference in juvenile production between infected and uninfected snails (uninfected mean = 6.954, St. Dev = 3.690, infected mean = 8.726, St. Dev. = 4.471, ANOVA: F (1,141) = 6.079, P = .015). There was a marginally significant trend for infected snails to grow less (uninfected mean = 0.257, St. Dev = 0.046, infected mean = .241, St. Dev. = 0.041, ANOVA: F (1,141) = 3.7, P = 0.056).

DISCUSSION

Infection by trematodes was common in *M. tuberculata* populations at some lake and stream sites in the Isthmus of Panama. *Centrocesus formosanus* was the most common parasite, representing more than 70% of all infected snails. Juveniles early in their development were rarely found in snails infected by *C. formosanus*, suggesting that infection effectively stopped the production of new juveniles. While infection by *C. formosanus* eventually stops production of new juveniles, infected snails were found commonly harboring juvenile snails of intermediate to

late development stages, suggesting that after infection, snails can provide residual maternal investment to juvenile development. However, snails infected by *C. formosanus* had significantly fewer juveniles developing in the brood pouch compared to uninfected snails, probably as a consequence of castration. I found no evidence that the other species of trematodes infecting *M. tuberculata* in field surveys affected brood size. Therefore, I focus the discussion of field surveys and laboratory experiments to the effects of *C. formosanus* on *M. tuberculata.*

In laboratory experiments I examined phenotypic plasticity in life-history traits in response to infection. At the end of the infection experiment, the number of juveniles released by infected *M. tuberculata* snails did not differ significantly compared to uninfected snails. While I found no evidence that trematode infection produced a change in the number of juveniles, I found evidence in my laboratory experiment that infected snails produced juveniles that were larger than those produced by uninfected snails. This increase in snail size could be the product of a plastic response in life-history strategies that provides a boost in maternal investment to juvenile offspring. Infected snails also exhibited decreased growth rates compared to uninfected snails over the course of my experiment, suggesting that there may be a trade-off between the energetics allocated to growth and reproduction, such that when a snail becomes infected, energy is reallocated to boost maternal investment in offspring. However, additional experiments are needed to elucidate the nature of the potential trade-offs that contribute to increased juvenile size.

Variability in juvenile body size, particularly at early life-stages, has strong implications for survivorship, as juvenile mortality rates in many organisms decrease with increasing body size (Sogard 1997, Blanckenhorn 2000, Swain et al 2007) due to a greater resistance to starvation, increased tolerance to environmental extremes and decreased vulnerability to

predation (Peterson and Wroblewski 1984, Houde 1987, Miller et al 1988, Reznick et al 1996, Sinclair et al 2002). Body size is particularly important in freshwater snails with respect to tolerance to desiccation as the snail's surface-to-volume ratio decreases with increasing shell size (Pointier 2001). *M. tuberculata* is very susceptible to desiccation, especially at juvenile stages, and is a major constraint to its abundance and distribution and likely affects its invasion potential in unstable or temporary environments (Pointier 2001). Therefore, plasticity in the size of juveniles when they are released into the environment could have implications for its ability to tolerate abiotic stress and could contribute to its ability to maintain high population densities in unstable environments.

Maternal investment in brood production could be important for infected *M. tuberculata*, especially as it relates to increases in juvenile size, as life-history strategies are an important fator in the ability of this snail to invade new environments. *M. tuberculata* is a species complex of highly invasive parthenogenic snails (Guimarães et al 2002, Genner 2008) whose invasion potential are tightly linked to life-history traits associated with reproductive strategies (Berry and Kadiri 1974, Pointier et al 1992), including asexual reproduction, high fecundity and tolerance of environmental extremes. These clonal snails are ovoviviparous; eggs are passed into a cephalic brood pouch where they develop into juveniles inside the snail's shell until released into the environment. Previous studies (Pointier et al. 1993, Facon et al. 2005, Patrice David - personal communication, Frankel unpublished data) have shown that genetic varieties within the *M. tuberculata* species complex have high variance in life history traits such that some clonal lineages produce many broods of small size, and some produce fewer broods of larger sizes. Additionally, larger juvenile size at birth is associated with the invasion success of the different clonal lineages of *M. tuberculata* (Pointier 1998). Perhaps modifying life-history strategies

increases fitness by boosting juvenile size (and thus survivorship) when they are infected by parasitic castrators that eventually stop reproduction.

Trematodes are a diverse group of parasites that can be very abundant in the populations of their first intermediate snail hosts (Hechinger et al 2005, Kuris et al 2008). In many cases, trematodes castrate snail hosts and could thus be important regulatory drivers of snail population dynamics (Kuris 1973, 1974) through their impacts on local host densities (Lafferty 1993) and thus impose selection pressures on snail life-history strategies to maximize fitness. In this study, I found that an invasive snail, *M. tuberculata* is frequently infected by a trematode parasite, *Centrocestus formosanus*, that significantly affects snail reproduction and could have important regulatory effects on snail populations. Yet the ability of infected snails to provide residual reproduction after infection could provide the means to sustain high population densities in environments commonly infected by parasites, which could also feed back into the high transmission rates of parasites benefiting from high local densities of obligate host species. Infections by *C. formosanus* also produced plastic shifts in the size of juveniles, a change in life-history strategies that could enhance the ability of M. tuberculata to survive in stressful environmental conditions such as desiccation. By modifying life-history strategies associated with juvenile size, it is possible that hosts infected by parasitic castrators, faced with their eventual reproductive death, can "make the best (if not the most) of such a bad situation."

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TABLE 1

Summary statistics of infection totals and prevalence (percent) by five trematodes across 5 lake and 4 stream sites in the Isthmus of Panama. Formo = *Centrocestus formosanus*, Hetero = *Haplorchis pumillo*, Loxo = *Loxocentroides sp*., Micro = *Microphallus sp*., Philop = *Philopthalmus gralii*). 3,749 snails were collected in my field survey. Of these snails, 462 (or 12.3%) were infected by trematodes. The most common of five species of trematode found infecting *M. tuberculata* was *Centrocestus formosanus*, with infection prevalence (or the percent of snails infected) ranging from 0% to 29.4% across sites but representing more than 70% of all trematode infections.

Site	Uninf.	Formo # (%)	Hetero # (%)	Loxo # (%)	Micro # (%)	Philo # (%)	Total
Bayano1	146	28 (15.91)	0 (0.0)	0 (0.0)	0 (0.0)	2 (01.1)	176
Bayano2	138	58 (29.44)	0 (0.0)	1	0 (0.0)	0 (0.0)	197
BCI	162	0 (0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	162
Gatun1	1346	151 (10.01)	4 (0.003)	4 (0.003)	0 (0.0)	4 (0.003)	1509
Gatun2	200	1 (0.005)	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.01)	203
Colon1	744	54 (0.06)	0 (0.0)	9 (0.01)	4 (0.005)	29 (0.035)	840
Colon2	110	0 (0.0)	0 (0.0)	4 (0.03)	6 (0.05)	3 (0.02)	123
Langosta	297	34 (0.09)	0 (0.0)	1 (.003)	57 (0.15)	4 (.01)	393
Cascajal	144	0 (0.0)	0 (0.0)	0 (0.0)	2 (0.01)	0 (0.0)	146
Total	3287	326 (0.088)	4 (.001)	19 (0.005)	69 (0.019)	44 (0.011)	3749 (0.875)

Table 2:

Summary statistics of brood size (number of juveniles in brood pouch) in field surveys of uninfected M. tuberculata snails and snails infected by five species of trematodes (Formo = *Centrocestus formosanus*, Hetero = *Haplorchis pumillo*, Loxo = *Loxocentroides sp*., Micro = *Microphallus sp*., Philop = *Philopthalmus gralii*). I found that both infected and uninfected snails could harbor juvenile snails in the brood pouch but found that infections by *C. formosanus* significantly decreased brood size, measured as the number of juveniles found in the cephalic brood pouch of female snails (*C. formosanus*: N = 326, mean = 7.86, St. dev = 16.93, uninfected: N = 3,287, mean brood size = 62.44, St. dev. = 76.23, ANOVA: F (5, 3695) = 35.3, $P < .001$) but I found no evidence that the other four trematodes affected brood size compared to uninfected snails.

Infection Type	Brood size (Mean / St Dev)
Formo	7.86 / 16.93
Hetero	0 / 0
Loxo	68.39 / 60.21
Micro	40.75 / 41.97
Philop	48.48 / 60.27
Uninfected	62.44 / 76.23

Table 3: Pairwise comparisons of brood size across uninfected *Melanoides tuberculata* snails and those infected with 5 species of trematodes in the Isthmus of Panama in five lake sites and four stream sites in the Isthmus of Panama (Formo = *Centrocestus formosanus*, Hetero = *Haplorchis pumillo*, Loxo = *Loxocentroides sp*., Micro = *Microphallus sp*., Philop = *Philopthalmus gralii*)). I found that both infected and uninfected snails could harbor juvenile snails in the brood pouch but found that snails infected by M. tuberculata harbored significantly smaller brood sizes compared to uninfected snails. significantly decreased brood size, measured as the number of juveniles found in the cephalic brood pouch of female snails (ANOVA: F (5, 3695) = 35.3, $P <$ .001).

Uninfected	Formo	Hetero	Loxo	Micro	Philo
Uninfected	0.0000000	0.5098927	0.7908135	0.8285923	0.9999846
Hetero 1	X	0.9997325	0.2793414	0.0002276	0.0000521
Hetero 2		X	0.3647390	0.7283927	0.5269824
Loxo			X	0.5204828	0.9126617
Micro				X	0.9478091
Philo					X

Table 4:

Pairwise comparisons of brood size across uninfected *Melanoides tuberculata* snails and those infected at 5 levels of infection by *Centrocestus formosanus* in the Isthmus of Panama.(0 = uninfected, 1 = early stage, 5 = late stage). Infected and uninfected snails could harbor juvenile snails in the brood pouch but snails infected by *M. tuberculata* harbored significantly smaller brood sizes compared to uninfected snails (ANOVA: F (5, 3559) = 41.56, $P < .001$). This difference in brood size was significant across all infection levels (early vs late stage infections). However, pairwise comparisons of snails at each level of infection show that no evidence that the number of new juveniles found in early stage infections differed to the number of juveniles found in late stage infections.

0 (Uninfected)	1	2	3	4	5
0 (Uninfected)	0.0000022	0.0000000	0.0000000	0.0000018	0.0000108
1	X	0.9997423	0.9999984	0.9997938	0.9999348
2		X	0.9976283	0.9900739	1.0000000
3			X	0.9999552	0.9994540
4				X	0.9968716
5					X

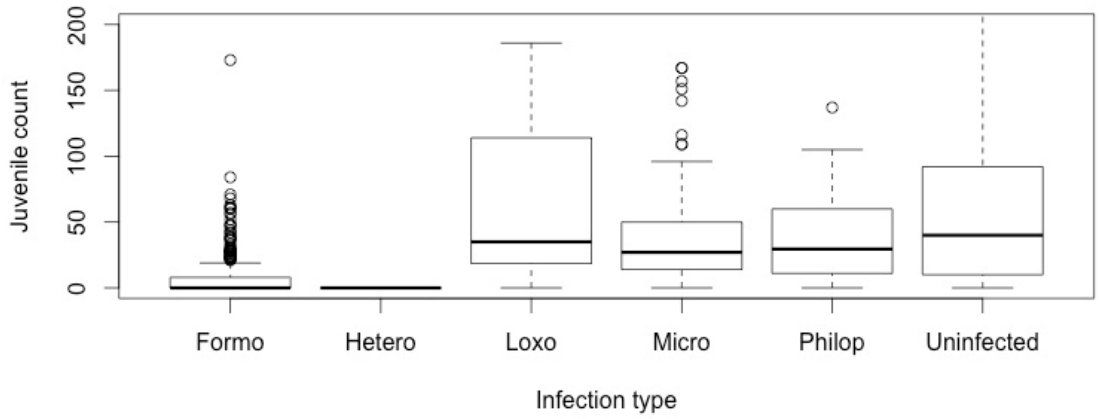

Figure 1: Brood size of *Melanoides tuberculata* snails infected by 5 species of trematodes compared to uninfected snails. Formo = *Centrocestus formosanus*, Hetero = *Haplorchis pumillo*, Loxo = *Loxocentroides sp*., Micro = *Microphallus sp*., Philop = *Philopthalmus gralii*) .3,749 snails were collected in my field survey of five lake sites and four stream sites in the Isthmus of Panama. Infected and uninfected snails could harbor juvenile snails in the brood pouch but infection significantly decreased brood size, measured as the number of juveniles found in the cephalic brood pouch of female snails, when I compared all infected snails, irrespective of the parasite species, with uninfected snails (ANOVA: F (5, 3695) = 35.3, $P < .001$). However, pairwise analysis of brood size of each trematode species with uninfected snails shows that the only parasite that significantly decreased brood size was *Centrocestus formosanus*.

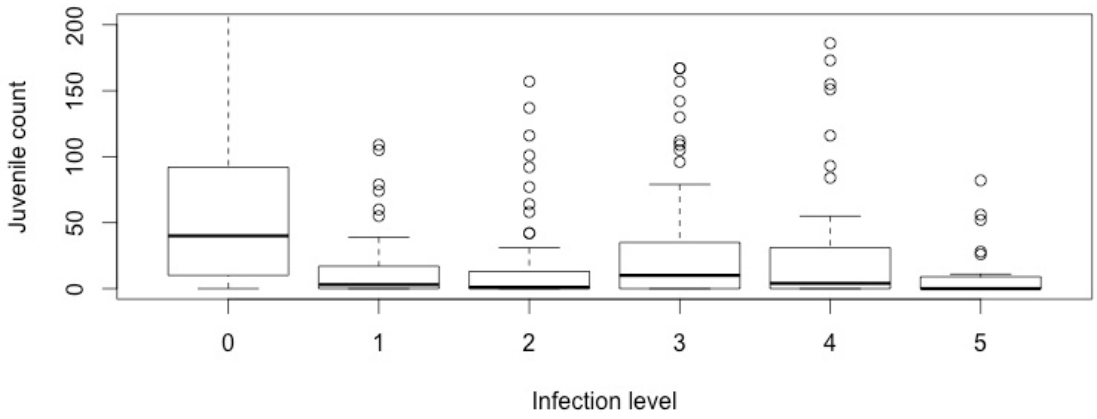

Figure 2: Brood size across uninfected *Melanoides tuberculata* snails and those infected at 5 levels of infection by *Centrocestus formosanus* in the Isthmus of Panama.(0 = uninfected, 1 = early stage, 5 = late stage). Infected and uninfected snails could harbor juvenile snails in the brood pouch but snails infected by *M. tuberculata* harbored significantly smaller brood sizes compared to uninfected snails (ANOVA: F (5, 3559) = 41.56, $P < .001$). This difference in brood size was significant across all infection levels (early vs late stage infections). However, pairwise comparisons of snails at each level of infection show that no evidence that the number of new juveniles found in early stage infections differed to the number of juveniles found in late stage infections.

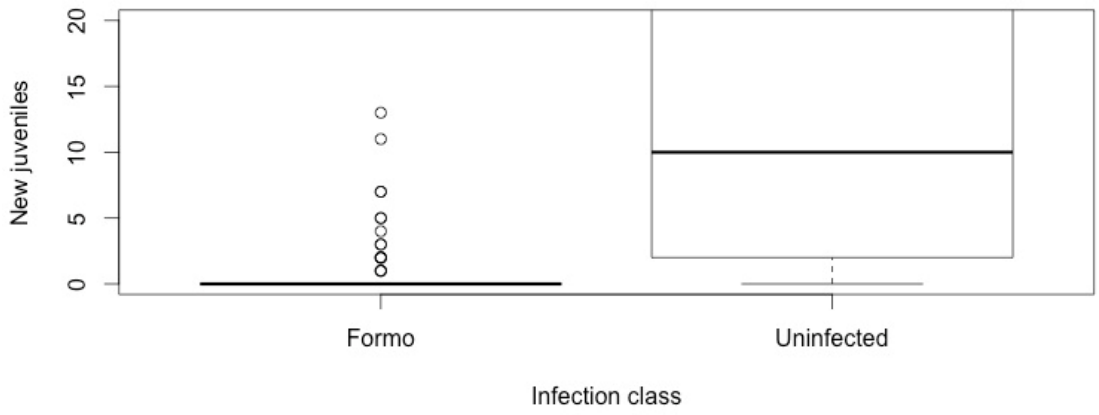

Figure 3: Number of juveniles of the smallest size class in snails infected by *Centrocestus formosanus* and uninfected snails. When found a significant difference in the number of juveniles of the smallest size class produced by infected *M. tuberculata* snails (N = 326, mean= 0.21, St. dev. = 1.21) compared to uninfected snails (N = 3,287, mean = 16.83, St. dev. = 20.80, ANOVA: F (1, 3563) = 208, $P < .001$) (see Figure 3). The absence of juveniles of the smallest size class in snails infected by *C. formosanus* suggests that this parasite stops reproduction in *M. tuberculata* soon after infection.

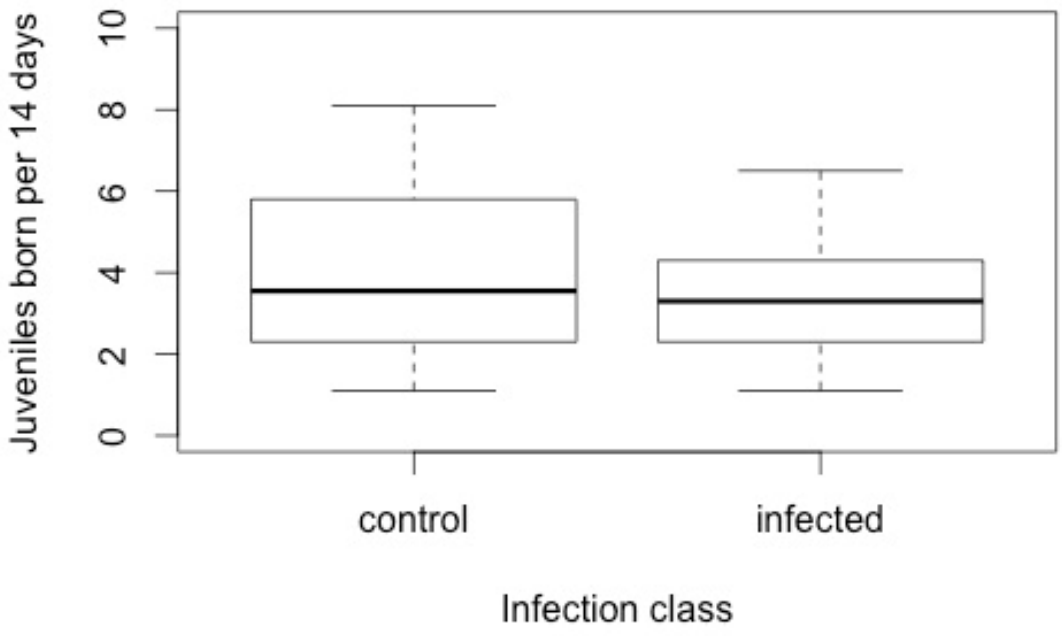

Figure 4: Brood production in control versus infected snails in a laboratory experiment. Experimental infections of *Melanoides tuberculata* snails with a trematode parasite, *Centrocestus formosanus*, show no significant affect in the number of juveniles born (fledged) from snaails after a 16 week post-infection period (uninfected mean = 3.939, St. Dev = 1.975, infected mean = 3.396 St. Dev. = 1.390, ANOVA: F (1,141) = 2.705, P = 0.102).

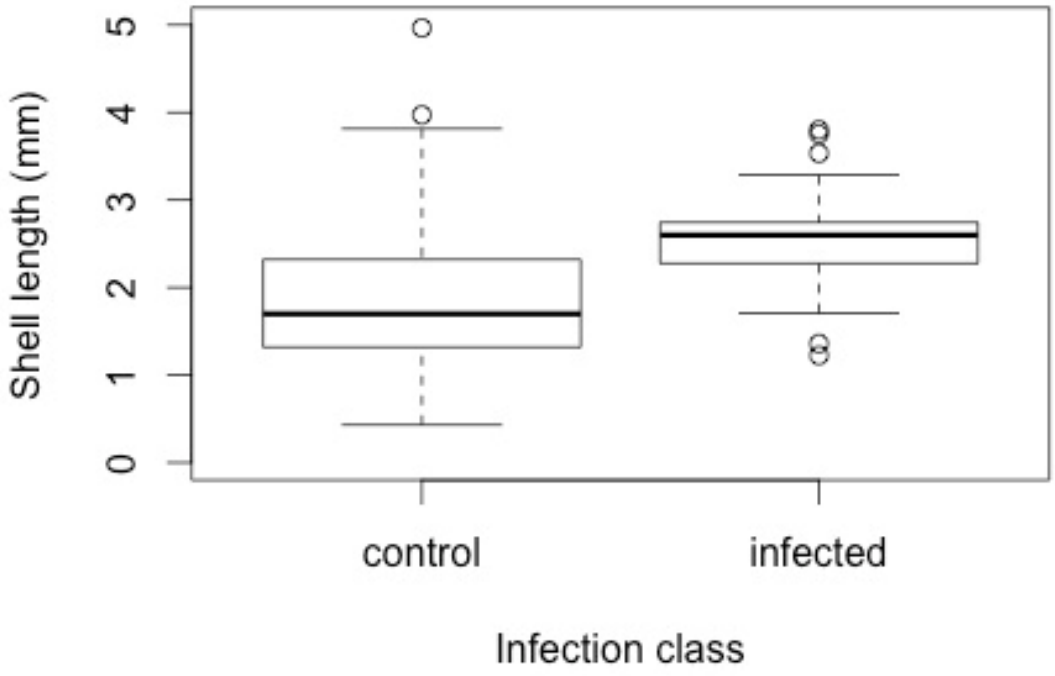

Figure 5: Size of juvenile snails in control versus infected snails in a laboratory experiment. Experimental infections of *Melanoides tuberculata* snails with a trematode parasite, *Centrocestus formosanus*, show a significant increase in juvenile size, measured as the length of the shell, in juveniles born (fledged) from infected snails after a 16 week post-infection period compared to uninfected snails (uninfected mean = 1.990, St. Dev = 1.114, infected mean = 2.534 St. Dev. = 0.51542, ANOVA: F (1,141) = 9.354, P = .003).

CONNECTING STATEMENT (Chapter 2 → Chapter 2)

The general objective of this thesis is to consider the entire life-cycle of trematode parasites and the ecological processes that are driving their transmission in introduced ranges. In the first chapter, I consider the ecological factors that structure parasite assemblages in populations of first intermediate snail hosts, evaluating the influence of native bird hosts and habitat features on the abundance and species richness of trematode parasites infecting *M. tuberculata* in an invaded range. In the second chapter, I investigate how these trematodes interact with first intermediate snail hosts and consider the effect of parasitism by an invasive trematode, *Centrocestus formosanus*, on host reproduction and investigate the extent to which snail hosts exhibit plasticity in life-history strategies in response to parasitic castration.

The third chapter of this thesis follows the life-cycle of *Centrocestus formosanus*, the parasite that was most abundant in first intermediate snail hosts (described in chapter 2) and explores transmission patterns from snail hosts to fish hosts. In this chapter, I conducted field surveys at various sites in the Isthmus of Panama to explore to what extent *C. formosanus*, which is considered a generalist parasite in second intermediate fish hosts, infects potential cichlid fish with which this parasites does not share a common evolutionary history. By considering various aspects of the parasite's life-cycle in natural field surveys and laboratory experiments, I am able to gain a better understanding of the ecological and evolutionary drivers of host-parasite associations in invaded communities.

CHAPTER 3

HOST PREFERENCE OF A "GENERALIST" PARASITE FOR A NON-NATIVE HOST

ABSTRACT

Parasites can invade new ecosystems if they are introduced with their native hosts or if they successfully infect and colonize new hosts upon arrival. Here, I ask to what extent an introduced parasite demonstrates specialization among novel host species. Infection surveys across three field sites in Gatun Lake, Panama, revealed that the invasive peacock bass, *Cichla monoculus*, was more commonly infected by the introduced trematode parasite *Centrocestus formosanus* than were three other common cichlid fishes. Laboratory infection experiments were conducted to determine if parasitism might be driven by differential encounter/exposure to parasites or by differential infection susceptibility/preference across different host species. These experiments were performed by controlling for parasite exposure in single host (compatibility) experiments and in mixed host (preference) experiments. In all cases, the peacock bass exhibited higher infection rates of viable metacercariae relative to the other potential fish hosts. My experiments thus provide support that an introduced generalist parasite shows apparent specialization on a specific novel host. Further studies are needed to determine if these patterns of specialization are the result of local adaptation following invasion by the parasite.

1 INTRODUCTION

The widespread introduction of non-native species is responsible for major environmental and economic impacts worldwide (Vitousek et al., 1997). Of particular concern are introduced parasites and pathogens, which can lead to emerging diseases of humans (Daszak, 2000), economically valuable species (Cleaveland et al., 2001) and wildlife (Dobson and Foufopoulos, 2001). When parasites and pathogens are introduced into regions where their original "native" hosts are already established, a ready-made biotic environment exists for the parasite/pathogen to persist and spread (Woolhouse and Gowtage-Sequeria, 2005). However, some introduced parasites/pathogens establish and spread in new communities lacking hosts from their native range, which they accomplish by infecting novel hosts (Woolhouse et al., 2001). Yet to establish, persist, and spread without their native hosts, introduced parasites must either be generalist species that can infect a broad variety of new hosts with some degree of success or they must specialize on new hosts in a novel community (Combes, 1981; Font, 2003; Holt, 2003, Holt et al., 2003). Here I ask to what extent does an introduced generalist parasite specialize on specific novel hosts in a new environment?

The relative contribution of host species to parasite reproduction and transmission, $R_{0,}$ (Anderson and May, 1981), will depend on their encounter rates and infection compatibility with different hosts, and can also be affected by host preference exhibited by the parasite (Combes, 1991). These properties (e.g. encounter, compatibility and preference), jointly determine a parasite's host range, also expressed as the extent to which a parasite specializes on a given set of potential host species. Patterns of host range are invariably shaped by the biogeographical and evolutionary history of both parasites and hosts, and influence the extent to which introduced

parasites can infect host species in novel ecological communities and persist in new ranges. Host specificity is thus a central feature of the ecology and evolution of host-parasite interactions (Combes, 2001; Poulin and Keeney, 2008), yet little is known about the factors underpinning a parasite's host range (Perlman and Jaenike, 2003). This is because it is typically impossible to discern the relative contributions of differences in encounter rates and compatibility across different potential host species in natural conditions (Kuris et al., 2007). Thus, in order to investigate drivers of host specialization, laboratory experiments that manipulate host-parasite encounter rates are needed to disentangle the likely drivers of parasite-host specificity (Bush and Clayton 2006; Kuris et al 2007). Here, I evaluate the extent to which a generalist species can demonstrate increased specialization across potential host species, and attempt to discern the proximate causes (e.g., encounter, compatibility or preference) eliciting natural patterns of infection with laboratory experiments.

My study is unique in two ways. First, attempts to elucidate ecological and evolutionary drivers of host specificity typically focus on native species and long-established host-parasite associations (Little et al., 2006; Sears et al., 2014; Simkova et al., 2006). In contrast, my use of non-native parasites and novel hosts is more informative for inferring interactions during the early stages of host-parasite community assembly. Second, laboratory experiments testing for patterns of host specificity typically focus on parasites thought to be highly host-specific, and often document infection compatibility with an unexpectedly broad range of novel host species typically not encountered in nature (King and Cable, 2007; Perlman and Jaenike, 2003; Poulin and Keeney, 2008). Conversely, my experiments consider whether a parasite putatively considered a generalist (from its native range) shows unexpectedly high host-specificity in its introduced range among a set of host species with which the parasite does not share a common

evolutionary history. Overall, my combined survey-experimental approach for introduced parasites on novel hosts should provide theoretical and practical insights concerning the ecological and evolutionary processes underpinning host-parasite associations in expanded geographical ranges.

Study system and experiments

The global spread of the trematode *Centrocestus formosanus* (Nishigori, 1924; Price, 1932) is of concern because of its ability to infect and cause disease in wild and endangered fishes (Mitchell et al., 2000), and commercially valuable species (Vélez-Hernández et al., 1998). This parasite has spread to freshwater habitats around the world following the invasion of its first intermediate snail host (Salgado-Maldonado et al., 1995), the Asian red-rimmed melania, *Melanoides (Thiara) tuberculata* (Muller, 1774), which was first reported in Panama in 2003 (Garcés and Garcia, 2004). *C. formosanus* has a complex life cycle, free-swimming larval cercariae emerge from snail hosts and encyst as metacercariae in the gills of second intermediate fish hosts (Scholz and Salgado-Maldonado, 2000). The parasite is then trophically transmitted to a piscivorous avian or mammalian definitive host (where the parasite sexually reproduces) when the infected fish is eaten, thereby completing its life cycle (Chen, 1942). The parasite, while highly specialized to its first intermediate snail host, has been reported to infect dozens of fish species across different families throughout its broad geographic range (Scholz and Salgado-Maldonado, 2000).

While this parasite is reported to have a broad host range throughout its global distribution, its potential to specialize on particular fish host species in its native or introduced ranges has not been examined. In order to measure specialization in nature, a robust comparison

of parasite prevalence and abundances across multiple co-occurring host species is necessary (Poulin, 2011). In order to gain some insight into the possibility of *C. formosanus* to specialize on a particular host species in a novel habitat, I evaluated natural patterns of parasitism across four potential cichlid fishes common in Gatun Lake, Panama, the native cichlid "vieja" (*Vieja maculicauda*, Regan, 1905) and three introduced cichlids, peacock bass (*Cichla monoculus*, Agassiz, 1831) which is native to the Oronoco Basin in South America , oscar (*Astronotus ocellatus*, Agassiz, 1831) which is native to South America and Nile tilapia (*Oreochromis niloticus*, Linnaeus, 1758) which is native to Africa. These four species are the most common cichlids in the Gatun Lake (Gonzalez-Gutierrez, 2000) and, importantly, none of these species shares an evolutionary/biogeographical history with *C. formosanus*, which is native to Southeast Asia (Scholz and Salgado-Maldonado, 2000).

I first compared prevalences and abundances of *C. formosanus* infection across the four fish species to evaluate natural patterns of parasitism of this introduced parasite. This is the first report of *C. formosanus* in Panama and unlike reports from elsewhere, my field comparison suggests that the parasite is not broadly distributed across the fish hosts but rather appears to be specialized on the peacock bass, a novel fish host with which the parasite does not a share a long term co-evolutionary history. I hypothesize that the patterns of infection in the field could be driven by differences in encounter/exposure rate or susceptibility of the fishes to the parasite or host preference of *C. formosanus*. To distinguish these hypotheses, I used laboratory experiments that (a) held encounter rates constant to test for differences in infection compatibility in single-species trails and (b) test host preference in mixed-species experiments. My laboratory experiments corroborate field comparisons suggesting that higher infection in the peacock bass are due to both greater host preference of peacock bass by *C. formosanus* and increased

compatibility of that host compared to the other fishes. The potential specialization by this introduced parasite on a common introduced host could provide further insight into how introduced parasites establish, integrate and potentially evolve in novel assemblages of hosts in recently expanded geographic ranges. However, additional research is needed to discern whether this potential specialization is the result of local adaptation of the parasite to a common introduced host.

2 MATERIALS AND METHODS

2.1 Field comparisons

Gatun Lake, part of the Panama Canal, was formed when the Chagres River was dammed early in the 20th century. The lake now has a biotic community of native and introduced species with broad biogeographic origins at all tropic levels, including aquatic plants, invertebrates such as snails and clams, and vertebrates such as fishes and reptiles (Zaret and Paine, 1973; Gonzalez-Gutierrez, 2000). The Asian red-rimmed melania snail, *M. tuberculata*, was first reported in Panama in 2003 as one of the two most abundant introduced mollusks in Gatun Lake (Garcés and Garcia, 2004), yet the pathway and chronology of the introduction and invasion (and that of its trematode parasite, *Centrocestus. formosanus)* in Panama is unknown.

In February and March of 2010, I quantified prevalences, abundances and intensities (Bush et al., 1997) of *Centrocestus formosanus* in four common and co-occurring cichlid fish species (*C. monoculus*, *A. ocellatus*, *O. niloticus* and *V. maculicauda*) across four sites within Gatun Lake: Gamboa (09° 09′ 22″ N 79° 51′ 22″ W, Barro Colorado Island (09° 10′ 02″ N 79°

50′ 07″ W), Rio Gatun (09° 15′ 21″ N 79° 46′ 46″ W) and Rio Chagres (09° 12′ 23″ N 79° 38′ 07″ W). All of the fish were sampled near shore where they were likely exposed to *C. formosanus* cercariae shed from *M. tuberculata* in the littoral zone. I caught fish using a 30-meter monofilament gill net (divided into three panels with 1.5 cm, 3 cm, and 4.5 cm width filaments). The net was set 3-4 times each morning at each site over a 60 day period to reach a comparable sample size (N = 15) for each species at each site. Approximately an hour after the nets were set, live fish were removed and transported in oxygenated 50-gallon coolers to STRI's Naos Marine Laboratory in Panama City where they were euthanized by spinal incision and dissected for parasites. I removed and examined gill arches with a stereomicroscope immediately after the fish were euthanized. Encysted *C. formosanus* metacercariae were identified using a compound microscope that allowed visual inspection of diagnostic features of this parasite (Yanohara and Kagei, 1983).

2.2 Experimental design

To test for infection susceptibility and host preference, I used two experiments that exposed the four focal fish species to *C. formosanus* in aquaria. All experimental fish were collected from a location near Barro Colorado Island, where *C. formosanus* is absent in first intermediate snail hosts (n = 1,000+ VMF, personal observations) and native and introduced second intermediate cichlid fish hosts (Roche et al., 2010) including peacock bass (n = 42, VMF, personal observations). Fish were caught and transported to Naos Marine Laboratory in Panama City as described above.

The first laboratory experiment examined infection susceptibility across the four cichlid fish species using each fish species as a separate treatment (hereafter referred to as single species

experiments). For this experiment, I placed four individuals of each species of comparable size (mean 22.67 cm, SD 3.71, standard length) into the same aquarium using 8 - 10 replicate aquaria per species. I then exposed the fishes to approximately 500 larval trematode cercariae shed from *M. tuberculata* (Lo and Lee, 1996). The second experiment tested whether *C. formosanus* cercariae preferentially infected particular fish species by simultaneously exposing the four target species to *C. formosanus* cercariae (hereafter referred to as mixed species trails). Here, one individual of each species of similar size (mean 21.74 cm, SD 2.44) was placed into the same aquarium, replicated 10 times. Fishes were exposed to *C. formosanus* cercariae as above.

2.3 Experimental details

Prior to the experiment, fish were kept for two weeks in 1 m² 50 gallon glass tanks filled with filtered tap water and de-chlorinated by running an air pump for 24 hours before the fish were introduced. The tanks were maintained under ambient conditions (23.5°C - 26.0°C, dissolved oxygen 8.1-8.5 mg/l, pH 6.7 - 7.2). All fish were fed once a week with comparable amounts of live fish (1g for peacock bass and the oscar) or fresh fruit (1g for tilapia and vieja). At the beginning of the holding period, each tank was treated with 5 ml of API STRESS COAT® and API STRESS ZYME® (Aquarium Pharmaceuticals) to treat possible tissue damage from netting and transport. After 24 hours of this treatment, a 25% water change was conducted each day for five consecutive days to clear these chemicals from the water. A week after the last water change, the fish were transferred to the experimental aquaria (see below) where they were housed for the rest of the experiment.

I used 1-m² 50-gallon glass aquaria, each divided into four 0.5 m² quadrants, each quadrant containing a single fish. The quadrants were divided using plastic screening (1 cm mesh

size) that allowed free passage of trematode cercariae, but not fish. The fish were acclimated for 24 hour before I introduced 500 cercariae to the center of the tank. After a 24-hour period I changed 25% of the water daily for two weeks, after which the fish were dissected for parasites as above. I allowed two weeks for *C. formosanus* to develop into metacercariae in the experimentally exposed fishes. I then haphazardly sampled *C. fomrosanus* metacercariae in each of the experimentally infected fishes to confirm viability of trematodes inside the cysts. Specifically, I visually observed living worms in the cysts and measured metacercariae, all which were within the size range (0.145 and 0.200mm diameter) of *C. formosanus* metacercariae described across various fish species (Mitchell et al., 2002, Salgado-Maldonado et al., 1995; Scholz and Salgado-Maldonado, 2000).

2.4 Statistical analysis

Comparisons of *C. formosanus* prevalences and abundances were conducted using generalized linear mixed models (GLMMs) with a log-linear link function and binomial-distributed (prevalence) or Poisson-distributed (abundance) errors. Field and laboratory experiments were analyzed separately. All models were fit using maximum likelihood (Bates and Maechler, 2010) and Poisson models incorporated an observation-level random effect to account for over-dispersion (Elston et al., 2001) Confidence intervals around model coefficients were obtained through N = 1000 model simulation runs (Gelman and Hill, 2006).

All experiments were analyzed with GLMMs that incorporated fish species as a fixed effect and body size as a covariate. I set peacock bass as the reference group (intercept) with the species prediction, such that all other species were evaluated by comparison. For the field data, GLMMs also incorporated a species-by-site interaction in the random structure to allow site-

specific inferences of species differences. For the single species trials, GLMSs incorporated a species-within-tank random effect, as well as an individual level random effect. GLMMs for the mixed species trails incorporated a tank-level random effect as well as an individual-level random effect. Significance of random structures was estimated based on likelihood ratio tests that compared the full model to a reduced model without the random structure in question (Gelman and Hill, 2006). For single and mixed species experiments, I accounted for infection levels in individual fish as replicates. As individual fish were grouped into groups of four into infection tanks in both single species and mixed species trails, I accounted for non-independence by treating species within tanks as a random effect, as well as by incorporating an individual-level random effect.

3 RESULTS

3.1 Field comparisons

At all four sites (Gamboa, Gatun, Chagres and Barro Colorado Island), peacock bass was by far the most commonly caught fish, being captured 4 to 5 times more frequently (mean = 2.36 CPUE, s.d. 1.61) than the second most abundant fish, the oscar (mean = .53 CPUE, s.d. .31), followed by the Nile tilapia (mean = .38 CPUE, s.d. .24). The native vieja was the least frequently caught fish (mean =.29 CPUE, s.d. .47). The prevalences, abundances and intensities of *C. formosanus* were highest in the peacock bass at each of the three sites where the parasite occurred (see Table 1). I did not recover *C. formosanus* in any of the fishes in Barro Colorado Island, nor did I find *C. formosanus* in the Nile tilapia or vieja at any of the sites. I only found *C.*

formosanus infecting the oscar at one site, Gamboa, at low prevalence and intensities (see Fig. 1, Table 1). Fish species (fixed effect) had a significant effect on both infection prevalence (χ^2 = 13.679, P = .003) and mean abundance (χ^2 = 50.180, P < .001). Host body size (covariate) did not significantly affect prevalence (χ^2 = 2.553, P = .110) but did have a significant effect on abundance (χ^2 = 6.167, P = .013) and parasites were more abundant on larger fish. Site (random effect) significantly improved the model (ΔAIC = 31.01, χ^2 = 51.01, P = < 0.001), so I report site-specific estimates for species differences in parasite abundance (see insert plots, Fig. 1). Overall, peacock bass were more heavily infected compared the other fish species at across the sites (Fig 1).

3.2 Laboratory experiments

Laboratory experiments testing infection susceptibility and host preference corroborated the patterns observed in field surveys (Table 2). In the laboratory experiments, all four fish species were infected by *C. formosanus* but peacock bass were infected twice as frequently in single-species experiments and over eight times as frequently in mixed-species experiments compared to the next most heavily infected fish species, the oscar (Fig. 2). Peacock bass were, on average, infected by about 70% of the 500 introduced cercariae in the single-species experiments compared to about 25% for the oscar. Over 50% of the 500 *C. formosanus* cercariae in the mixed-species experiments infected peacock bass compared to only10% for all other species combined (Table 3). Fish size did not affect infection rates in the experiments and aquarium (random effect) did not significantly improve the model (single-species trials: ΔAIC = 2, χ^2 = 0, P = 0.99 ΔAIC = 2, X2 = 0, P = .99, mixed-species trials: ΔAIC = 0.36, χ^2 = 1.64, P = 0.21).

4 DISCUSSION

4.1 Disentangling factors limiting host range

The degree of a parasite's host specificity, or host range, is a primary determinant of its ability to spread to novel host species and expand into new biogeographical ranges (Cleaveland et al., 2001). For this reason, generalist parasites are typically considered to be more successful in invading and establishing in new ranges compared to specialist species, yet parasites that specialize on locally common hosts species can benefit from associations with these species that can become widespread in new geographic ranges (Font, 2003). The introduced trematode *Centrocestus formosanus* is considered to be a generalist parasite with an extensive global distribution and broad host range in second intermediate fish species (Mitchell et al., 2002; Salgado-Maldonado et al., 1995; Scholz and Salgado-Maldonado, 2000). By contrast, my field survey suggested that, where it is introduced in Panama, *C. formosanus* can be specializing on peacock bass, a particularly common species that is also introduced, but with which *C. formosanus* does not share a long evolutionary history. In particular, *C. formosanus* prevalence in Gatun Lake was seven times greater, and its intensity 30 times higher, in the peacock bass, than in three other common cichlid fish species (Table 1). Patterns of parasitism in two laboratory experiments that controlled encounter rates were consistent with the field surveys. These experiments yielded infection rates in peacock bass that were more than twice as high (relative to the other potential fish species) in single host species experiments and more than eight times as high in mixed host species experiments. In short, an introduced parasite that is normally considered a generalist can actually specialize on a particular novel host. This outcome

can arise owing to a combination of differential encounter rates, host compatibility (susceptibility), and infection preference, effects that I now consider in turn.

4.1a Encounter rates

Encounter rates are an ecological "filter" for host-parasite interactions because, all else being equal, parasites should be more abundant on hosts they encounter more frequently (Combes, 2001). Encounter rates are certainly likely to vary among potential fish hosts given that peacock bass are the most abundant cichlid in near shore environments in Gatun Lake (Sharpe et al. unpubl. data) and were captured in my nets more than five times more frequently than the other cichlids as described above (V. Frankel, unpubl. data). Differential encounter rates are unlikely to be the sole reason for differential parasitism rates in nature because my laboratory experiments controlled exposure and yet yielded qualitatively similar patterns, as I will discuss below. However, I suggest that encounter rates might be an important contributor as the differential infection rates were more extreme in nature (30 times great in peacock bass) than in the laboratory experiments (two times greater in peacock bass in infection compatibility experiments and eight times greater in host preference experiments).

4.2b Host compatibility

Host compatibility is an intrinsic physiological "filter" because, all else being equal, parasites should be more abundant on susceptible hosts in which their likelihood of successful establishment and transmission is higher (Combes, 2001). In my experiments, *C. formosanus* was found to infect and develop into viable metacercariae in all four exposed cichlid species. However, peacock bass were more susceptible to infection, with infection rates twice as high as

the oscar, the next most heavily infected fish. One reason for these differences in susceptibility across the four cichlid species could be variation in immunological responses to parasite infection (Mitchell et al., 2002). However, infected fish did not display obvious visible reactions to the parasites (such as gill cartilage filament distortion or epithelial hyperplasia) or immune responses around the metacercariae cysts, as have been observed in other species infected by *C. formosanus* (Mitchell et al., 2002). Thus, the differences in infection rates might instead be the result of differences in parasite compatibility to different host species.

4.2c Parasite preference

Parasite preference for particular host species can also underpin patterns of host specialization (Combes, 1991; Esbérard et al., 2005) and should be evolutionarily shaped by both encounter and compatibility (Combes, 2001). In my experiments, more than half of the 500 cercariae in the mixed-species experiments infected peacock bass, whereas I would expect no more than 25% if parasites showed no preference across the four potential host species. This difference in host preference patterns could be achieved through host recognition (Ohhashi et al., 2007) and chemotaxis (Gerardo et al., 2006) of free-swimming larval cercariae. Indeed, other species of trematode cercariae have been shown to actively swim toward the most susceptible host species when given multiple alternatives (Sears et al., 2012). Alternatively, the pattern could be explained by differential attachment of cercariae to host gills. Additional behavioral experiments could discriminate among these possibilities.

4.2 Next steps

Using the conceptual framework of encounter and compatibility filters for host-parasite interactions (Euzet and Combes 1980, Combes 2001), I combined a series of field observations and laboratory experiments to begin to disentangle the apparent specialization of the introduced trematode, *C. formosanus*, on a non-native host, the peacock bass. A particularly intriguing possibility is that *C. formosanus* has become locally adapted to infect peacock bass in Gatun Lake, but further experiments are needed to formally test this possibility. Such experiments should include testing host susceptibility and parasite preferences across different host and parasites populations from different locations and evolutionary histories. If it turns out that that *C. formosanus* have indeed become locally adapted to peacock bass since their introduction, the logical next question is what factors have favored this adaptation. One possibility is variation in evolutionary time (Cornell and Hawkins, 1994; Torchin and Mitchell, 2004) but this seems unlikely in my case given that the obligate snail host for *C. formosanus* (*M. tuberculata*) was first recorded (2003) long after all the fish species were well established in the lake: vieja (native), peacock bass (1967, Zaret and Paine, 1973), tilapia (1976, Gonzalez-Gutierrez, 2000; Roche et al., 2010) and (1991, Gonzalez Gutierrez, 2000). Another possibility is that density mediated transmission rates are driving selection to the peacock bass through differential encounter rates with this highly invasive fish. Indeed, as noted earlier, the peacock bass is the most abundant cichlid in Gatun Lake (Sharpe et al, in prep, VMF unpublished data). A third possibility is that frequency mediated transmission rates are driving selection that drives local adaptation to the peacock bass. In particular, the peacock bass is the most frequently captured fish caught by fishermen and after processing the day's catch, fish remains, including infected gill tissue, are commonly consumed by birds (V. Frankel unpubl. data) that are their final hosts

(Scholtz and Salgado-Maldonado, 2000). Of course, much more work will be needed to discriminate among these possibilities.

4.3 General implications

Host specificity is a central facet of a parasite's life history, a feature that can determine the ability of a parasite to spread to new habitats and invade novel communities of hosts in new geographic ranges (Holt, 2003; Holt et al.; 2003). Yet the extent to which introduced parasites interact with a given set of novel host species in an expanded geographic range is often unknown, as evaluations of host range in natural communities require the collection of robust parasitological data of multiple host species that co-occur and potentially interact with a given parasite species (Poulin, 2011). Clearly, research investigating the introduction of parasite species to new habitats provides both practical insights on the species that are most affected by the geographic expansion of parasite species and the transmission pathway of these parasites in novel habitats (Ruiz-Gonzalez and Brown 2006; Olstead et al. 2007). In addition to these practical considerations, research investigating a parasite's host range in an expanded geographic range can also provide important natural experiments with which to test patterns of host range of specialist and generalist parasite species, and can allow scientists to test theoretical predictions concerning the ecological and evolutionary drivers of host-parasite associations and the success of introduced species in expanded geographic ranges.

Biological invasions are ultimately an irreversible component of human induced environmental change, a process that continues to reshuffle species distributions, re-shapes biotic communities, and thus facilitates novel biotic interactions (Vitousek et al., 1996), including host-parasite associations, in ecosystems around the world. Yet basic epidemiological data on

emerging host-parasite associations in nature, accompanied by simple laboratory experiments, can provide important insights concerning ecological and evolutionary processes underpinning host-parasite interactions, the dominant life-style on Earth, in an increasingly globalized and rapidly changing world.

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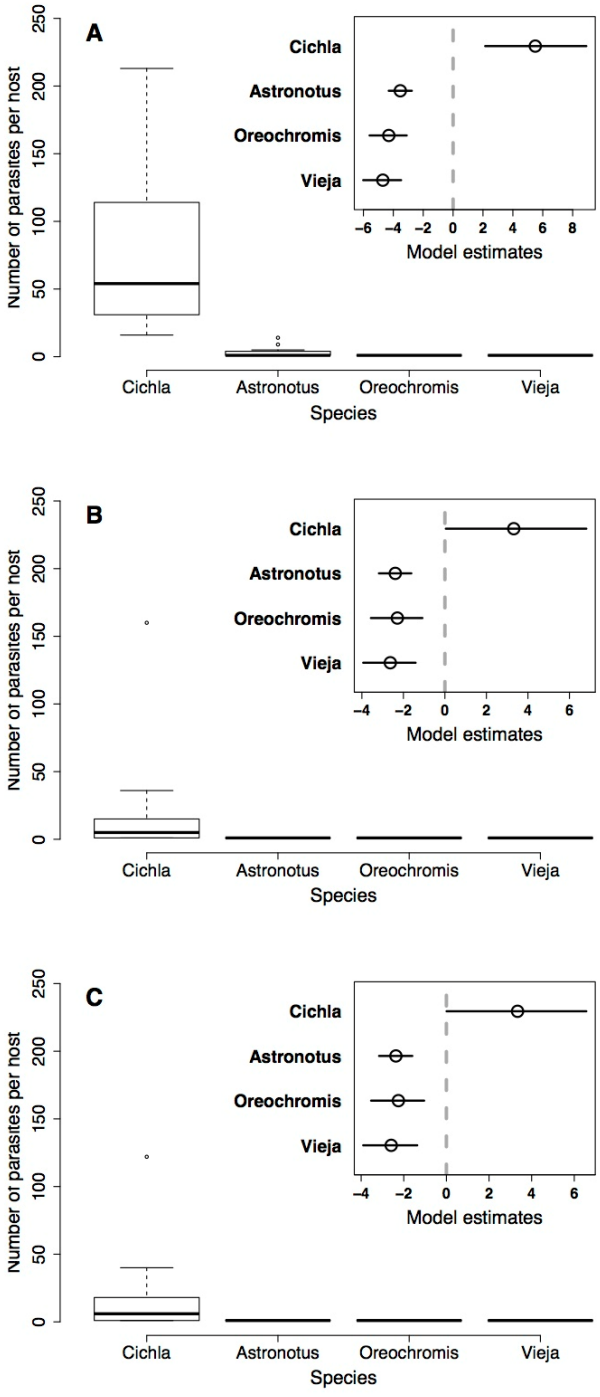
A
B
C
Number of parasites per host
0
50
100
150
200
250
Cichla
Astronotus
Oreochromis
Vieja
Species
Model estimates
−6 −4 −2 0 2 4 6 8
−4 −2 0 2 4 6

Figure 1: Field survey of parasitism by *Centrocestus formosanus* across four cichlids at three sites in Gatun Lake, Panama: A) Gamboa, B) Chagres, C) Gatun. Box-plots for three sites depicting the number of parasites per host demonstrate significant differences in parasitism, calculated as the mean infection abundance (n =15 fish per species) for each of four species (two outlier data points were omitted from the Gamboa plot for visual purposes). My data indicate that parasitism in nature was significantly higher in the peacock bass (*Cichla monoculus*) compared to three other species consistently sampled at all three sites: oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*), and vieja (*Vieja maculicauda*). Insert plots (top right of each box-plot) depict model estimates from GLMM with 95% confidence boundaries

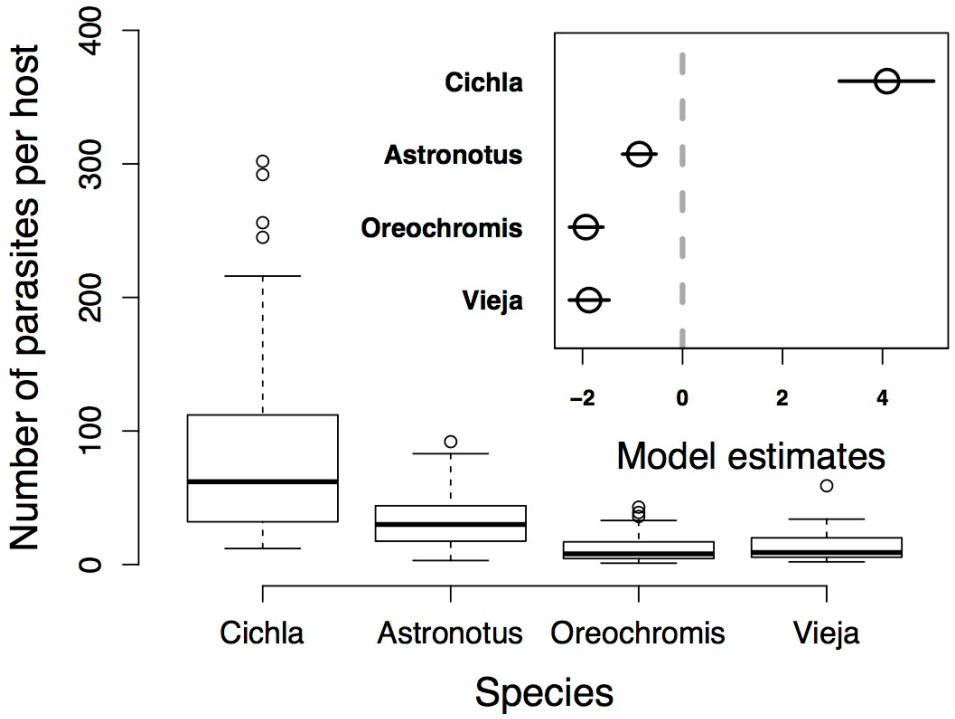

Figure 2: Experimental infection of four cichlids in single-species trials. Box-plots demonstrate that all four species can be infected by *Centrocestus formosanus*, but the invasive peacock bass, *Cichla monoculus*, is the most susceptible host for infection than three other species included in this study: oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*), and vieja (*Vieja maculicauda*). Insert plot (top right) depicts model estimates from GLMM with 95% confidence boundaries.

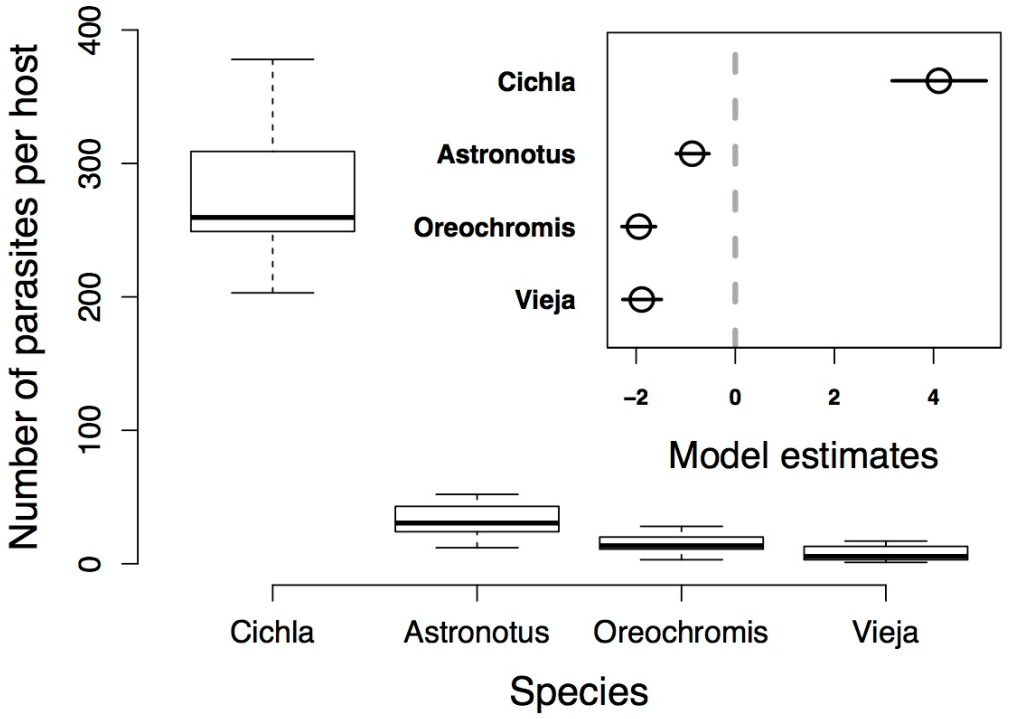

Figure 3: Experimental infection of four cichlids in mixed-species trials. Box-plots demonstrate significant differences in infection preference for the peacock bass over other three species tested: oscar (*Astronotus ocellatus*), Nile tilapia (*Oreochromis niloticus*), and vieja (*Vieja maculicauda*). Insert plot (top right) depicts model estimates from GLMM with 95% confidence boundaries.

Site	Host species	Prevalence (%)	Infection load (Mean / StDev)	Intensity (mean / StDev)	Body size (cm) / StDev
Gamboa	Cichla	100	133.27 / 190.77	133.27 / 190.77	27.8 / 5.48
	Astronotus	33.3	2.07 / 3.81	6.2 / 4.32	26.93 / 2.52
	Oreochromis	0	0	0	30.31 / 2.55
	Vieja	0	0	0	21.85 / 3.11
Chagres	Cichla	66.7	18.53 / 40.31	27.8 / 47.36	28.26 / 7.03
	Astronotus	0	0	0	25.72 / 3.86
	Oreochromis	0	0	0	28.46 / 4.67
	Vieja	0	0	0	19.98 / 3.23
Gatun	Cichla	66.7	16.87 / 31.36	25.3 / 35.96	26.63 / 7.30
	Astronotus	0	0	0	24.55 / 2.68
	Oreochromis	0	0	0	28.17 / 2.77
	Vieja	0	0	0	22.65 / 2.87

Table 1: Summary statistics of field surveys investigating parasitism by *C. formosanus* in four cichlid fish species at three field sites in Gatun Lake, Panama. Prevalence (%) is the proportion of all sampled hosts infected by at least one parasite. Parasite load is the mean number of parasites for each fish species at each of three sites, which includes 0 values for uninfected individuals. Intensity, similar to parasite load, is the mean number of parasites, but it does not include uninfected individuals. Across all sites, parasitism in the peacock bass, *Cichla monoculus*, measured in terms of infection prevalence, parasite load and intensity, was higher than in the three other co-occurring cichlid species investigated (N = 15 for each species at each of three sites).

	Prevalence		Intensity	
	χ^2	P	χ^2	P
Species	13.679	0.003	50.180	< 0.001
Size	2.553	0.110	6.167	0.013

Table 2: Type-II ANOVA table for overall effects of fish species and body size in the field surveys. Depicted are the significance (based on Wald χ^2 tests) for fixed effects from binomial GLMMs fitted to parasite infection prevalence and Poisson GLMMs fitted to infection load.

	Field experiments			Lab experiments (single)			Lab experiments (mixed)		
	Coef	z	P	Coef	z	P	Coef	z	P
Cichla	3.96	5.05	< 0.001	4.09	8.58	< 0.001	5.43	8.26	< 0.001
Astronotus	-2.72	-6.83	< 0.001	-0.87	-4.96	< 0.001	-2.19	-15.69	< 0.001
Oreochromis	-2.87	-4.59	< 0.001	-1.94	-10.77	< 0.001	-2.98	-19.44	< 0.001
Vieja	-3.20	-4.94	< 0.001	-1.87	-9.08	< 0.001	-3.66	-21.19	< 0.001

Table 3: Poisson GLMM estimates for infection intensity among fish species in the field experiment and in both laboratory experiments. In all trials, peacock bass, *Cichla monoculus* sported significantly higher infection rates then all other species.

Host species	Parasite load (mean / StDev)	Infection success (%)	N (trials)
Cichla	89.5 / 79.78	71.60	10
Astronotus	32.4 / 21.33	25.12	10
Oreochromis	11.35 / 10.60	9.48	10
Vieja	12.34 / 12.44	9.86	8

Table 4: Summary statistics for single species infection experiment. Four individuals of each of four cichlid fish species were exposed to 500 infective parasite cercaria in a 50 gallon tank. Parasite load is the mean number of parasites successfully established in each fish. Infection success is defined as the sum of successfully encysted parasites (metacercaria) in each tank containing four fish, divided by the number of parasites introduced into each tank. Parasite load and infection success was significantly higher in the peacock bass compared to other three cichlid species investigated in this experiment.

Host species	Parasite load (mean / StDev)	Proportion of parasites establishment of total introduced (%)	N (trials)
Cichla	276.5 / 49.23	55.3	10
Astronotus	31.6 / 12.87	6.32	10
Oreochromis	13.7 / 7.01	2.74	10
Vieja	6.99 / 6.44	1.40	10

Table 5: Summary statistics for mixed species infection experiment. (One individual of each of four cichlid fish species were exposed to 500 cercaria in a 50 gallon tank.) Parasite load is the mean number of parasites successfully established in each fish. I also calculated the proportion of parasites established in each fish of four species when given three other alternatives as an estimate of the level of infection preference that larva cercaria had for each of four species. Remarkably, more than half of all parasites introduced into each tank established on the peacock bass.

CONCLUSION:

In this research, I investigate the ecological factors driving the transmission of parasites in an invaded community by considering the interactions of trematodes with different host species throughout their life cycle. I specifically consider the ecological processes that affect the extent to which biological invasions facilitate the transmission of introduced parasites in expanded geographic ranges.

In the first chapter, I test the idea that "host diversity begets parasite diversity" in an introduced range by evaluating the influence of native bird hosts and habitat features on the abundance and species richness of trematode parasites infecting *M. tuberculata* in an invaded range. I show that the abundance and diversity of trematodes in *M. tuberculata* is strongly associated with the abundance and diversity of potential bird hosts in lake sites but found no evidence for this association from field surveys in stream sites. This system therefore shows how the assembly of host-parasite associations in new ranges is a complex mosaic of historical processes, novel associations and environmental effects. In the second chapter, I investigate how these trematodes described in the previous chapter interact with first intermediate snail hosts. Specifically, I consider the effect of parasitism by an invasive trematode, *Centrocestus formosanus*, on snail reproduction and investigate the extent to which infected hosts exhibit plasticity in life-history strategies in response to parasitic castration. Field surveys showed that infected snails are castrated and unable to produce new offspring. However, infected snails could harbor juvenile snails in their brood pouch and thus provide maternal investment in brood development post-infection. Snails infected in laboratory had depressed growth rates and produced larger juveniles compared to uninfected snails. These results support the idea that trematode infection induces a plastic response in the energetic budget allocated to reproduction

to boost reproduction at the cost of growth. I discuss the implications of the ability of snails to "make the best, if not the most, of a bad situation " In the third chapter, I consider how *C. formosanus*, which is transmitted by snails to fish, interacts with a community of potential fish hosts with which this parasite does not share a common evolutionary history. Infection surveys across three field sites in Gatun Lake, Panama, revealed that the invasive peacock bass, *Cichla monoculus*, was more commonly infected by *C. formosanus* than were three other common cichlid fishes. Laboratory infection experiments were conducted to determine if parasitism might be driven by differential encounter to parasites or by differential infection susceptibility/preference across different host species. In all cases, the peacock bass exhibited higher infection rates relative to other potential fish hosts. These data provide support that an introduced 'generalist' parasite shows specialization on a novel host, which could be the product of rapid local adaptation, post-invasion.

As a whole, this research illustrates the ecological complexity associated with the establishment of parasites with complex life cycles in new environments, creating a mosaic of interactions with different host species that are driven by different ecological and evolutionary processes.