TRANS-BOUNDARY ECOSYSTEM EFFECTS ON VECTOR COMMUNITY DIVERSITY: IMPLICATIONS FOR DILUTION AND AMPLIFICATION IN MULTI-SPECIES HOST-PATHOGEN SYSTEMS

By

SARAH E. BOWDEN

(Under the Direction of John M. Drake)

ABSTRACT

The importance of biodiversity to ecosystem health and functioning at global, regional, and local scales is becoming increasingly evident in the ecological and biological sciences. Biodiversity provides key ecosystem services, promotes ecosystem, human, and wildlife health, and can provide a buffer to the introduction and spread of infectious diseases. Specifically, high host diversity is known to decrease pathogen transmission of vector-borne pathogens, a phenomenon known as the *dilution effect*. In recent studies, the effects of biodiversity on disease systems have been explored primarily with respect to the host community. However, many multi-host vector-borne pathogens, like West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Chikungunya virus, are also transmitted by diverse vector communities. The effects of vector community biodiversity on pathogen transmission have been much neglected compared with effects of host community diversity. As mosquitoes are the most abundant vector of arboviruses

in the world, a deeper understanding of the ecology and transmission dynamics of vector communities will serve as a model for understanding the ecology of multi-host pathogens in general, as well as important zoonoses like Dengue virus and Zika virus. With this in mind, my dissertation research aimed to answer the following questions: (1) *How do interspecific interactions during the larval life stage affect population growth and coexistence?*; (2) *Are the effects of interspecific larval competition temperature-dependent*?; and (3) *How does competition between vector species affect pathogen transmission*?

INDEX WORDS: Trans-boundary ecosystem effects; vector ecology; vector-borne disease; interspecific competition; thermal niche; stage-structured population model; multi-vector transmission model

TRANS-BOUNDARY ECOSYSTEM EFFECTS ON VECTOR COMMUNITY DIVERSITY: IMPLICATIONS FOR DILUTION AND AMPLIFICATION IN MULTI-SPECIES HOST-PATHOGEN SYSTEMS

By

SARAH E. BOWDEN

B.S. Ecology, University of Georgia, 2009

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GEORGIA

2016

TRANS-BOUNDARY ECOSYSTEM EFFECTS ON VECTOR COMMUNITY DIVERSITY:

IMPLICATIONS FOR DILUTION AND AMPLIFICATION IN MULTI-SPECIES

HOST-PATHOGEN SYSTEMS

by

SARAH E. BOWDEN

Major Professor:	John M. Drake
Committee:	Alan Covich
	Daniel Mead
	Andrew Park

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
August 2016

DEDICATION

To Jacob and Chris – I love you through and through

ACKNOWLEDGEMENTS

First and foremost I would like to thank coffee, without which none of this would have been possible (h/t Jittery Joe's, Two Story Coffee, and Independent Baking Co. for all the tasty lattes and cappuccinos).

Many thanks to John Drake for taking me in as an undergraduate and letting me stick around for the next nine years. I transferred to UGA so that I would have the opportunity to do research, but I stayed for graduate school to continue working with John; it is undoubtedly one of the best decisions I've ever made.

I am so appreciative of the guidance provided by my committee – Alan Covich, Danny Mead, and Andrew Park – as I navigated six years of graduate school, from forming and cementing my research questions, to experimental and modeling implementation, to writing. Thank you for sharing your experience and expertise along the way.

To the many members of the Drake lab, both past and present (Andrea Silletti, Drew Kramer, Marcus Zokan, Barbara Han, Sean Maher, Heather Barton, J.P. Schmidt, Reni Kaul, Suzanne O'Reagan, Tad Dallas, Tom Pulliam, Chris Dibble, Eamon O'Dea, Paige Miller, Michelle Evans, and many others): you have all helped to shape who I am as a scientist, and I'm fortunate to count you among my colleagues and friends. To my fellow Drake lab grad students and officemates (Tad, Marcus, Reni, Paige, Michelle) – thank you for keeping me sane, driving me crazy, laughing and/or crying with me, and providing science fuel in the form of stimulating conversations and coffee. I don't miss the yoga ball, but I miss all of you.

I owe a lifetime of support to my parents, Larry and Bonnie, and my sister Samantha. Thank you for always believing that I could and would do great things.

To my dear friends Charlie Braman, Zach Sabo, Zach Yarbrough, and Jessica Gostin – thank you for seeing me through the past 7, 11, 12, and 16(!) years, respectively. I wouldn't have made it without you.

To my husband Chris – you've never known me when I wasn't a student. Thank you for always supporting my love of learning. Now it's your turn.

And finally, to our son Jacob – you've changed my life in ways you'll never know. I am a stronger, wiser, and kinder person because you are my son. I can't wait to see the person that you grow up to be.

TABLE OF CONTENTS

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

CHAPTER 1

ECOLOGY OF MULTI-HOST PATHOGENS OF ANIMALS

Introduction

Pathogens are often characterized as *specialists* or *generalists* based on the number of different host species they infect, as well as the *phylogenetic relatedness* among hosts. *Host range* is can be associated with several factors, including the geographic ranges of pathogens and hosts, host and pathogen phylogeny, and life history traits (Cleaveland et al. 2001, Malpica et al. 2006). Some studies also point to the influence of mode of transmission in determining host specificity of different types of pathogens (Pedersen et al. 2005). Additionally, a pathogen's host range can be specific to a certain strain or subtype of the pathogen, which is the case for various subtypes of influenza A. It is worth noting that the designation of a narrow or wide host range is relative. For example, White Nose syndrome (WNS), a disease caused by a pathogenic fungus infecting several bat species, could be said to have a narrow host range because it only infects one class of animals. One the other hand, it could be considered to have a wide host range compared to a pathogen like *Plasmodium falciparum*, the protozoan that causes malarial illness and only infects humans.

The majority of pathogens of animals are generalists that infect multiple host species, referred to as *multi-host pathogens* or *multi-host parasites*. Some multi-host pathogens are maintained in a *sylvatic* transmission cycle where the pathogen is maintained completely in multiple wildlife species. Among domesticated animal species, roughly 77% of pathogens of livestock and 90% of pathogens of domestic carnivores are known to be multi-host pathogens (Cleaveland et al. 2001). Over 60% of all known human pathogens are *zoonotic* (Taylor et al.

2001), meaning they originate in animals but can cross-infect humans. In some cases, humans can go on to infect humans or other animals (e.g., plague), while in others (e.g. West Nile virus) humans are *dead-end hosts*. In the latter case, the pathogen causes disease in an individual human but further transmission to other hosts or vectors does not occur.

Why and how do pathogens infect multiple host species?

The ability to infect multiple host species is not limited to pathogens of a specific type (virus, bacteria, helminth, etc.) or pathogens employing a particular mode of transmission (Figure 1). The following are only a small subset of multi-host pathogens (or diseases caused by multi-host pathogens) listed by their mode of transmission:

- Close contact/direct transmission (including direct contact, airborne, aerosol, bite, or sexual transmission): SARS, rabies, monkeypox, influenza, hantavirus, herpes, SIV (simian immunodeficiency virus)
- Non-close/indirect transmission (including fomites, environmental transmission): cholera, avian influenza, anthrax, brucellosis
- Intermediate host: Schistosomiasis, *Dicrocoelium dendriticum*
- Vector-borne transmission: West Nile virus, Lyme disease, Chikungunya

Depending on the mode of transmission, some pathogens are considered to be obligate multi-host pathogens or parasites; these include parasites with complex life cycles and vector-borne pathogens. Parasites that exhibit a *complex life cycle* require a definitive host for reproduction and one or more intermediate host species for growth and development. Vector-borne pathogens are transmitted between hosts by an intermediate organism, often an arthropod like mosquitoes or ticks, referred to as a *vector*.

Several factors can enable a pathogen to infect multiple host species (e.g., Pulliam and Dushoff 2009). For example, genetic change in the pathogen can occur through selection or through random mutations, allowing the pathogen to become better adapted to infect a new host species (Pepin et al. 2010). It is generally believed that the higher a pathogen's mutation rate, the more genetically diverse it will be and therefore the more likely it is that the pathogen is a generalist. For example, RNA viruses mutate ~300 times faster than DNA viruses, and directly transmitted RNA viruses of humans are more likely to be zoonotic than directly transmitted DNA viruses of humans (Drake 1993, Woolhouse et al. 2001). Host speciation is another mechanism by which a pathogen that originally infects an ancestral host comes to infect multiple new host species (e.g., Garamszegi 2009). Generally, pathogens tend to infect host species that are phylogenetically similar to each other because these host species share traits that make them susceptible to the same pathogens (e.g., immunologic, antigenic, or ecological similarities). Conversely, more distantly related host species do not share as many traits, decreasing the chances that they will share pathogen species (Freeland 1983, Davies and Pedersen 2008). Recently speciated hosts share genetic similarities, potentially allowing a pathogen to infect both species. Introductions of non-native hosts and pathogens can also result in the infection of a new host species by providing new opportunities for infectious contact between pathogens and naïve hosts (e.g., Peeler et al. 2011).

Infecting a wide range of host species is one way in which a pathogen’s chance of persistence is increased. The ability to infect multiple host species is not always adaptive, however, and several ecological trade-offs are associated with the benefit of a broad host range. For example, while single-host pathogens tend to evolve an intermediate level of *virulence* in their host, virulence evolution in multi-host pathogens is more complex. A multi-host pathogen could be

highly virulent in one host while exhibiting low virulence in another. The optimal virulence in each host will depend on how each host contributes to pathogen *fitness* (Regoes et al. 2000, Gandon 2004, Rigaud et al. 2010). Another cost of infecting multiple host species is the degree to which a pathogen can adapt to a host's immune system. If a pathogen only infects one host species, the pathogen can evolve to become highly proficient at evading the immune system of that host. In multi-host pathogens, however, an adaptation in one host species may be maladaptive in another host species (Elena et al. 2009). For example, many vector-borne pathogens are viruses, and thus are expected to have a great deal of genetic diversity due to high mutation rates (Cooper and Scott 2001, Ciota et al. 2007). However, experimental research has shown that viral genetic sequences are largely unchanged after multiple transmissions between very different species (i.e., a serial passage experiment between an invertebrate vector and a vertebrate host). Moreover, viruses that were experimentally allowed to transmit between only one species rapidly adapted to that species, with coinciding loss of fitness often observed in the bypassed species (Romanova et al. 2007, Coffey et al. 2008, Vasilakis et al. 2009). This *host alternation* is, therefore, a potential constraint on the genetic diversity of multi-host pathogens.

Invasion and population dynamics of multi-host pathogens

The invasion of a naïve population of hosts and subsequent epidemiological dynamics of multi-host pathogens are inherently different from single host systems because multiple host species provide multiple invasion pathways, as well as multiple transmission routes. That is, if infection is unsuccessful in one host species, the presence of another host species provides an alternative route for the pathogen to invade a community. Both invasion and persistence are related to a theoretical quantity, R_0, referred to as the basic reproductive number and defined as the number

of secondary infections resulting from a single primary infection in a completely susceptible population. If R_0>1, then an introduced pathogen is likely to persist and may go on to cause an epidemic in the host population (Anderson and May 1991). In a community comprised of multiple host species, R_0 may be greater than one for one species, but less than one for another species. In this case, the *community composition* would determine whether or not the pathogen will persist at the community level (discussed further below). The form of transmission (i.e., *density-dependent* versus *frequency-dependent*) also has implications for population dynamics of multi-host pathogens (Dobson 2004). Single-host pathogens that rely on density-dependent transmission rarely drive their host to extinction because the host population will drop below a threshold size such that pathogen transmission can no longer be maintained (Grenfell and Dobson 1995, Hudson et al. 2002). Utilizing mechanisms that give rise to frequency dependent transmission (e.g., sexually transmitted or vector-borne pathogens), as well as infecting multiple host species that may act as reservoirs, increases the chance of pathogen-induced host extinction because the threshold density for pathogen persistence is eliminated (de Castro and Bolker 2005).

In host-pathogen systems with multiple hosts, disease dynamics can also be dependent on the *competence* of each host species for harboring and transmitting the pathogen, as well as the relative frequency of transmission between host and vector species (LoGuidice et al. 2003). Thus, the community composition of potential hosts can have a large effect on pathogen dynamics, especially when there is substantial variation in competence within the host community (Holt et al. 2003, LoGuidice et al. 2008). Specifically, theory suggests that, in multi-host vector-borne pathogen systems, more diverse host communities may reduce pathogen transmission by decreasing contacts between infected vectors and highly competent hosts compared with single-species host systems (Schmidt and Ostfeld 2001, Keesing et al. 2006). This phenomenon, referred

to as the *dilution effect*, has been studied primarily in the Lyme disease system of the Northeast U.S., but has also been demonstrated in other multi-host pathogen systems (Figure 2; Swaddle and Calos 2008, Dizney and Ruedas 2009, Hall et al. 2009). Some scientists have argued that while empirical evidence exists for the dilution effect in several multi-host pathogen systems, we often do not know the mechanism by which disease dilution is occurring (e.g., dilution versus density effects; Begon 2008).

Multi-host pathogens in a changing climate

As many pathogens are associated with tropical or equatorial areas of the world, it has been suggested that increased temperatures accompanying climate change will lead to the emergence, re-emergence, or persistence of many more pathogens (Harvell et al. 2002). Changes in climate are predicted to lead to range expansion and range shifts of pathogens, their hosts, and their vectors, making precise climate-associated changes in disease dynamics difficult to predict (Lafferty et al. 2009, Harvell et al. 2009). Adverse effects of climate warming have already been discovered in some multi-host pathogen systems, like chytridiomycosis outbreaks (a fungal infection caused by *Batrachochytrium dendrobatidis*) in amphibian communities. Severe declines in amphibian diversity have been linked to warmer temperatures, which are thought to increase the growth of the fungus (e.g., Bosch et al. 2007). Changes in climate are also known to alter certain animal behaviors, like the timing or spatial course of migration, which has the potential to alter multi-host pathogen transmission by changing when and where pathogens and parasites encounter their hosts, affecting both the time and size of disease outbreaks (Altizer et al. 2011).

Why should we study multi-host pathogens?

Multi-host pathogen systems are intrinsically complex, shaped by pathogen and host dynamics as well as evolutionary, environmental, and climatic interactions. Understanding multi-host pathogens from an ecological perspective provides a variety of potential applications. Multi-host pathogens can, for instance, affect organisms and ecological dynamics far outside their host range. Depending on their effect on a host species (high virulence/mortality, behavioral modification, reduced fitness/reproduction, etc.), multi-host pathogens may regulate not only populations and communities of host species, but also predator, prey, or competitor populations (Hatcher et al. 2006 and references therein). Understanding the ecology and evolution of multi-host pathogens may also be important for species conservation and biodiversity preservation (McCallum and Dobson 1995, Smith et al. 2006). Some species that are now declining due at least in part to multi-host pathogens include bird species infected by avian malaria in Hawaii (Van Riper et al. 1986) and WNV in the continental U.S. (LaDeau et al. 2007), bat species in the U.S. infected with the pathogenic fungus (*Geomyces destructans*) that causes White-nose Syndrome (Frick et al. 2010), and seals infected with phocine distemper virus in Europe (Swinton et al. 1998, Jensen et al. 2002). Lastly, understanding the ecology of multi-host pathogens, particularly those that are zoonotic, can provide important information needed for shaping human health policy, and may contribute to outbreak detection and other warning systems, or be central to programs aimed at preventing or reducing transmission and human infections by multi-host pathogens.

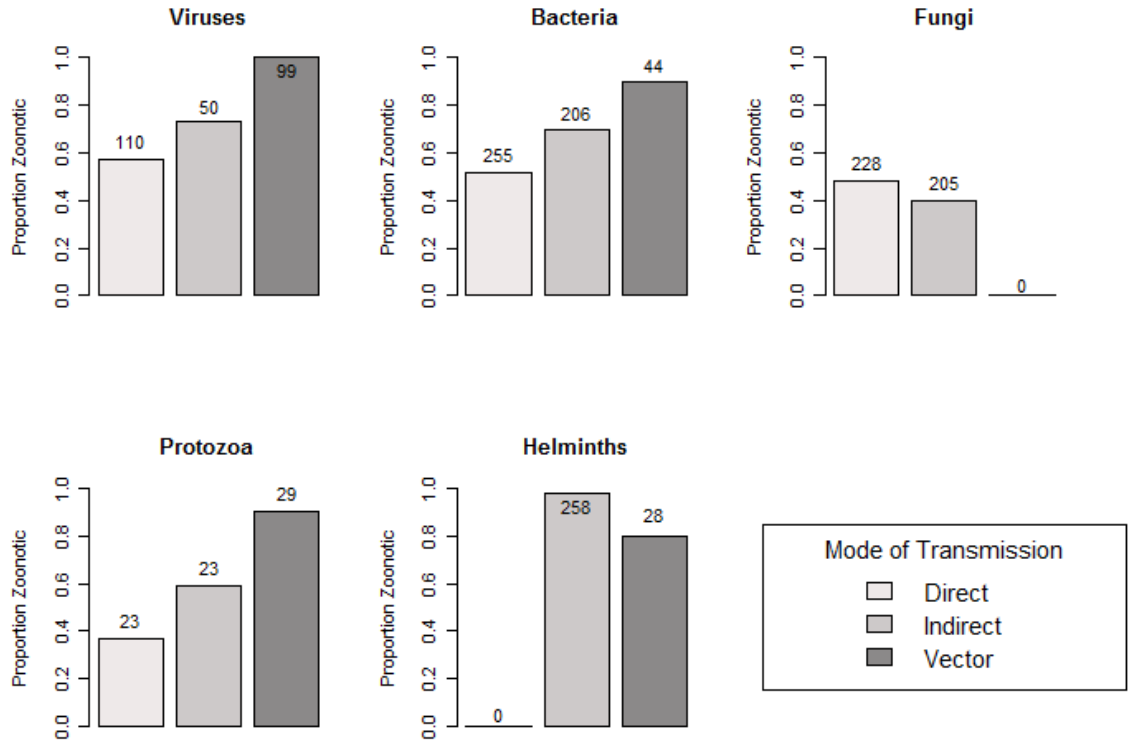

Figure 1.1: Proportion of pathogens known to be zoonotic, stratified by pathogen class and mode of transmission (adapted from Woolhouse et al. 2001). As demonstrated by this figure, multi-host pathogens (in this case, pathogens that infect at least one non-human animal species in addition to humans) are abundant regardless of the type of pathogen or mode of transmission.

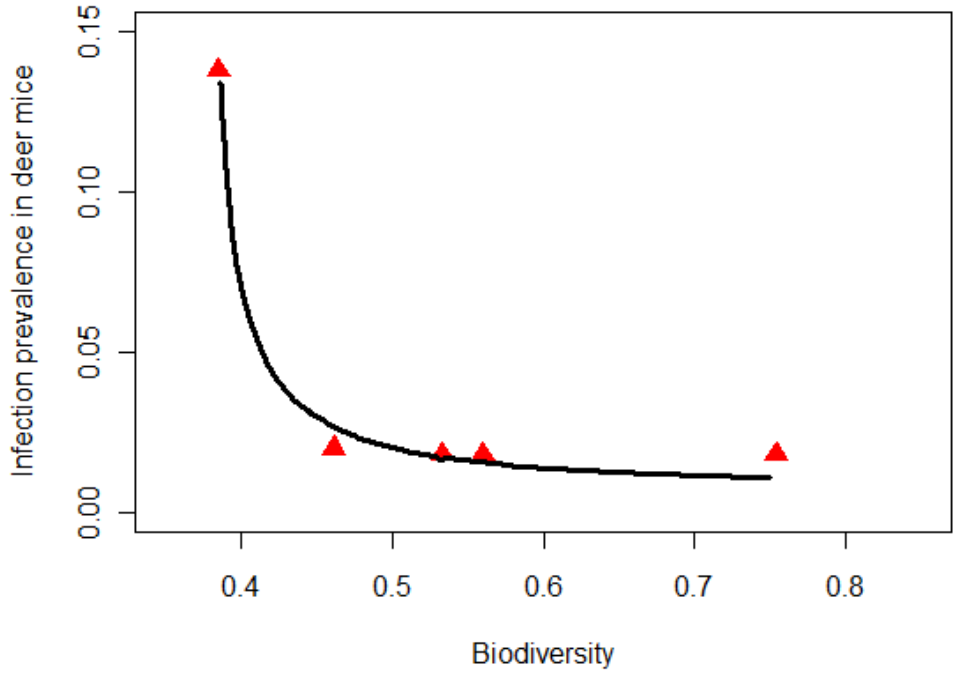

Figure 1.2: Evidence of the dilution effect, shown here as a decline in prevalence of Sin Nombre virus in deer mice with increasing biodiversity of the surrounding community (adapted from Dizney and Reudas 2009). Biodiversity was measured using Simpson's diversity index, which accounts for both species richness (number of species in a community) and species evenness (relative abundance of each species in a community).

Glossary

Multi-host pathogen: A pathogen that infects multiple host species

Sylvatic transmission: Transmission cycle of a pathogen maintained completely in non-human animals

Zoonotic: Referring to a pathogen that infects humans, but originates from a non-human animal species

Dead-end host: A host in which a pathogen can cause disease, but not maintain transmission

Phylogenetic relatedness: Evolutionary distance among species; organisms that share a recent common ancestor and typically have genetic similarities

Host range: The set of host species that a pathogen or parasite can infect, described by both the number of host species and the phylogenetic relatedness between host species.

Direct transmission: Occurring from direct or close contact with infectious individuals, including aerosol/airborne transmission, sexual transmission, and transmission via a bite

Indirect transmission: Occurring from non-close contact with infectious individuals, including fomites and environmental transmission

Complex life cycle: A parasite life cycle that requires a definitive host for reproduction and one or more intermediate hosts for growth and development

Vector: Organisms (primarily arthropods like mosquitoes, ticks, and fleas) that transmit a pathogen between host species

Virulence: Pathogen-induced mortality or other decline in the fitness of a host caused by infection

Fitness: The potential for an organism to survive and reproduce

Host alternation: Pathogen transmission between two or more (often disparate) host species, which constrains pathogen adaptation to one host species over another

R_0*:* The basic reproductive number; the number of secondary infections arising from an initial infection in a completely susceptible population

Community composition: The number and relative abundance of host species in a community

Density-dependent transmission: Transmission rate that increases with host density

Density-independent transmission: Transmission rate that functions independent of host density

Competence: The differential ability of an organism to harbor and transmit a pathogen

Dilution effect: A net reduction in pathogen transmission from increasing host species diversity

References

Cleaveland S, Laurenson MK, Taylor LH, 2001. Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. Phil Trans R Soc Lond B 356(1411): 991—999.

Malpica JM, Sacristán S, Fraile A, García-Arenal F, 2006. Association and host selectivity in multi-host pathogens. PLoS ONE 1(1): e41.

Pedersen AB, Altizer S, Poss M, Cunningham AA, Nunn CL, 2005. Patterns of host specificity and transmission among parasites of wild primates. International Journal for Parasitology 35(6): 647—657.

Taylor LH, Latham SM, Woolhouse MEJ, 2001. Risk factors for human disease emergence. Phil Trans R Soc Lond B 356(1411): 983—989.

Pulliam JRC, Dushoff J, 2009. Ability to replicate in the cytoplasm predicts zoonotic transmission of livestock viruses. J Infect Dis 199(4): 565—568.

Pepin KM, Lass S, Pulliam JRC, Read AF, Lloyd-Smith JO, 2010. Identifying genetic markers of adaptation for surveillance of viral host jumps. Nature Reviews Microbiology 8: 802—813.

Drake JW, 1993. Rates of spontaneous mutation among RNA viruses. PNAS 90(9): 4171—4175.

Woolhouse MEJ, Taylor LH, Haydon DT, 2001. Population biology of multihost pathogens. Science 292(5519): 1109—1112.

Garamszegi LZ, 2009. Patterns of co-speciation and host switching in primate malaria parasites. Malaria Journal 8: 110.

Freeland WJ, 1983. Parasites and the coexistence of animal host species. Am Nat 121(2): 223—236.

Davies TJ, Pedersen AB, 2008. Phylogeny and geography predict pathogen community similarity in wild primates and humans. Proc R Soc B 275(1643): 1695—1701.

Peeler EJ, Oidtmann BC, Midtlyng PJ, Moissec L, Gozlan RE, 2011. Non-native aquatic animal introductions have driven disease emergence in Europe. Biological Invasions 13(6): 1291—1303.

Regoes RR, Nowak MA, Bonhoeffer S, 2000. Evolution of virulence in a heterogeneous host population. Evolution 54(1): 64—71.

Gandon S, 2004. Evolution of multihost parasites. Evolution 58(3): 455—469.

Rigaud T, Perrot-Minnot MJ, Brown MJF, 2010. Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. Proc R Soc B 277(1701): 3693—3702.

Elena SF, Agudelo-Romero P, Lalić J, 2009. The evolution of viruses in multi-host fitness landscapes. Open Virol J 3: 1—6.

Cooper LA, Scott TW, 2001. Differential evolution of Eastern Equine Encephalitis virus populations in response to host cell type. Genetics 157(4): 1403—1412.

Ciota AT, Lovelace AO, Ngo KA, Le AN, Maffei JG, Franke MA, Payne AF, Jones SA, Kauffman EB, Kramer LD, 2007. Cell-specific adaptation of two flaviviruses following serial passage in mosquito cell culture. Virology 357(2): 165—174.

Romanova LI, Gmyl AP, Dzhivanian TI, Bakhmutov DV, Lukashev AN, Gyml LV, Rumyantsev AA, Burenkova LA, Lashkevich VA, Karganova GG, 2007. Microevolution of tick-borne encephalitis virus in course of host alternation. Virology 362(1): 75—84.

Coffey LL, Vasilakis N, Brault AC, Powders AM, Tripet F, Weaver SC, 2008. Arbovirus evolution *in vivo* is constrained by host alternation. PNAS 105(19): 6970—6975.

Vasilakis N, Deardorff ER, Kenney JL, Rossi SL, Hanley KA, Weaver SC, 2009. Mosquitoes put the brake on arbovirus evolution: experimental evolution reveals slower mutation accumulation in mosquito than vertebrate cells. PLoS Pathog 5(6): e1000467.

Anderson RM, May RM (Eds.). Population biology of infectious diseases. Springer-Verlag, New York: 1982.

Dobson A, 2004. Population dynamics of pathogens with multiple host species. Am Nat 164(S5): S64—S78.

Grenfell BT, Dobson AP (Eds). Ecology of infectious diseases in natural populations. Cambridge University Press, Cambridge: 1995.

Hudson PJ, Rizzoli A, Grenfell BT, Heesterbeek H, Dobson AP (Eds). The ecology of wildlife diseases. Oxford University Press, Oxford: 2002.

De Castro F, Bolker B, 2005. Mechanisms of disease-induced extinction. Ecology Letters 8(1): 117—126.

LoGiudice K, Ostfeld RS, Schmidt KA, Keesing F, 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. PNAS 100(2): 567—371.

Holt RD, Dobson AP, Begon M, Bowers RG, Schauber EM, 2003. Parasite establishment in host communities. Ecology Letters 6(9): 837—842.

LoGiudice K, Duerr STK, Newhouse MJ, Schmidt KA, Killilea ME, Ostfeld RS, 2008. Impact of host community composition on Lyme disease risk. Ecology 89(10): 2841—2849.

Schmidt KA, Ostfeld RS, 2001. Biodiversity and the dilution effect in disease ecology. Ecology 82(3): 609—619.

Keesing F, Holt RD, Ostfeld RS, 2006. Effects of species diversity on disease risk. Ecology Letters 9(4): 485—498.

Swaddle JP, Calos SE, 2008. Increased avian diversity is associated with lower incidence of human West Nile virus infection: observation of the dilution effect. PLoS ONE 3(6): e2488.

Dizney LJ, Ruedas LA, 2009. Increased host species diversity and decreased prevalence of Sin Nombre virus. Emerg Infect Dis 15(7): 1012—1018.

Hall SR, Becker CR, Simonis JL, Duffy MA, Tessier AJ, Cáceres CE, 2009. Friendly competition: evidence for a dilution effect among competitors in a planktonic host-parasite system. Ecology 90(3): 791—801.

Begon M. Effects of host diversity on disease dynamics. Infectious disease ecology: effects of ecosystems on disease and of disease on ecosystems. Ostfeld RS, Keesing F, Eviner VT (Eds). Princeton University Press, Princeton, New Jersey: 2008.

Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson AP, Ostfeld RS, Samuel MD, 2002. Climate warming and disease risks for terrestrial and marine biota. Science 296(5576): 2158—2162.

Lafferty KD, 2009. The ecology of climate change and infectious diseases. Ecology 90(4): 888—900.

Harvell D, Altizer S, Carradori IM, Harrington L, Weil E, 2009. Climate change and wildlife diseases: when does the host matter the most? Ecology 90(4): 912—920.

Bosch J, Carrascal LM, Durán L, Walker S, Fisher MC, 2007. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of Central Spain; is there a link? Proc R Soc B 274(1607): 253—260.

Altizer S, Bartel R, Han BA, 2011. Animal migration and infectious disease risk. Science 331(6015): 296—302.

Hatcher MJ, Dick JTA, Dunn AM, 2006. How parasites affect interactions between competitors and predators. Ecology Letters 9(11): 1253—1271.

McCallum H, Dobson A, 1995. Detecting disease and parasite threats to endangered species and ecosystems. TREE 10(5): 190—194.

Smith KF, Sax DF, Lafferty KD, 2006. Evidence for the role of infectious disease in species extinction and endangerment. Conserv Biol 20(5): 1349—1357.

van Riper III C, van Riper SG, Goff ML, Laird M, 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs 56(4): 327—344.

LaDeau SL, Kilpatrick AM, Marra PP, 2007. West Nile virus emergence and large-scale declines of North American bird populations. Nature 447: 710—713.

Frick WF, Pollock JF, Hicks AC, Langwig KE, Reynolds DS, Turner GG, Butchkoski CM, Kunz TH, 2010. An emerging diseases causes regional population collapse of a common North American bat species. Science 329(5992): 679—682.

Swinton J, Harwood J, Grenfell BT, Gilligan CA, 1998. Persistence thresholds for phocine distemper virus infection in harbour seal *Phoca vitulina* metapopulations. Journal of Animal Ecology 67: 54—68.

Jensen T, van de Bildt M, Dietz HH, Andersen TH, Hammer AS, Kuiken T, Osterhaus A, 2002. Another phocine distemper outbreak in Europe. Science 297(5579): 209.

CHAPTER 2

TRANS-BOUNDARY ECOSYSTEM EFFECTS OF LARVAL MOSQUITO COMPETITION ON ADULT VITAL RATES, INTRINSIC GROWTH RATE, AND SPECIES COEXISTENCE OF DISEASE VECTORS[1]

[1]Bowden, Sarah E. and Drake, John M. To be submitted to *Ecology and Evolution*.

Abstract

Mosquitoes undergo an ontogenetic niche shift from the aquatic to terrestrial ecosystem during their life cycle. This niche shift makes it possible for interactions during the aquatic life stages (e.g., larval competition) to impact population dynamics of terrestrial life stages through changes in vital rates, a phenomenon termed *trans-boundary ecosystem effects*. The potential for mosquitoes to produce such effects is of interest to both ecologists and epidemiologists because many mosquito species serve as disease vectors during the adult life stage. We performed laboratory microcosm experiments to quantify the effects of larval competition on adult population growth and species coexistence of three mosquito vectors (*Culex quinquefasciatus*, *Aedes albopictus*, and *Ae. aegypti*). Our results suggest a competitive heirarchy among the three species under the specific physical conditions of this experiment (e.g., 27°C, animal-based food source), with *Ae. aegypti* expected to competitively exclude both *Cx. quinquefasciatus* and *Ae. albopictus*. Our research highlights the potential for coupled dynamics between ecosystems due to species with complex life cycles.

Introduction

Species which undergo ontogenetic niche shifts may couple the dynamics of two ecosystems, whereby ecological processes such as nutrient acquisition or interaction with another species occurring during one life stage in one ecosystem may carry over to affect processes or interactions in another ecosystem during another stage of development (Werner and Gilliam 1984). Such "trans-boundary" ecosystem effects, coupling the dynamics of two or more ecosystems, is often brought about by species with complex life cycles (Schreiber and Rudolf 2008, McCoy et al. 2009). For example, a study by (Knight et al. 2005) found evidence of a trans-boundary tropic cascade between freshwater aquatic ecosystems and the adjacent terrestrial environment. A survey

of permanent ponds, some of which contained fish and some of which did not, showed that there were fewer adult dragonflies in the terrestrial environment surrounding ponds containing fish due to predation by fish on larval dragonflies. Important pollinators, such as bees, serve as prey for adult dragonflies; thus, this reduction in adult dragonfly abundance resulted in an increase in pollinator abundance and increased terrestrial plant reproduction (Knight et al. 2005). Mosquitoes are a paradigmatic example of such a species, shifting from the aquatic larval habitat to the terrestrial adult habitat during their life cycle, suggesting that they are capable of producing these trans-boundary effects. The potential for mosquitoes to produce such effects is of interest to both ecologists and epidemiologists because many mosquito species serve as disease vectors during the adult life stage. Understanding the processes by which mosquitoes produce trans-boundary ecosystem effects could lead to better models of mosquito population dynamics and vector-borne disease risk.

For mosquitoes, interactions which occur during the larval stage, such as intraspecific and interspecific competition, are known to alter larval survival (Schneider et al. 2000), development time (Agnew et al. 2000), adult size (Gimnig et al. 2002), and adult longevity (Reiskind and Lounibos 2009, Alto 2011). Changes in these vital rates translate to changes in population dynamics (Juliano 2007) that affect the relative abundance of a species and the overall adult (vector) community composition. As vectors vary in their ability to harbor and transmit a pathogen (competence) (Turell et al. 2001), changes in vector community composition may also alter the transmission risk to the host community through disease dilution or amplification (Keesing et al. 2006). In addition to its effect on vital rates and population dynamics, interspecific competition has also been shown to alter the vector competence of some species, usually through reductions in body size (Alto et al. 2005, 2008, Bevins 2008). While many studies of interspecific competition

in larval mosquitoes exist, most focus exclusively on a small subset of vector species, such as the invasive Asian tiger mosquito (*Aedes albopictus*) and anopheline vectors of malaria (Juliano 2009). However, vector species breed in a variety of habitat types and may interact with several other species during the larval stage. Thus, it is important to study larval competition in the context of the larger ecological community, and not just with respect to pairwise interactions.

We propose that trans-boundary coupling occurs in mosquito communities through the effects of interspecific larval competition on adult community composition. To test this hypothesis, we performed laboratory microcosm experiments to measure the effects of interspecific competition on larval survival, development time, and body size (which relates linearly to fecundity (Armbruster and Hutchinson 2002)) of three ecologically and medically important mosquito species – *Culex quinquefasciatus*, *Aedes albopictus*, and *Aedes aegypti*. We then estimated population growth rates for each species under different competitive scenarios across a density gradient. While previous research has shown that *Aedes albopictus* is a superior competitor to *Aedes aegypti* (Juliano 1998, Juliano et al. 2004, Juliano and Philip Lounibos 2005), the competitive interaction of either Aedes species with *Culex quinquefasciatus* and with all three species together is unknown. Our results indicate a competitive hierarchy among these three species, with *Aedes aegypti* as the superior competitor and *Culex quinquefasciatus* as the inferior competitor.

Methods

Experimental design. The three mosquito species used in this experiment were selected because they overlap in geographic range in the southeast U.S. (Darsie and Ward 2005); share similar oviposition and larval habitat preferences, making it possible encounter one another during the larval stage; and are all known vectors of zoonotic diseases. *Culex quinquefasciatus* is a

competent West Nile virus (WNV) vector and is highly abundant in urban areas. *Aedes albopictus* and *Aedes aegypti* are both vectors of Dengue virus (DENV), Chikungunya virus (CHIKV), and Zika virus (ZIKV).

We used a full factorial design to explore the additive effects of increasing density (30, 60, or 90 individuals) simultaneously with the substitutive effects of increasing species richness (1, 2, or 3 species) for all combinations of the three mosquito species described above. An experimental design that is both additive and substitutive allowed us to parse apart density-dependence of vital rates from the effects of interspecific competitors, as well as to determine if the effects of interspecific competition were density dependent (Scheiner and Gurevitch 2001). This resulted in a total of 21 treatments: nine single-species treatments (3 species x 3 densities), nine two-species treatments (3 combinations x 3 densities), and three three-species treatments (1 combination x 3 densities). This design was replicated five times.

First instar larvae of each species were synchronously hatched within 24 hours of the start of each experiment using eggs from laboratory-maintained colonies at the University of Georgia. *Culex quinquefasciatus* larvae were hatched live from egg rafts, while *Aedes albopictus* and *Aedes aegypti* larvae were hatched from dried eggs. Larvae were placed in BioQuip Mosquito Breeder microcosms with 200 mL of de-ionized (DI) water. Each treatment received 0.1 g of ground fish flake food suspended in 10 mL of DI water, after which microcosms were incubated at 27 °C with a 16:8 hour L:D cycle. Microcosms were monitored daily for 15 days; eclosed adults were removed every 24 hours and frozen for further data collection.

Data collection. We used a dissecting microscope with an integrated camera to determine the species and sex of all eclosed adults (n=3,453), as well as measured abdominal length (from the metapostnotum to the end of the cerci). Development time was calculated as the number of

days from the start of the experiment to emergence. Egg-to-adult survival was calculated under the assumption that each treatment initially contained an equal number of individual of each sex. Mean vital rates (development time, body size, egg-to-adult survival) for each treatment within a replicate were used for statistical analyses.

Estimating population growth rate. Using experimental data on development time, survival, and body size of females, we estimated population growth rates using the estimator of Livdahl and Sugihara (1984):

$$r' = \frac{\ln\frac{1}{N_0}\sum_x A_x f(\bar{w}_x)}{D+\frac{\sum_x xA_x f(\bar{w}_x)}{\sum_x A_x f(\bar{w}_x)}} \quad \text{(Eq. 1)},$$

where N_0 is the initial number of females (assumed to be half of the total initial population size), A_x is the number of females eclosing on day x, and D is the time (in days) to reproduction after emergence. The function $f(w_x)$ relates the average body size of females emerging on day x to fecundity (egg production). We used abdominal length as a proxy for wing length due to feasibility of measurement. Species-specific allometric functions were taken from the primary literature: for *Culex quinquefasciatus* (McCann et al. 2009),

$$f(w_x) = 90.31w_x - 123.88 \quad \text{(Eq. 2)};$$

for *Aedes albopictus* (Farjana and Tuno 2012),

$$f(w_x) = 104.8w_x - 201.37 \quad \text{(Eq. 3)};$$

and for *Aedes aegpyti* (Farjana and Tuno 2012),

$$f(w_x) = 79.30w_x - 144.08 \quad \text{(Eq. 4)}.$$

Analysis of vital rates and r'. To determine if competitors have different effects on the same target species, we performed analysis of variance (ANOVA, type II) using the vital rates (development time, body size, and egg-to-adult survival) for each species as dependent variables.

To account for variation due to replication, we used linear mixed-effects models with competitor species identity, density, and their interaction as fixed effects and replicate as the random effect. To evaluate model fit, we calculated both the marginal and conditional R^2 values for each model. The marginal R^2 describes the amount of variation in the data that is accounted for by the fixed effects, while the conditional R^2 describes the amount of variation accounted for by the whole model (fixed and random effects). Analysis of estimated intrinsic rates of increase followed the same method as the vital rates analysis, using a linear mixed-effects model with competitor identity and density as fixed effects and replicate as the random effect. To compare the relative effect size of a competitor on different vital rates and estimated intrinsic rate of increase, we calculated the standardized mean difference (hedges' g) of each treatment relative to intraspecific competition.

Estimating carrying capacity and competition coefficients. To determine if and when a species is competitively excluded, we plotted zero net growth isoclines (ZNGI) using the Lotka-Volterra competition model (Volterra 1926, Lotka 1932). This model, when generalized to two or more species, takes the form:

$$\frac{dN_i}{dt} = r_i N_i \left(1 - \frac{N_i + \sum \alpha_{ij} N_j}{K_i}\right) \quad \text{(Eq. 5)},$$

where r_i is the population growth rate for species i; N_i is the population size of species i; K_i is the carrying capacity for species i in the absence of interspecific competition; and α_{ij} is the competition coefficient describing the effect of species j on species i. The ZNGI for a species is the line:

$$N_i = K_i - \alpha_{ij} N_j \quad \text{(Eq. 6)}.$$

ZNGIs that do not overlap indicate competitive exclusion of one species. Overlapping ZNGIs where $K_i > K_j/\alpha_{ji}$ and $K_j > K_i/\alpha_{ij}$ indicate an unstable equilibrium where the two ZNGIs cross. In this case, the result of competition is dependent on each species' initial population size.

Overlapping ZNGIs where $K_i < K_j/\alpha_{ji}$ and $K_j < K_i/\alpha_{ij}$ indicate a stable equilibrium (coexistence) where the two ZNGIs cross.

To parameterize the model described above for each species pair, we fit linear models to population growth rate estimates for each species-competitor combination. The response variable for these models was mean estimated intrinsic rate of increase and the predictors were density, competitor species, and their interaction. Carrying capacity (K_i) for each species in the absence of interspecific competition was obtained from the estimated x-intercept of the linear model under intraspecific competition. Competition coefficients (α_{ij}) were estimated by relating the slope of the intraspecific linear model to that of each interspecific linear model (i.e., $\alpha_{ij} = m_{ij}/m_{ii}$, where m is the slope of the linear regression). When $\alpha_{ij} > 1$, the strength of intraspecific competition is less than interspecific competition; for $\alpha_{ij} < 1$, the strength of intraspecific competition is greater than interspecific competition.

To visualize the coexistence-exclusion parameter space for each species pair, we evaluated the Lotka-Volterra competition model over all combinations of α_{ij} and α_{ji} between 0-3 in increments of 0.01. We also plotted the competition coefficients (with 95% confidence intervals) estimated from our experimental data.

Results

Out of 6,300 first-instar larvae, 3,560 adults eclosed; of those, 41 were damaged during storage or processing (unable to identify species, sex, or body size) and were excluded from analyses. An additional 66 individuals were excluded due to an error during treatment setup. Of the remaining 3,453 individuals, 1,484 were female (Table 1). We omitted males from this analysis

since their vital rates do not contribute to the growth rate of the population. Additionally, males are not of public health importance as they do not blood-feed.

Table 2.1: Total eclosed adults from experiments, stratified by species and sex. Only data on female vital rates were used for these analyses.

	Culex quinquefasciatus	***Aedes albopictus***	***Aedes aegypti***	**Total**
Females	693	567	224	1484
Males	746	868	355	1969
Total	1439	1435	579	3453

Culex quinquefasciatus vital rates. There were significant differences between competitors' effects on *Culex quinquefasciatus* development time, but these differences depended on treatment density (χ^2 = 21.719, p-value = 0.001). Development time under interspecific competition was not significantly different from intraspecific development time at a low density. Multi-species competition significantly increased development time at a medium density, while competition with *Aedes aegypti* significantly increased development time at a high density (Figure 1A). Fixed effects accounted for 37.4% of the variation in development time; the full model (fixed + random effects) accounted for 64.4% of the variation in development time (Table 3).

Culex quinquefasciatus body size decreased significantly with increasing density (χ^2 = 85.925, p-value < 0.0001). Competition with *Aedes aegypti* significantly decreased body size (compared to intraspecific body size) at a low density, while competition with *Aedes albopictus* significantly decreased body size at a medium density (Figure 1B). Fixed effects accounted for 69.9% of the variation in body size; the full model accounted for 72.1% of the variation in body size (Table 3).

Egg-to-adult survival of *Culex quinquefasciatus* decreased significantly with increasing density (χ^2 = 69.594, p-value < 0.0001). All interspecific competition treatments significantly reduced egg-to-adult survival at medium and high densities compared to intraspecific survival (Figure 1C). Fixed effects accounted for 64.1% of the variation in egg-to-adult survival, with no variation due to random effects (Table 3).

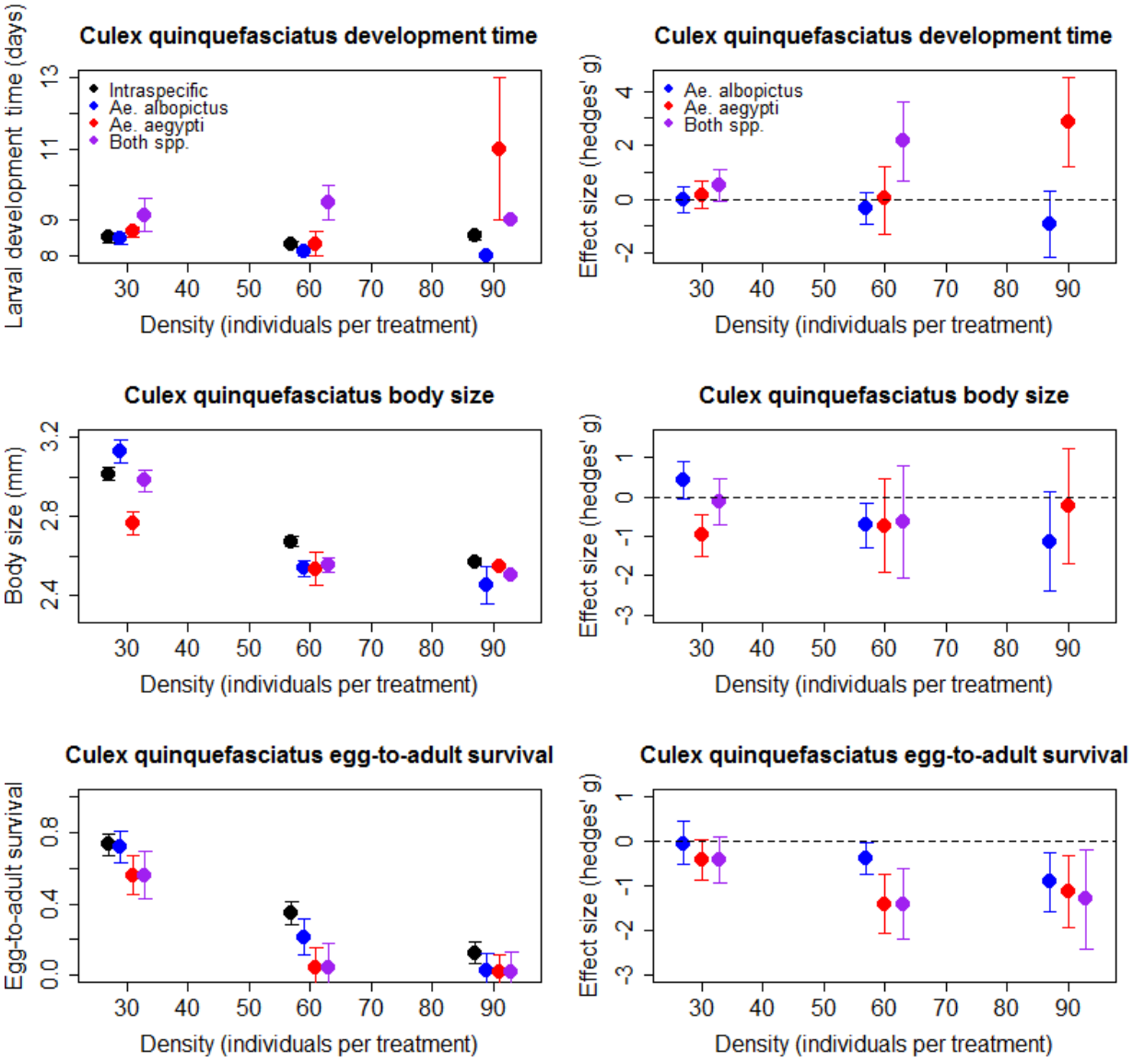

Figure 2.1: Vital rates (mean and standard error; left) and effect sizes (with 95% confidence intervals; right) by treatment for *Culex quinquefasciatus* females.

Table 2.2: Mean and standard error for development time (days), body size (mm), and egg-to-adult survival for *Culex quinquefasciatus* females, stratified by treatment.

Competitor	Density	n	Development time	Body size	Survival
Intraspecific	Low	55	8.509 (0.149)	3.016 (0.035)	0.733 (0.060)
	Med	52	8.308 (0.075)	2.674 (0.027)	0.347 (0.066)
	High	28	8.571 (0.120)	2.570 (0.018)	0.124 (0.062)
Aedes albopictus	Low	27	8.481 (0.145)	3.131 (0.056)	0.720 (0.086)
	Med	16	8.125 (0.125)	2.538 (0.042)	0.213 (0.102)
	High	3	8.000 (0.000)	2.454 (0.096)	0.027 (0.093)
Aedes aegypti	Low	21	8.667 (0.159)	2.765 (0.056)	0.560 (0.108)
	Med	3	8.333 (0.333)	2.534 (0.084)	0.040 (0.113)
	High	2	11.000 (2.000)	2.547 (0.003)	0.018 (0.093)
Both spp.	Low	14	9.143 (0.455)	2.983 (0.055)	0.560 (0.133)
	Med	2	9.500 (0.500)	2.553 (0.036)	0.040 (0.139)
	High	1	9.000 (NA)	2.502 (NA)	0.013 (0.115)

Table 2.3: Results from type II ANOVA for LMM of each *Culex quinquefasciatus* female vital rate. Bolded terms indicate statistical significance (R_m^2 = marginal; R_c^2 = conditional).

***Cx. quinquefasciatus* females**		**Development time**		**Body size**		**Survival**	
Model term	df	X^2	p-value	X^2	p-value	X^2	p-value
dens	2	2.075	0.354	**85.925**	**<0.0001**	**69.594**	**<0.0001**
comp	3	**11.601**	**0.009**	6.643	0.084	6.270	0.099
dens*comp	6	**21.719**	**0.001**	8.855	0.182	1.319	0.971
R_m^2/ R_c^2		0.374/0.644		0.699/0.721		0.641/0.641	

Table 2.4: Effect size (standardized mean difference; hedges' g) with 95% confidence intervals of different competitors on *Culex quinquefasciatus* female development time, body size, and egg-to-adult survival. Bolded numbers indicate effects that are statistically different from zero.

Density	Competitor	Development time	Body size	Survival
Low	*Ae. albopictus*	-0.03 (-0.49, 0.44)	0.43 (-0.04, 0.89)	-0.04 (-0.52, 0.45)
	Ae. aegypti	0.15 (-0.35, 0.66)	**-0.97 (-1.50, -0.44)**	-0.42 (-0.88, 0.03)
	Both *Aedes* spp.	0.50 (-0.09, 1.10)	-0.13 (-0.72, 0.46)	-0.42 (-0.94,0.10)
Medium	*Ae. albopictus*	-0.34 (-0.91, 0.23)	**-0.72 (-1.30, -0.15)**	**-0.37 (-0.73, -0.01)**
	Ae. aegypti	0.05 (-1.31, 1.22)	-0.73 (-1.91, 0.46)	**-1.40 (-2.06, -0.73)**
	Both *Aedes* spp.	**2.15 (0.66, 3.63)**	-0.63 (-2.06, 0.80)	**-1.40 (-2.20, - 0.59)**
High	*Ae. albopictus*	-0.91 (-2.14, 0.32)	-1.13 (-2.37, 0.12)	**-0.91 (-1.58, -0.24)**
	Ae. aegypti	**2.88 (1.23, 4.52)**	-0.24 (-1.70, 1.22)	**-1.13 (-1.94, -0.33)**
	Both *Aedes* spp.	NA	NA	**-1.29 (-2.41, -0.18)**

Aedes albopictus vital rates. Development time did not differ significantly across density for *Aedes albopictus* (χ^2 = 3.484, p-value = 0.175). Multi-species competition significantly increased development time at a high density compared to intraspecific development time (Figure 2A). Fixed effects accounted for 12.4% of the variation in development time, while the full model (fixed + random effects) accounted for 50.2% (Table 6).

Aedes albopictus body size decreased with increasing density (χ^2 = 327.605, p-value < 0.0001) and differed significantly between competitors (χ^2 = 12.941, p-value = 0.005). Competition with *Aedes aegypti* decreased *Aedes albopictus* body size at a high density compared to intraspecific body size (Figure 2B). The full model accounted for 86.9% of the variation in body size, with fixed effects alone accounting for 77.6% (Table 6).

The effect of different competitors on egg-to-adult survival of *Aedes albopictus* differed significantly, but differences were dependent on treatment density (χ^2 = 15.303, p-value = 0.018). Competition with *Aedes aegypti* significantly increased survival at a low density compared to intraspecific survival, but decreased survival at medium and high densities. Competition with *Culex quinquefasciatus* significantly increased *Aedes albopictus* survival at medium and high densities (Figure 2C). Fixed effects account for 55.4% of the variation in egg-to-adult survival, with no variation due to random effects (Table 6).

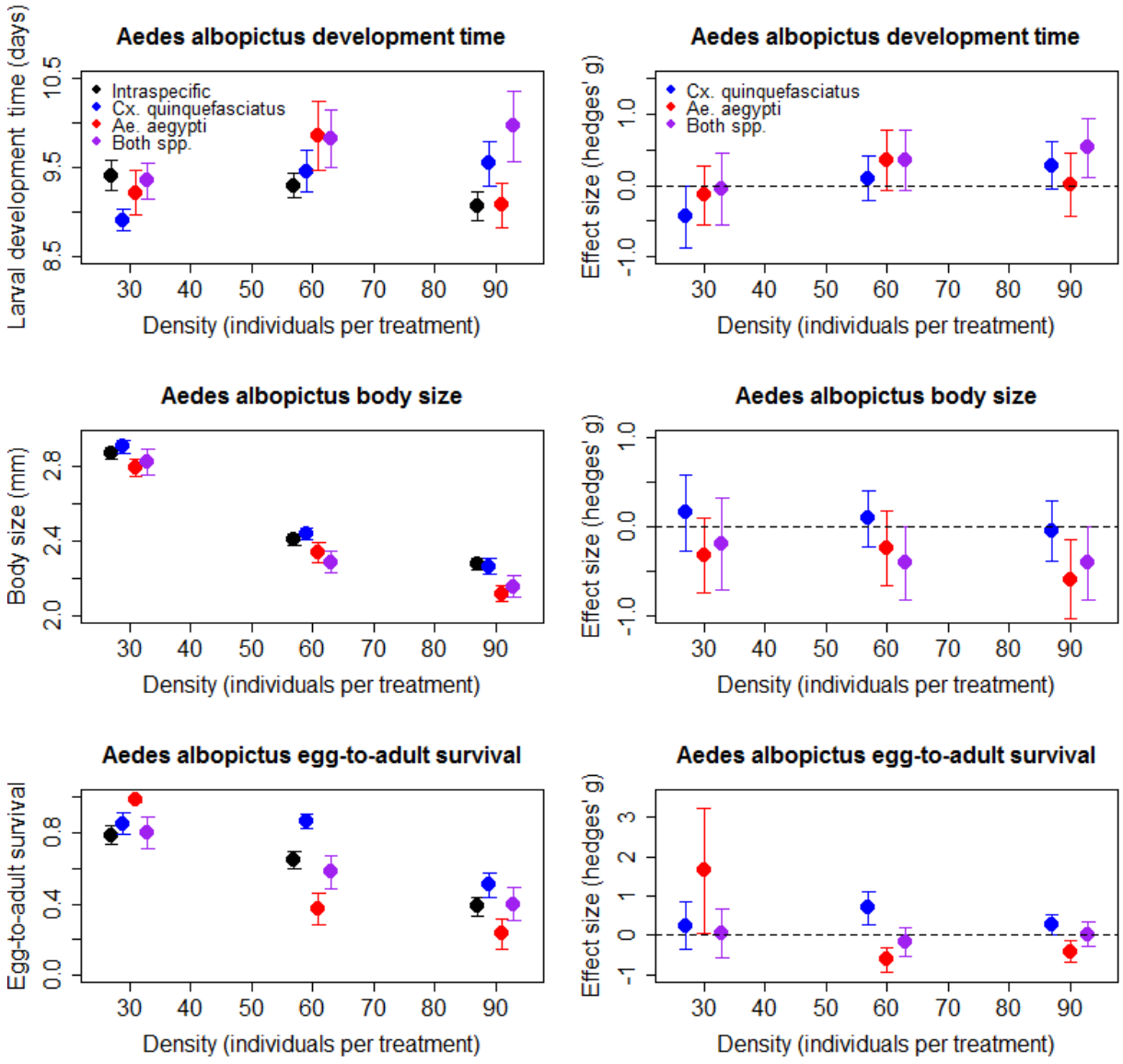

Figure 2.2: Vital rates (mean and standard error; left) and effect sizes (with 95% confidence intervals; right) by treatment for *Aedes albopictus* females.

Table 2.5: Mean and standard error for development time (days), body size (mm), and egg-to-adult survival for *Aedes albopictus* females, stratified by treatment.

Competitor	Density	n	Development time	Body size	Survival
Intraspecific	Low	59	9.407 (0.169)	2.869 (0.029)	0.787 (0.053)
	Med	97	9.299 (0.144)	2.409 (0.030)	0.647 (0.049)
	High	87	9.069 (0.161)	2.277 (0.030)	0.387 (0.052)
Culex quinquefasciatus	Low	32	8.906 (0.122)	2.903 (0.032)	0.853 (0.063)
	Med	65	9.462 (0.228)	2.436 (0.031)	0.867 (0.042)
	High	57	9.544 (0.255)	2.263 (0.041)	0.507 (0.066)
Aedes aegypti	Low	37	9.216 (0.252)	2.790 (0.046)	0.987 (0.019)
	Med	28	9.857 (0.387)	2.338 (0.052)	0.373 (0.091)
	High	26	9.077 (0.254)	2.117 (0.044)	0.231 (0.083)
Both spp.	Low	20	9.350 (0.196)	2.821 (0.071)	0.800 (0.089)
	Med	29	9.828 (0.318)	2.286 (0.057)	0.580 (0.092)
	High	30	9.967 (0.394)	2.156 (0.059)	0.400 (0.089)

Table 2.6: Results from type II ANOVA for LMM of each *Aedes albopictus* female vital rate. Bolded terms indicate statistical significance (R_m^2 = marginal; R_c^2 = conditional).

***Ae. albopictus* females**		**Development time**		**Body size**		**Survival**	
Model term	df	X^2	p-value	X^2	p-value	X^2	p-value
dens	2	3.484	0.175	**327.605**	**<0.0001**	**48.478**	**<0.0001**
comp	3	2.801	0.423	**12.941**	**0.005**	**8.278**	**0.041**
dens*comp	6	8.075	0.233	3.517	0.742	**15.303**	**0.018**
R_m^2/ R_c^2		0.124/0.502		0.776/0.869		0.554/0.554	

Table 2.7: Effect size (standardized mean difference; hedges' g) with 95% confidence intervals of different competitors on *Aedes albopictus* female development time, body size, and egg-to-adult survival. Bolded numbers indicate effects that are statistically different from zero.

Density	Competitor	Development time	Body size	Survival
Low	*Cq. quinq.*	-0.44 (-0.88, 0.00)	0.16 (-0.27, 0.59)	0.25 (-0.34, 0.84)
	Ae. aegypti	-0.14 (-0.55, 0.28)	-0.32 (-0.74, 0.10)	**1.64 (0.07, 3.22)**
	Both spp.	-0.05 (-0.56, 0.46)	-0.19 (-0.71, 0.32)	0.04 (-0.58, 0.67)
Medium	*Cq. quinq.*	0.10 (-0.21, 0.42)	0.10 (-0.22, 0.41)	**0.70 (0.28, 1.11)**
	Ae. aegypti	0.35 (-0.07, 0.78)	-0.24 (-0.66, 0.18)	**-0.62 (-0.93, -0.30)**
	Both spp.	0.35 (-0.07, 0.77)	-0.41 (-0.83, 0.01)	-0.15 (-0.52, 0.21)
High	*Cq. quinq.*	0.28 (-0.06, 0.62)	-0.05 (-0.38, 0.29)	**0.27 (0.02, 0.52)**
	Ae. aegypti	0.01 (-0.43, 0.45)	**-0.60 (-1.04, -0.15)**	**-0.41 (-0.69, -0.12)**
	Both spp.	**0.53 (0.11, 0.95)**	-0.41 (-0.83, 0.01)	0.03 (-0.26, 0.33)

Aedes aegypti vital rates. *Aedes aegypti* development time increased significantly with density (χ^2 = 12.380, p-value = 0.002). Competition with *Aedes albopictus*, as well as multi-species competition, significantly decreased development time at a medium density compared to intraspecific development time. Fixed effects explained only 8.6% of the variation in development time, while the full model (fixed + random effects) accounted for 82.8% (Table 9).

Body size decreased significantly with increasing density (χ^2 = 271.940, p-value < 0.0001). All interspecific treatments significantly increased body size at a medium density compared to intraspecific body size. Multi-species competition also increased body size at a high density (Figure 3B). Fixed effects accounted for 83.9% of the variation in body size, with no variation explained by random effects (Table 9).

Aedes aegypti egg-to-adult survival decreased significantly with increasing density (χ^2 = 19.653, p-value < 0.0001). All interspecific treatments significantly increased survival at medium and high densities compared to intraspecific survival (Figure 3C). Fixed effects accounted for 45.3% of the variation in egg-to-adult survival, while the full model (fixed + random effects) accounted for 58.1% (Table 9).

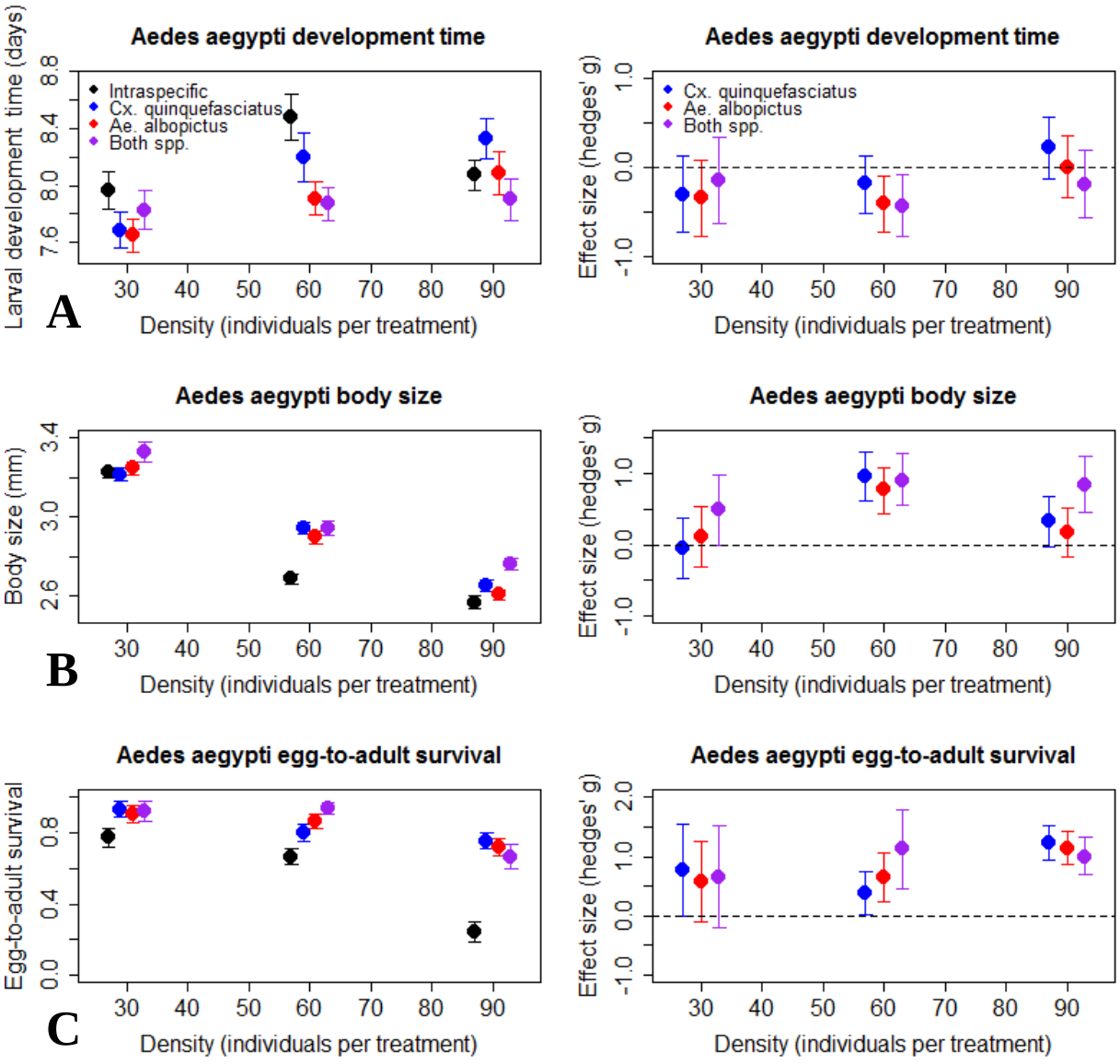

Figure 2.3: Vital rates (mean and standard error; left) and effect sizes (with 95% confidence intervals; right) by treatment for *Aedes aegypti* females.

Table 2.8: Mean and standard error for development time (days), body size (mm), and egg-to-adult survival for *Aedes aegypti* females, stratified by treatment.

Competitor	Density	n	Development time	Body size	Survival
Intraspecific	Low	58	7.966 (0.135)	3.225 (0.027)	0.773 (0.055)
	Med	100	8.480 (0.161)	2.685 (0.029)	0.667 (0.047)
	High	55	8.073 (0.107)	2.567 (0.034)	0.244 (0.058)
Culex quinquefasciatus	Low	35	7.686 (0.128)	3.216 (0.033)	0.933 (0.042)
	Med	60	8.200 (0.171)	2.943 (0.029)	0.800 (0.052)
	High	85	8.329 (0.141)	2.654 (0.030)	0.756 (0.047)
Aedes albopictus	Low	34	7.647 (0.119)	3.248 (0.032)	0.907 (0.050)
	Med	65	7.908 (0.118)	2.899 (0.032)	0.867 (0.042)
	High	81	8.086 (0.156)	2.606 (0.024)	0.720 (0.050)
Both spp.	Low	23	7.826 (0.136)	3.332 (0.051)	0.920 (0.057)
	Med	47	7.872 (0.116)	2.942 (0.038)	0.940 (0.035)
	High	50	7.900 (0.149)	2.761 (0.028)	0.667 (0.067)

Table 2.9: Results from type II ANOVA for LMM of each *Aedes aegypti* female vital rate. Bolded terms indicate statistical significance (R_m^2 = marginal; R_c^2 = conditional).

***Ae. aegypti* females**		**Development time**		**Body size**		**Survival**	
Model term	df	X^2	p-value	X^2	p-value	X^2	p-value
dens	2	**12.380**	**0.002**	**271.940**	**<0.0001**	**19.653**	**<0.0001**
comp	3	6.272	0.099	**19.786**	**0.0002**	**30.679**	**<0.0001**
dens*comp	6	10.056	0.122	7.601	0.269	11.156	0.084
R_m^2/ R_c^2		0.086/0.828		0.839/0.839		0.453/0.581	

Table 2.10: Effect size (standardized mean difference; hedges' g) with 95% confidence intervals of different competitors on *Aedes aegypti* female development time, body size, and egg-to-adult survival. Bolded numbers indicate effects that are statistically different from zero.

Density	Competitor	Development time	Body size	Survival
Low	*Cq. quinq.*	-0.30 (-0.72, 0.13)	-0.05 (-0.47, 0.38)	0.77 (0.00, 1.54)
	Ae. albopictus	-0.34 (-0.77, 0.08)	0.11 (-0.31, 0.54)	0.57 (-0.11, 1.25)
	Both spp.	-0.15 (-0.63, 0.34)	0.49 (0.00, 0.98)	0.66 (-0.19, 1.52)
Medium	*Cq. quinq.*	-0.18 (-0.51, 0.14)	**0.96 (0.62, 1.30)**	**0.38 (0.02, 0.75)**
	Ae. albopictus	**-0.41 (-0.73, -0.09)**	**0.77 (0.44, 1.09)**	**0.65 (0.23, 1.06)**
	Both spp.	**-0.43 (-0.78, -0.08)**	**0.91 (0.55, 1.28)**	**1.13 (0.46, 1.80)**
High	*Cq. quinq.*	0.23 (-0.12, 0.57)	0.33 (-0.02, 0.67)	**1.24 (0.95, 1.53)**
	Ae. albopictus	0.01 (-0.33, 0.36)	0.17 (-0.17, 0.51)	**1.14 (0.86, 1.42)**
	Both spp.	-0.19 (-0.57, 0.20)	**0.85 (0.45, 1.25)**	**1.00 (0.69, 1.32)**

Estimated intrinsic growth rates. Estimated intrinsic growth rates for *Culex quinquefasciatus* decreased with increasing density (χ^2 = 104.805, p < 0.0001), and there was a significant difference in growth rate between competitors (χ^2 = 11.696, p = 0.008). Competition with *Aedes aegypti* significantly decreased growth rate relative to intraspecific competition at medium densities (Figure 4A). Effect sizes for multi-species competition at medium and high densities, as well effect sizes for competition with *Aedes aegypti* at high densities, were unable to be calculated due to low survival in those treatments. The model explained 73.9% of the variation in intrinsic growth rate, all of which was due to fixed effects (Table 11).

The effect on estimated *Aedes albopictus* growth rate was significantly different between competitors, and the effect of competitor identity was density-dependent (χ^2 = 25.687, p = 0.0003). Competition with *Aedes aegypti* significantly decreased growth rate compared to intraspecific competition at medium densities (Figure 4B). The model explained 84.2% of the variation in intrinsic growth rate, most of which was accounted for by fixed effects (marginal R^2 = 0.782, Table 11).

Estimated *Aedes aegypti* growth rate was also significantly different between competitors, and the effect of competitor identity was density-dependent (χ^2 = 32.008, p < 0.0001). Competition with *Aedes albopictus*, as well as multi-species competition, significantly increased growth rate at medium densities. All interspecific treatments significantly increased growth rate at high densities (Figure 4C). The model explained 76.2% of the variation in intrinsic growth rate, most of which was attributable to fixed effects (marginal R^2 = 0.712, Table 11).

Zero net growth isoclines for each species pair indicate that *Aedes albopictus* excludes *Culex quinquefasciatus* (Figure 5A), while *Aedes aegypti* excludes both other species (Figure 5B/C).

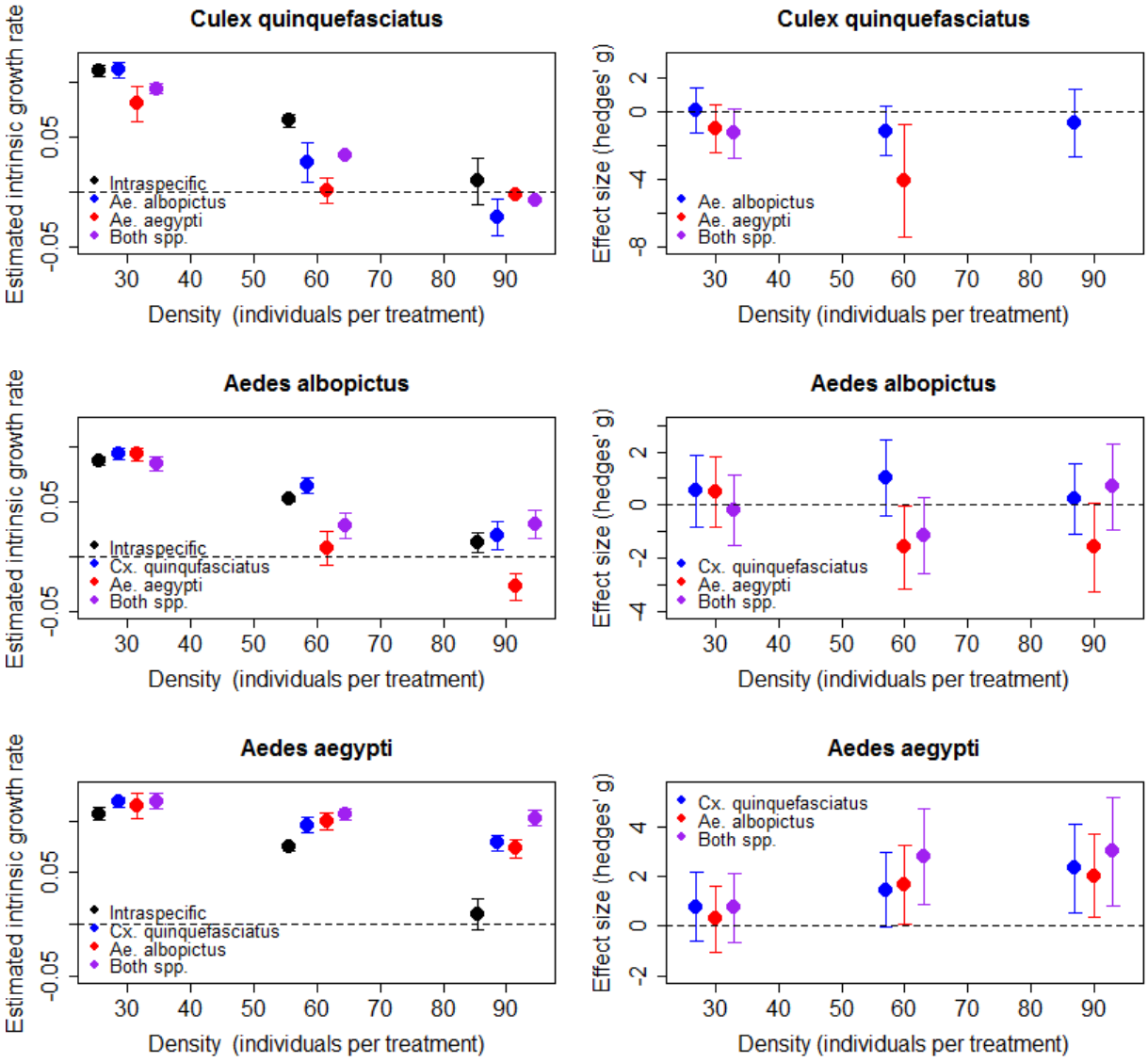

Figure 2.4: Estimated intrinsic growth rate (mean and standard error; left) and effect sizes (with 95% confidence intervals; right) for each species.

Table 2.11: Results from type II ANOVA for LMM of estimated intrinsic growth rate for each species. Bolded terms indicate statistical significance (R_m^2 = marginal; R_c^2 = conditional).

		Cq. quinquefasciatus		***Ae. albopictus***		***Ae. aegypti***	
Model term	df	X^2	p-value	X^2	p-value	X^2	p-value
dens	2	**104.805**	**<0.0001**	**225.715**	**<0.0001**	**82.611**	**<0.0001**
comp	3	**11.696**	**0.008**	**29.749**	**<0.0001**	**55.442**	**<0.0001**
dens*comp	6	4.935	0.552	**25.687**	**0.0003**	**32.008**	**<0.0001**
R_m^2/ R_c^2		0.739/0.739		0.782/0.842		0.712/0.762	

Table 2.12: Mean and standard error of estimated intrinsic growth rate for each species, stratified by treatment.

Target species	Competitor	Density	n	r'
Culex quinquefasciatus	Intraspecific	Low	5	0.111 (0.006)
		Med	5	0.065 (0.005)
		High	4	0.010 (0.021)
	Aedes albopictus	Low	5	0.111 (0.007)
		Med	5	0.027 (0.018)
		High	2	-0.023 (0.017)
	Aedes aegypti	Low	5	0.081 (0.016)
		Med	2	0.001 (0.012)
		High	1	-0.002 (NA)
	Both spp.	Low	5	0.094 (0.004)
		Med	1	0.034 (NA)
		High	1	-0.007 (NA)
Aedes albopictus	Intraspecific	Low	5	0.088 (0.004)
		Med	5	0.052 (0.002)
		High	5	0.012 (0.009)
	Culex quinquefasciatus	Low	5	0.094 (0.005)
		Med	5	0.065 (0.007)
		High	5	0.019 (0.013)
	Aedes aegypti	Low	5	0.094 (0.006)
		Med	5	0.008 (0.016)
		High	4	-0.028 (0.013)
	Both spp.	Low	5	0.085 (0.007)
		Med	5	0.029 (0.011)
		High	3	0.029 (0.013)
Aedes aegypti	Intraspecific	Low	5	0.088 (0.004)
		Med	5	0.052 (0.002)
		High	5	0.012 (0.009)
	Culex quinquefasciatus	Low	5	0.094 (0.005)
		Med	5	0.065 (0.007)
		High	5	0.019 (0.013)
	Aedes albopictus	Low	5	0.094 (0.006)
		Med	5	0.008 (0.016)
		High	5	-0.028 (0.013)
	Both spp.	Low	5	0.085 (0.007)
		Med	5	0.029 (0.011)
		High	4	0.029 (0.013)

Table 2.13: Effect size (standardized mean difference; hedges' g) with 95% confidence intervals of different competitors on estimated intrinsic growth rate of each species, stratified by treatment. Bolded numbers indicate effects that are statistically different from zero.

Species	Density	Competitor	Effect size (95% CI)
Culex quinquefasciatus	Low	*Ae. albopictus*	0.06 (-1.26, 1.38)
		Ae. aegypti	-1.01 (-2.42, 0.41)
		Both spp.	-1.31 (-2.79, 0.17)
	Medium	*Ae. albopictus*	-1.16 (-2.60, 0.29)
		Ae. aegypti	**-4.12 (-7.47, -0.76)**
		Both spp.	NA
	High	*Ae. albopictus*	-0.68 (-2.68, 1.32)
		Ae. aegypti	NA
		Both spp.	NA
Aedes albopcitus	Low	*Cx. quinquefasciatus*	0.53 (-0.82, 1.87)
		Ae. aegypti	0.48 (-10.86, 1.82)
		Both spp.	-0.19 (-1.52, 1.13)
	Medium	*Cx. quinquefasciatus*	1.01 (-0.40, 2.43)
		Ae. aegypti	**-1.60 (-3.16, -0.05)**
		Both spp.	-1.16 (-2.60, 0.29)
	High	*Cx. quinquefasciatus*	0.23 (-1.10, 1.55)
		Ae. aegypti	-1.59 (-3.26, 0.07)
		Both spp.	0.69 (-0.92, 2.30)
Aedes aegpyti	Low	*Cx. quinquefasciatus*	0.79 (-0.59, 2.17)
		Ae. albopictus	0.29 (-1.03, 1.62)
		Both spp.	0.74 (-0.63, 2.11)
	Medium	*Cx. quinquefasciatus*	1.46 (-0.06, 2.98)
		Ae. albopictus	**1.67 (0.10, 3.25)**
		Both spp.	**2.81 (0.85, 4.77)**
	High	*Cx. quinquefasciatus*	**2.35 (0.56, 4.14)**
		Ae. albopictus	**2.04 (0.36, 3.73)**
		Both spp.	**3.02 (0.82, 5.21)**

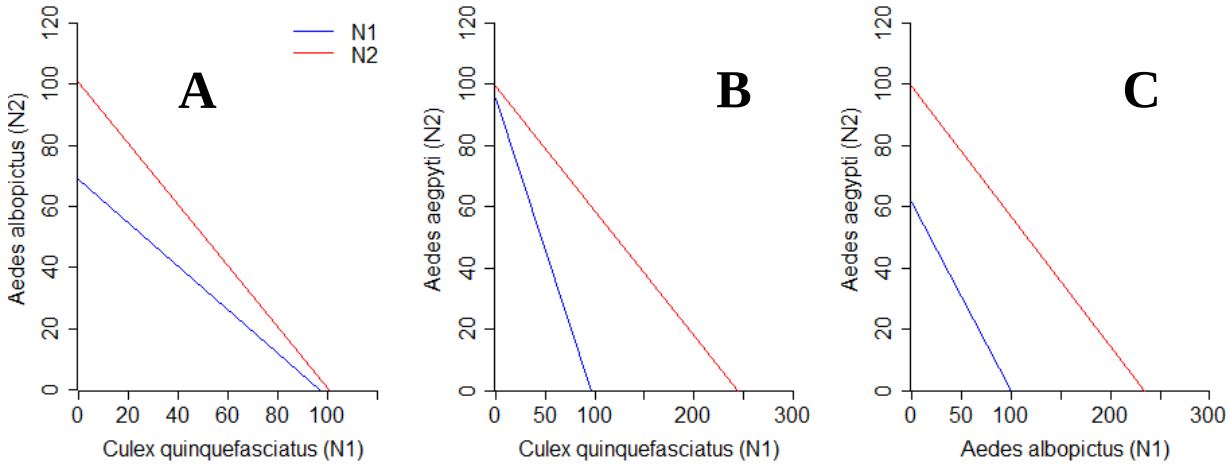

Figure 2.5 (A-C): Zero net growth isoclines (ZNGIs) for each species pair. The ZNGI for the species on the x-axis is plotted in blue; the ZNGI for the species on the y-axis is plotted in red.

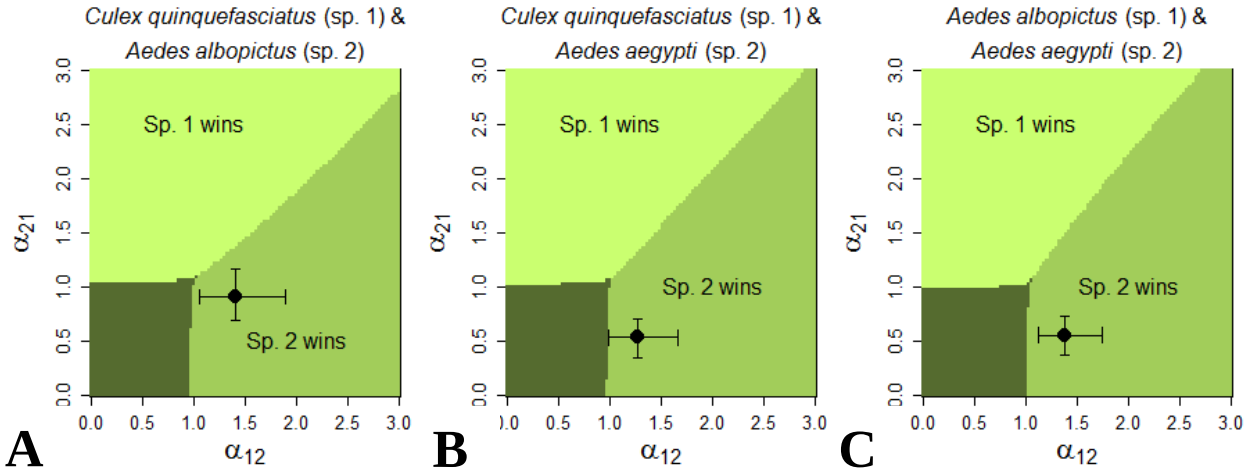

Figure 2.6 (A-C): Coexistence-exclusion plots for each species-pair. The light green area represents the parameter space in which species 1 excludes species 2; the medium green area represents the parameter space in which species 2 excludes species 1; and the dark green area represents the parameter space in which both species can coexist. Competition coefficients (with 95% confidence intervals) estimated from our experimental data are plotted in black.

Discussion

Our results provide evidence of trans-boundary ecosystem effects in mosquito communities. Interspecific larval competition altered development time, body size (fecundity), and egg-to-adult survival, which translated to significant changes in the intrinsic growth rate of each species. Interspecific competition with one or both *Aedes* species resulted in lower estimated intrinsic growth rates for *Culex quinquefasciatus*. While competition with *Aedes aegypti* reduced the estimated population growth rate of *Aedes albopictus*, competition with *Culex quinquefasciatus*, as well as competition with both interspecific competitors simultaneously, increased estimated population growth rates. Compared to intraspecific competition, *Aedes aegypti* estimated growth rates were increased by competition with either or both interspecific competitors. These results suggest a competitive hierarchy in which *Culex quinquefasciatus* is the inferior competitor and *Aedes aegypti* is the superior competitor.

Additionally, our results show that the effects of multiple competitors on vital rates and estimated population growth rate are often non-additive, can be antagonistic or synergistic, and can be density-dependent. For example, the effect of interspecific competition with either *Aedes aegypti* or *Culex quinquefasciatus* on *Aedes albopictus* growth rate was positive, but competition with both interspecific competitors simultaneously resulted in a small negative effect on *Aedes albopictus* population growth rate. However, we only saw this antagonistic effect of multiple competitors on *Aedes albopictus* population growth rate in low density treatments. In high density treatments, interspecific competition with *Culex quinquefasciatus* had a small positive effect on *Aedes albopictus* population growth rate, while interspecific competition with *Aedes aegypti* had a large negative effect on population growth rate. The effect of interspecific competition with both species simultaneously, however, resulted in a large positive (and thus synergistic) effect on

population growth rate. These results emphasize the need to study the dynamics of multi-species communities in addition to paired species experiments, which allows for the quantification of both direct and indirect effects.

Although our results suggest a competitive hierarchy among these three species with *Aedes aegypti* being the superior competitor, there is a wealth of evidence from field observations and experiments indicating that *Aedes albopictus* outcompetes *Aedes aegypti*. However, it appears that the resource type (i.e., plant or animal) available during the larval stage can influence which species is competitively superior. For example, *Aedes albopictus* tends to be the dominant competitor when the available resource is plant-based, while *Aedes aegypti* is the superior competitor when fed animal-based nutrients (Barrera 1996, Juliano 1998, Murrell and Juliano 2008). The food resource used in this experiment was animal-based, providing a possible explanation for the competitive dominance of *Aedes aegypti*. These species are also known to differ in their foraging behavior, with *Aedes albopictus* primarily browsing on leaf litter and detritus and *Aedes aegypti* primarily filtering (Yee et al. 2004). The resource used for this experiment consisted of a finely-ground powder suspended in water which may have given *Aedes aegypti* an advantage. Additionally, most studies of competition between these two *Aedes* species are conducted with field populations of mosquitoes, while our experiments used species from laboratory colonies (Juliano 2009). Specifically, our *Aedes aegypti* population had been reared in a laboratory colony for several years which may have allowed for the optimization of vital rates (development time, egg-to-adult survival, fecundity) that determine population growth rates, providing a possible explanation for why our results differed from previous research.

While our results indicate the competitive exclusion of one species in each pair of competitors, co-occurrence of these species in the field has been documented (Juliano et al. 2004,

Costanzo et al. 2005b, Rey et al. 2006). What might alter these competitive relationships to allow for coexistence in natural habitats? (Juliano et al. 2004) suggest that a higher tolerance for extreme conditions in competitively inferior species may allow for coexistence in some environments. There is some evidence that other life stages, like eggs (Costanzo et al. 2005a) and adults (Alto 2011), may also contribute to coexistence. Photoperiod has been known to alter vital rates, like adult size and longevity, that contribute to population dynamics (Costanzo et al. 2015), indicating that the ability for species to coexist may depend on day length and, thus, could vary by season or latitude. Interpopulation variability could explain why we see co-occurrence in some areas and not others (Leisnham et al. 2009, Leisnham and Juliano 2010). Differences in phenology between species could lead to priority effects that allow for coexistence if the inferior competitor establishes first. For example, larval growth rate and survival in *Aedes albopictus* was shown to decrease in the presence of fourth instar *Wyeomyia* spp., but not in the presence of first instars (Lounibos et al. 2003). Finally, temperature has been shown to be the most important determinant of larval development time (Couret and Benedict 2014), so one species could be competitively dominant at lower temperatures while the other may be the superior competitor at higher temperatures (Carrieri et al. 2003).

Our research highlights the potential for coupled dynamics between ecosystems due to species with complex life cycles, like mosquitoes. We demonstrate that interspecific larval competition alters the vital rates that determine population growth rate and species coexistence in mosquito communities. The inconsistency in competitive outcomes among our study and several others underscores the need for broad field sampling of larval habitats to determine where and under what biotic and abiotic conditions coexistence can occur between two or more mosquito species. While we have a solid understanding of how larval interactions alter population dynamics,

future research should focus on how larval interactions might affect vectorial capacity. Experimental studies of the effects of larval competition on host feeding preferences, biting rate, adult survival, and vector competence will help us model potential changes in the R_0 of vector-borne diseases in different vector communities.

References

Agnew, P., C. Haussy, and Y. Michalakis. 2000. Effects of Density and Larval Competition on Selected Life History Traits of Culex pipiens quinquefasciatus (Diptera: Culicidae). Journal of Medical Entomology 37:732–735.

Alto, B. W. 2011. Interspecific Larval Competition Between Invasive Aedes japonicus and Native Aedes triseriatus (Diptera: Culicidae) and Adult Longevity. Journal of Medical Entomology 48:232–242.

Alto, B. W., L. P. Lounibos, S. Higgs, and S. A. Juliano. 2005. Larval competition differentially affects arbovirus infection in aedes mosquitoes. Ecology 86:3279–3288.

Alto, B. W., L. P. Lounibos, C. N. Mores, and M. H. Reiskind. 2008. Larval competition alters susceptibility of adult Aedes mosquitoes to dengue infection. Proceedings of the Royal Society of London B: Biological Sciences 275:463–471.

Armbruster, P., and R. A. Hutchinson. 2002. Pupal Mass and Wing Length as Indicators of Fecundity in Aedes albopictus and Aedes geniculatus (Diptera: Culicidae). Journal of Medical Entomology 39:699–704.

Barrera, R. 1996. Competition and resistance to starvation in larvae of container-inhabiting Aedes mosquitoes. Ecological Entomology 21:117–127.

Bevins, S. 2008. Invasive mosquitoes, larval competition, and indirect effects on the vector competence of native mosquito species (Diptera: Culicidae). Biological Invasions 10:1109–1117.

Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003. On the Competition Occurring Between Aedes albopictus and Culex pipiens (Diptera: Culicidae) in Italy. Environmental Entomology 32:1313–1321.

Costanzo, K. S., B. Kesavaraju, and S. A. Juliano. 2005a. Condition-Specific Competition in Container Mosquitoes: The Role of Noncompeting Life-History Stages. Ecology 86:3289–3295.

Costanzo, K. S., K. MORMANN, and S. A. JULIANO. 2005b. Asymmetrical Competition and Patterns of Abundance of Aedes albopictus and Culex pipiens (Diptera: Culicidae). Journal of medical entomology 42:559–570.

Costanzo, K. S., S. Schelble, K. Jerz, and M. Keenan. 2015. The effect of photoperiod on life history and blood-feeding activity in Aedes albopictus and Aedes aegypti (Diptera: Culicidae). Journal of Vector Ecology 40:164–171.

Couret, J., and M. Q. Benedict. 2014. A meta-analysis of the factors influencing development rate variation in Aedes aegypti (Diptera: Culicidae). BMC Ecology 14:3.

Darsie, R. F., and R. A. Ward. 2005. Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico. University of Florida Press, Gainesville, FL.

Farjana, T., and N. Tuno. 2012. Effect of body size on multiple blood feeding and egg retention of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae). Medical Entomology and Zoology 63:123–131.

Gimnig, J. E., M. Ombok, S. Otieno, M. G. Kaufman, J. M. Vulule, and E. D. Walker. 2002. Density-Dependent Development of Anopheles gambiae (Diptera: Culicidae) Larvae in Artificial Habitats. Journal of Medical Entomology 39:162–172.

Juliano, S. A. 1998. Species Introduction and Replacement Among Mosquitoes: Interspecific Resource Competition or Apparent Competition? Ecology 79:255–268.

Juliano, S. A. 2007. Population Dynamics. Journal of the American Mosquito Control Association 23:265–275.

Juliano, S. A. 2009. Species Interactions Among Larval Mosquitoes: Context Dependence Across Habitat Gradients. Annual review of entomology 54:37–56.

Juliano, S. A., L. P. Lounibos, and G. F. O'Meara. 2004. A field test for competitive effects of Aedes albopictus on A. aegypti in South Florida: differences between sites of coexistence and exclusion? Oecologia 139:583–593.

Juliano, S. A., and L. Philip Lounibos. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. Ecology Letters 8:558–574.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–498.

Knight, T. M., M. W. McCoy, J. M. Chase, K. A. McCoy, and R. D. Holt. 2005. Trophic cascades across ecosystems. Nature 437:880–883.

Leisnham, P. T., and S. A. Juliano. 2010. Interpopulation differences in competitive effect and response of the mosquito Aedes aegypti and resistance to invasion by a superior competitor. Oecologia 164:221–230.

Leisnham, P. T., L. P. Lounibos, G. F. O'Meara, and S. A. Juliano. 2009. Interpopulation divergence in competitive interactions of the mosquito Aedes albopictus. Ecology 90:2405–2413.

Livdahl, T. P., and G. Sugihara. 1984. Non-Linear Interactions of Populations and the Importance of Estimating Per Capita Rates of Change. Journal of Animal Ecology 53:573–580.

Lounibos, L. P., G. F. O'Meara, and N. Nishimura. 2003. Interactions with native mosquito larvae regulate the production of Aedes albopictus from bromeliads in Florida. Ecological Entomology 28:551–558.

McCann, S., J. F. Day, S. Allan, and C. C. Lord. 2009. Age modifies the effect of body size on fecundity in Culex quinquefasciatus Say (Diptera: Culicidae). Journal of Vector Ecology 34:174–181.

McCoy, M. W., M. Barfield, and R. D. Holt. 2009. Predator shadows: complex life histories as generators of spatially patterned indirect interactions across ecosystems. Oikos 118:87–100.

Murrell, E. G., and S. A. Juliano. 2008. Detritus Type Alters the Outcome of Interspecific Competition Between Aedes aegypti and Aedes albopictus (Diptera: Culicidae). Journal of Medical Entomology 45:375–383.

Reiskind, M. H., and L. P. Lounibos. 2009. Effects of intraspecific larval competition on adult longevity in the mosquitoes Aedes aegypti and Aedes albopictus. Medical and Veterinary Entomology 23:62–68.

Rey, J. R., N. Nishimura, B. Wagner, M. A. H. Braks, S. M. O'Connell, and L. P. Lounibos. 2006. Habitat Segregation of Mosquito Arbovirus Vectors in South Florida. Journal of Medical Entomology 43:1134–1141.

Scheiner, S., and J. Gurevitch. 2001. Design and Analysis of Ecological Experiments. Oxford University Press, USA.

Schneider, P., W. Takken, and P. J. Mccall. 2000. Interspecific competition between sibling species larvae of Anopheles arabiensis and An. gambiae. Medical and Veterinary Entomology 14:165–170.

Schreiber, S., and V. H. W. Rudolf. 2008. Crossing habitat boundaries: coupling dynamics of ecosystems through complex life cycles. Ecology Letters 11:576–587.

Turell, M. J., M. L. O'Guinn, D. J. Dohm, and J. W. Jones. 2001. Vector Competence of North American Mosquitoes (Diptera: Culicidae) for West Nile Virus. Journal of Medical Entomology 38:130–134.

Werner, E. E., and J. F. Gilliam. 1984. The Ontogenetic Niche and Species Interactions in Size-Structured Populations. Annual Review of Ecology and Systematics 15:393–425.

Yee, D. A., B. KESAVARAJU, and S. A. JULIANO. 2004. Interspecific Differences in Feeding Behavior and Survival Under Food-Limited Conditions for Larval Aedes albopictus and Aedes aegypti (Diptera: Culicidae). Annals of the Entomological Society of America 97:720–728.

CHAPTER 3

TEMPERATURE-DEPENDENT EFFECTS OF INTERSPECIFIC LARVAL COMPETITION ON VITAL RATES AND POPULATION GROWTH RATES OF THREE MOSQUITO VECTORS[1]

[1]Bowden, Sarah E. and Drake, John M. To be submitted to *Ecology Letters*.

Abstract

The growth, development, and survival of mosquitoes, the most abundant vectors of disease worldwide, are highly dependent on temperature. These temperature-dependent vital rates are also sensitive to species interactions, like interspecific larval competition. In order to better predict how vectors and the diseases they transmit will respond to a changing climate, there is a need to study not only the thermal response of individuals and populations of mosquitoes, but also how communities of interacting mosquito species respond to different temperatures as well. We conducted laboratory microcosm experiments to determine the effects of interspecific larval competition on vital rates of three vector mosquito species across a range of temperatures (21-33°C), then fit curves to quantify the temperature range in which each species could maintain non-negative population growth ("thermal niche") under different competitive scenarios. Our results showed that temperature-dependent effects of interspecific larval competition on mosquito vital rates varied by target species and species identity of the competitor(s). Interspecific competition with *Ae. aegypti* significantly narrowed the thermal niche for population growth in both *Ae. albopictus* and *Cx. quinquefasciatus*. These findings provide evidence for temperature-dependent species interactions in larval mosquitoes, which may have important implications for predicting how vector communities and vector-borne disease transmission will respond to a changing climate.

Introduction

The response of infectious disease systems to climate change has important implications for both human and wildlife health, especially in vector-borne disease systems where mosquito vectors can respond quickly to environmental changes. Mosquitoes are the most abundant vectors of disease worldwide, and over 500,000 people die each year from Malaria alone. Despite the large public health burden mosquitoes pose, knowledge of mosquito ecology is often limited to a small

subset of species and is in constant need of expansion. Over the past decade, a great deal of research has explored how climate change may affect infectious diseases dynamics worldwide. There is considerable debate as to whether increasing temperatures will lead to range expansions (Harvell et al. 2009) or range shifts (Lafferty 2009) in disease systems, and the answer is likely to depend on the organism (host or vector) or pathogen in question. As ectotherms, the survival and reproduction of mosquitoes is highly dependent on temperature. Components of vector-borne disease transmission, such as vector competence (ability to harbor and transmit a pathogen) and pathogen incubation period, are known to have strong relationships with temperature as well (Richards et al. 2007, Muturi and Alto 2011, Muturi et al. 2012, Carrington et al. 2013, Bara et al. 2015). Thus, it is important to the forecasting of vector-borne disease dynamics that we further explore the ecology of vector mosquitoes in the context of climate change.

The majority of what is known about vector mosquitoes comes from studying individuals or populations. However, aquatic larval habitats can be highly diverse and larval mosquitoes have the potential to interact with several other species during larval development, including both intraspecific and interspecific competition with other mosquito larvae. Therefore, there is a need to study not only how individuals and populations of mosquitoes will respond to climate change, but how communities of interacting mosquito species will respond as well. Mosquitoes have temperature-dependent vital rates, such as larval development time, egg-to-adult survival, and adult longevity (Rueda et al. 1990, Delatte et al. 2009, Couret et al. 2014). The same vital rates are also sensitive to species interactions (e.g., interspecific competition (Kirby and Lindsay 2009, Farjana et al. 2012)), and they combine to determine population growth rates and population and community dynamics. Understanding how temperature alters species interactions between disease vectors will help us to better predict how vector abundance, distribution, and community

composition will change with temperature. Additionally, changes in vector abundance could amplify the burden of vector-borne diseases in tropical areas. Recent research by Mordecai et al. (2013) showed that life history traits of *Anopheles* mosquitoes (survival, development time, fecundity), as well as vector competence (ability to harbor and transmit a pathogen) and pathogen replication rate, all exhibit nonlinear thermal responses. However, few studies have looked at the thermal responses of larval mosquito interactions, such as interspecific competition (Lounibos et al. 2002, Farjana et al. 2012).

We conducted a study to determine if temperature modulates competitive interactions among three vector mosquito species – *Culex quinquefasciatus*, *Aedes albopictus*, and *Ae. aegypti*. To answer this question, we first performed laboratory microcosm experiments to determine the effects of interspecific competition on vital rates across a range of temperatures (21-33°C). From these measured vital rates, we constructed estimates of intrinsic growth rate, to which we then fit curves to quantify the temperature range in which each species could maintain non-negative population growth ("thermal niche") under different competitive scenarios. We hypothesized that the effects of interspecific competition on vital rates and intrinsic growth rate would be stronger at either end of the temperature gradient (i.e., 21°C and 33°C) because vital rates would be more vulnerable to the effects of competition at extreme temperatures (Figure 1). Our results showed that temperature-dependent effects of interspecific larval competition on mosquito vital rates varied by target species and species identity of the competitor(s). Overall, interspecific competition with *Ae. aegypti* significantly narrow the thermal niche for population growth in both *Ae. albopictus* and *Cx. quinquefasciatus*. These findings provide evidence for temperature-dependent species interactions in larval mosquitoes, which may have important implications for predicting how vector communities and vector-borne disease transmission will respond to a changing climate.

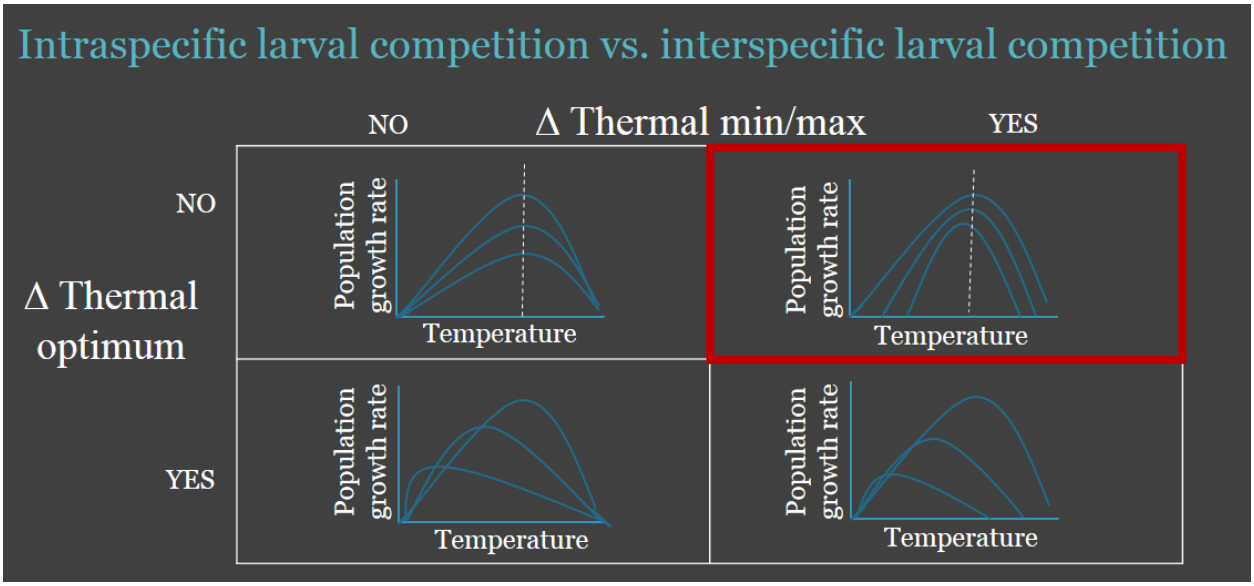

Figure 3.1: Diagram of potential effects of interspecific competition on mosquito population growth rate across a temperature gradient. In the top-left box, interspecific competition has no effect on neither the thermal extremes (minima or maxima) nor the thermal optimum of population growth. In the top-right box, interspecific competition alters population growth at the minimum and maximum, but has no effect on the thermal optimum. In the bottom-left box, interspecific competition alters the thermal optimum, but has no effect on the thermal minimum or maximum. Finally, the bottom-right box shows an effect of interspecific competition on both the thermal extremes and thermal optimum for population growth. We hypothesized that interspecific competition would alter growth rates at the thermal minimum and/or maximum (top-right, highlighted in red) because vital rates, like larval development and survival would, would be more vulnerable to the effects of competition at extreme temperatures.

Methods

To assess the effects of larval competition on the thermal niche of vector mosquitoes, we selected three species of ecological and medical importance. *Culex quinquefasciatus* is an abundant urban mosquito species known to be a highly competent vector of West Nile virus. The Asian tiger mosquito *Aedes albopictus* is an invasive species, introduced into the southern United States in the 1980s, and vector of Chikungunya, Dengue, and Zika viruses. Widely geographically distributed in the tropics and sub-tropics, *Aedes aegypti* is the primary vector of Yellow fever and Dengue viruses, as well as a competent vector of Chikungunya and Zika viruses. All three species prefer similar larval habitats – stagnant water in artificial containers, usually with high organic matter content – providing the potential to interact in the larval stage.

To quantify the temperature-dependent effects of interspecific competition on the vital rates, intrinsic growth rate, and thermal niche of these three species, we performed substitutive laboratory microcosm experiments across a wide, yet realistic, temperature gradient (21-33°C with 3° increments). Our experimental design consisted of all combinations of the three species (e.g., one (30 individuals), two (15 individuals each), and three (10 individuals each) species), with additional species added to maintain a total density of 30 individuals, for a total of seven treatments (Figure 2). This design was replicated five times at each temperature.

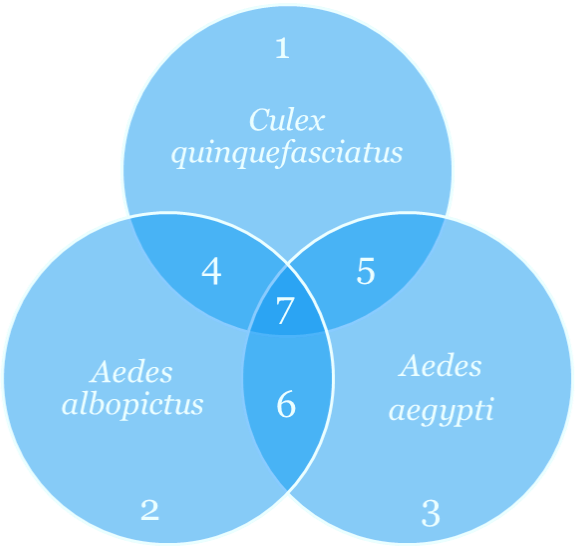

Figure 3.2: Conceptual diagram of factorial experimental design. All combinations of one, two, and three species were used for a total of seven treatments (corresponding to numbers in diagram). Each treatment included 30 individuals total, and species were added to the design in a substitutive manor (e.g., 30 individuals of species 1, 15 individuals each of species 1 and 2, or 10 individuals each of species 1, 2, and 3). Each treatment was replicated five times at each temperature (gradient from 21-33°C with treatments every 3°C).

Larvae were synchronously hatched approximately 24 hours prior to the start of the experiment from live (*Cx. quinquefasciatus*) or dried (*Aedes* spp.) egg rafts obtained from laboratory-maintained colonies at the University of Georgia. Larvae were placed in BioQuip Mosquito Breeders with 200 mL of de-ionized water and 0.1g ground fish flake food suspended in 10 mL of de-ionized water. Microcosms were placed in randomized locations within programmable Percival incubators set to a static temperature with a 16L:8D cycle. Experiments were monitored for 21 days. Emerged adults were removed every 24 hours and frozen for further data collection.

To estimate the intrinsic growth rate for each treatment, we measured individual and population-level vital rates: larval development time (days from experimental setup to emergence), adult body size (abdominal length from the metapostnotum to the end of the cerci), and egg-to-adult survival. We individually photographed each emerged adult using a dissecting microscope with an integrated camera to confirm the species and sex of each individual, as well as to measure body size. We used these vital rates to estimate the intrinsic growth rate for each treatment, using the Livdahl-Sugihara model (Livdahl and Sugihara 1984):

$$\hat{r} = \frac{ln\frac{1}{N_0}\sum_x A_x f(\bar{w}_x)}{D+\frac{\sum_x xA_x f(\bar{w}_x)}{\sum_x A_x f(\bar{w}_x)}} \quad \text{(Eq. 1)}$$

where N_0 is the initial number of female larvae (assumed to be half of total); A_x is the number of females emerging on day x; D is the time from emergence to first reproduction; and $f(w_x)$ is an empirically estimated allometric function relating the average body size of females emerging on day x to fecundity (number of eggs). Species-specific allometric fecundity functions were taken from the primary literature:

for *Cx. quinquefasciatus* (McCann et al. 2009),

$$f(w_x) = 90.31w_x - 123.88 \quad \text{(Eq. 2);}$$

for *Ae. albopictus* (Farjana and Tuno 2012),

$$f(w_x) = 104.8w_x - 201.37 \quad \text{(Eq. 3);}$$

and for *Ae. aegypti* (Farjana and Tuno 2012),

$$f(w_x) = 79.30w_x - 144.08 \quad \text{(Eq. 4).}$$

To determine if the effects of interspecific competition on vital rates and intrinsic growth rate varied with temperature, we calculated the effect size (standardized mean difference with pooled standard deviation, Hedges' g) of each treatment relative to intraspecific competition.

To estimate the thermal niche of each species under intraspecific and interspecific competition, we fit a Briére curve to intrinsic growth rate estimates for each treatment across a temperature gradient. For this study, we defined a species' thermal niche as the range of temperatures over which a species exhibits non-negative intrinsic growth. The Briére (Briere et al. 1999) function,

$$y = cT(T - T_0)(T_m - T)^{1/2} \quad \text{(Eq. 5),}$$

where T_0 is the thermal minimum, T_m is the thermal maximum, and c is a scaling parameter, is similar to a quadratic function, but with a long left tail that provides a better fit to temperature-dependent response variables. To quantify uncertainty around each thermal niche, we also fit Briére curves to 10,000 bootstraps of intrinsic growth rate estimates for each treatment.

Results

Out of 5,250 first-instar larvae, 3,717 adults eclosed; of those, nine were damaged during storage or processing (unable to identify species, sex, or body size) and were excluded from analyses. Of the remaining 3,708 individuals, 1,786 were female (Table 1). We omitted males from this analysis since their vital rates do not contribute the growth rate of the population, nor are they of public health importance as they do not blood-feed.

Table 3.1: Total eclosed adults, stratified by species and sex. Only data on female vital rates were used for analysis.

Species	Sex	21°C	24°C	27°C	30°C	33°C	Total
Ae. aegypti	F	151	170	157	157	129	764
	M	152	153	157	159	149	770
Ae. albopictus	F	86	128	133	125	93	565
	M	122	139	129	146	111	647
Cx. quinquefasciatus	F	83	119	127	110	0	439
	M	117	138	143	124	1	523
Total		711	847	846	821	483	3708

Mean *Cx. quinquefasciatus* development time ranged from 7.89 (± 0.14) days at 30°C to 16.40 (± 0.51) days at 21°C (Figure 3, top-left; Table 2). Competition with *Ae. aegypti* alone significantly increased *Cx. quinquefasciatus* development time at 21°C (Hedges' g = 0.68, p = 0.04), as did multi-species competition at the same temperature (Hedges' g = 0.63, p = 0.05). Competition with *Ae. albopictus* had no significant effect on *Cx. quinquefasciatus* development time (Table 3).

Mean *Cx. quinquefasciatus* body size ranged from 2.293 (± 0.047) mm at 21°C to 3.042 (± 0.037) mm at 27°C (Figure 3, middle-left; Table 2). Competition with *Ae. albopictus* significantly increased *Cx. quinquefasciatus* body size at 24°C (Hedges' g = 0.56, p = 0.04) and 27°C (Hedges' g = 0.47, p = 0.04). Competition with *Ae. aegypti* significantly decreased *Cx. quinquefasciatus* body size at 21°C (Hedges' g = -0.76, p = 0.02), 27°C (Hedges' g = -0.87, p < 0.01), and 30°C (Hedges' g = -0.89, p < 0.01). Multi-species competition significantly decreased *Cx. quinquefasciatus* body size at 21°C (Hedges' g = -0.78, p = 0.02) and 30°C (Hedges' g = -0.78, p = 0.01).

Mean *Cx. quinquefasciatus* egg-to-adult survival ranged from 0.347 (± 0.078) at 21°C to 0.827 (± 0.062) at 24°C (Figure 3, bottom-left; Table 2). Competition with *Ae. aegypti* significantly decreased *Cx. quinquefasciatus* egg-to-adult survival at 30°C (Hedges' g = -0.57, p = 0.01). Neither multi-species competition nor competition with only *Ae. albopictus* had a significant effect on *Cx. quinquefasciatus* egg-to-adult survival (Table 3).

Mean *Cx. quinquefasciatus* intrinsic growth rate ranged from 0.125 (± 0.008) at 21°C to 0.218 (± 0.005) at 30°C (Figure 6, top-left; Table 2). There were no significant effects of interspecific competition on *Cx. quinquefasciatus* intrinsic growth rate at any temperature (Figure 6, top-right; Table 3).

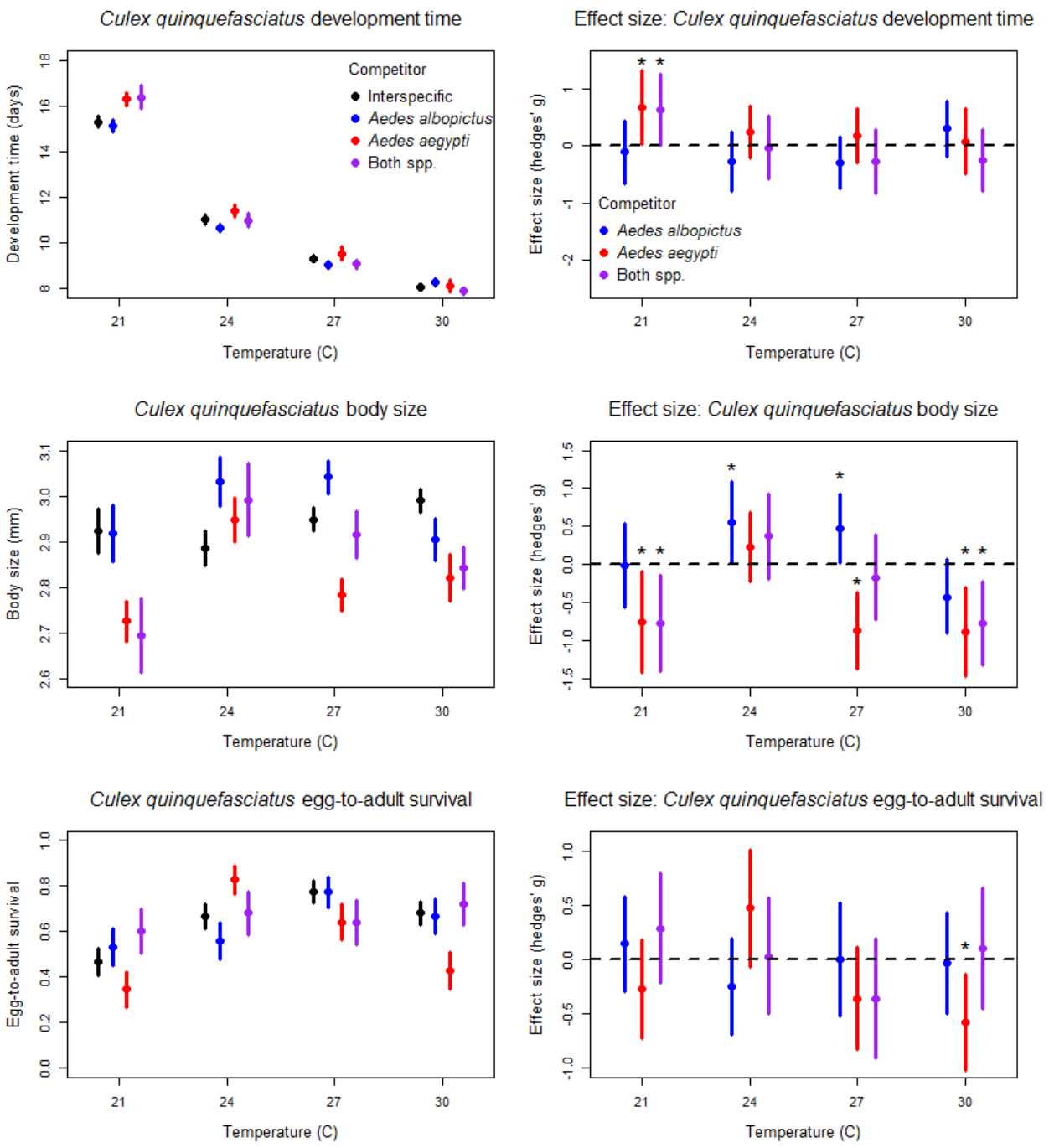

Figure 3.3: Mean and standard error (left) and effect size and standard error (right) of *Culex quinquefasciatus* development time, body size, and egg-to-adult survival.

Table 3.2: Mean and standard error of *Cx. quinquefasciatus* vital rates (*days; **millimeters).

Competitor	Temp. (°C)	N	Mean development time* (± SE)	Mean body size** (± SE)	Mean egg-to-adult survival (± SE)	Mean intrinsic growth rate (± SE)
Intraspecific	21	35	15.31 (0.26)	2.293 (0.047)	0.467 (0.058)	0.147 (0.005)
	24	50	11.04 (0.21)	2.886 (0.037)	0.667 (0.054)	0.187 (0.004)
	27	58	9.33 (0.14)	2.948 (0.026)	0.773 (0.048)	0.210 (0.006)
	30	51	8.06 (0.09)	2.990 (0.026)	0.680 (0.054)	0.218 (0.005)
	33	0	n/a	n/a	n/a	n/a
Ae. albopictus	21	20	15.15 (0.27)	2.918 (0.062)	0.533 (0.081)	0.151 (0.006)
	24	21	10.67 (0.17)	3.031 (0.053)	0.560 (0.081)	0.182 (0.012)
	27	29	9.03 (0.14)	3.042 (0.037)	0.773 (0.068)	0.214 (0.008)
	30	25	8.28 (0.16)	2.905 (0.045)	0.667 (0.077)	0.211 (0.007)
	33	0	n/a	n/a	n/a	n/a
Ae. aegypti	21	13	16.31 (0.31)	2.726 (0.044)	0.347 (0.078)	0.125 (0.008)
	24	31	11.42 (0.29)	2.947 (0.049)	0.827 (0.062)	0.193 (0.009)
	27	24	9.54 (0.32)	2.782 (0.035)	0.640 (0.078)	0.188 (0.015)
	30	16	8.13 (0.26)	2.820 (0.051)	0.427 (0.081)	0.185 (0.013)
	33	0	n/a	n/a	n/a	n/a
Both spp.	21	15	16.40 (0.51)	2.694 (0.080)	0.600 (0.098)	0.146 (0.002)
	24	17	11.00 (0.31)	2.992 (0.080)	0.680 (0.093)	0.190 (0.006)
	27	16	9.06 (0.17)	2.914 (0.051)	0.640 (0.096)	0.202 (0.006)
	30	18	7.89 (0.14)	2.843 (0.046)	0.720 (0.090)	0.215 (0.010)
	33	0	n/a	n/a	n/a	n/a

Table 3.3: Effect sizes with 95% CIs and p-values for *Cq. quinquefasciatus* vital rates at each temperature treatment. Effect sizes were calculated as the standardized mean difference with pooled standard deviation (Hedges' *g*) between vital rates under intraspecific vs. interspecific competition.

Competitor	Temperature	Development time	Body size	Egg-to-adult survival	Intrinsic growth rate
Ae. albopictus	21	-0.11 (-0.67, 0.44) p = 0.69	-0.02 (-0.57, 0.53) p = 0.94	0.15 (-0.29, 0.58) p = 0.51	0.33 (-1.00, 1.66) p = 0.58
	24	-0.27 (-0.79, 0.24) p = 0.29	0.56 (0.04, 1.08) p = 0.04	-0.25 (-0.69, 0.20) p = 0.27	-0.26 (-1.58, 1.07) p = 0.67
	27	-0.30 (-0.75, 0.15) p = 0.18	0.47 (0.02, 0.93) p = 0.04	0.00 (-0.52, 0.52) p = 1.00	0.25 (-1.07, 1.57) p = 0.67
	30	0.31 (-0.18, 0.79) p = 0.21	-0.43 (-0.91, 0.06) p = 0.08	-0.03 (-0.50, 0.43) p = 0.89	-0.40 (-1.73, 0.93) p = 0.51
Ae. aegypti	21	0.68 (0.02, 1.33) p = 0.04	-0.76 (-1.43, -0.10) p = 0.02	-0.27 (-0.72, 0.18) p = 0.23	-1.33 (-2.81, 0.16) p = 0.07
	24	0.24 (-0.21, 0.70) p = 0.29	0.23 (-0.23, 0.68) p = 0.32	0.48 (-0.06, 1.01) p = 0.08	0.32 (-1.01, 1.64) p = 0.60
	27	0.18 (-0.30, 0.65) p = 0.47	-0.87 (-1.37, -0.37) p < 0.01	-0.36 (-0.83, 0.12) p = 0.14	-0.77 (-2.15, 0.60) p = 0.23
	30	0.08 (-0.48, 0.65) p = 0.77	-0.89 (-1.47, -0.30) p < 0.01	-0.57 (-1.02, -0.13) p = 0.01	-1.35 (-2.83, 0.14) p = 0.07
Both spp.	21	0.63 (0.01, 1.26) p = 0.05	-0.78 (-1.41, -0.15) p = 0.02	0.29 (-0.21, 0.80) p = 0.25	-0.05 (-1.37, 1.27) p = 0.93
	24	-0.03 (-0.58, 0.53) p = 0.92	0.37 (-0.19, 0.93) p = 0.19	0.03 (-0.50, 0.57) p = 0.90	0.21 (-1.11, 1.54) p = 0.72
	27	-0.27 (-0.83, 0.29) p = 0.34	-0.17 (-0.73, 0.39) p =0.54	-0.36 (-0.90, 0.19) p = 0.20	-0.55 (-1.89, 0.80) p = 0.38
	30	-0.26 (-0.80, 0.29) p = 0.35	-0.78 (-1.33, -0.22) p = 0.01	0.10 (-0.45, 0.66) p = 0.71	-0.15 (-1.47, 1.17) p = 0.80

Mean *Ae. albopictus* development time ranged from 7.67 (± 0.24) days at 33°C to 19.04 (± 0.14) days at 21°C (Figure 4, top-left; Table 4). Competition with *Cx. quinquefasciatus* significantly increased *Ae. albopictus* development time at 24°C (Hedges' g = 0.56, p = 0.03). Competition with *Ae. aegypti* significantly decreased *Ae. albopictus* development time at 21°C (Hedges' g = -0.94, p = 0.01) and significantly increased development time at 24°C (Hedges' g = 0.48, p = 0.03). Multi-species competition did not have a significant effect on *Ae. albopictus* development time at any temperature (Figure 4, top-right; Table 5).

Mean *Ae. albopictus* body size ranged from 2.415 (± 0.043) mm at 21°C to 2.994 (± 0.032) mm at 27°C (Figure 4, middle-left; Table 4). Competition with *Cx. quinquefasciatus* significantly decreased *Ae. albopictus* body size 24°C (Hedges' g = -0.91, p < 0.01) and 27°C (Hedges' g = -0.55, p = 0.02), but significantly increased body size at 33°C (Hedges' g = 0.81, p = 0.01). Competition with *Ae. aegypti* significantly decreased *Ae. albopictus* body size at 24°C (Hedges' g = -0.58, p = 0.01) and 27°C (Hedges' g = -0.52, p = 0.03). Multi-species competition significantly decreased *Ae. albopictus* body size at 27°C (Hedges' g = -1.15, p < 0.01) only (Figure 4, middle-right; Table 5).

Mean *Ae. albopictus* egg-to-adult survival ranged from 0.240 (± 0.070) at 21°C to 0.920 (± 0.054) at 30°C (Figure 4, bottom-left; Table 4). Competition with *Cx. quinquefasciatus* significantly decreased *Ae. albopictus* egg-to-adult survival at 24°C (Hedges' g = -0.52, p = 0.03). Competition with *Ae. aegypti* significantly decreased *Ae. albopictus* egg-to-adult survival at 21°C (Hedges g = -0.98, p < 0.01), but had no significant effect at higher temperatures (Figure 4, bottom-right; Table 5). Multi-species competition significantly decreased *Ae. albopictus* egg-to-adult survival at 21°C (Hedges' g = -0.66, p = 0.01) and significantly increased egg-to-adult survival at 30°C (Hedges' g = 1.02, p = 0.02).

Mean *Ae. albopictus* intrinsic growth rate ranged from 0.070 (± 0.013) at 21°C to 0.186 (± 0.006) at 27°C (Figure 6, middle-left; Table 4). Competition with *Cx. quinquefasciatus* had no significant effect on *Ae. albopictus* intrinsic growth rate at any temperature (Figure 6, middle-right; Table 5). Competition with *Ae. aegypti* significantly decreased *Ae. albopictus* intrinsic growth rate at 21°C (Hedges' g = -1.85, p = 0.03). Multi-species competition also significantly decreased *Ae. albopictus* intrinsic growth rate at 21°C (Hedges' g = -1.74, p = 0.05).

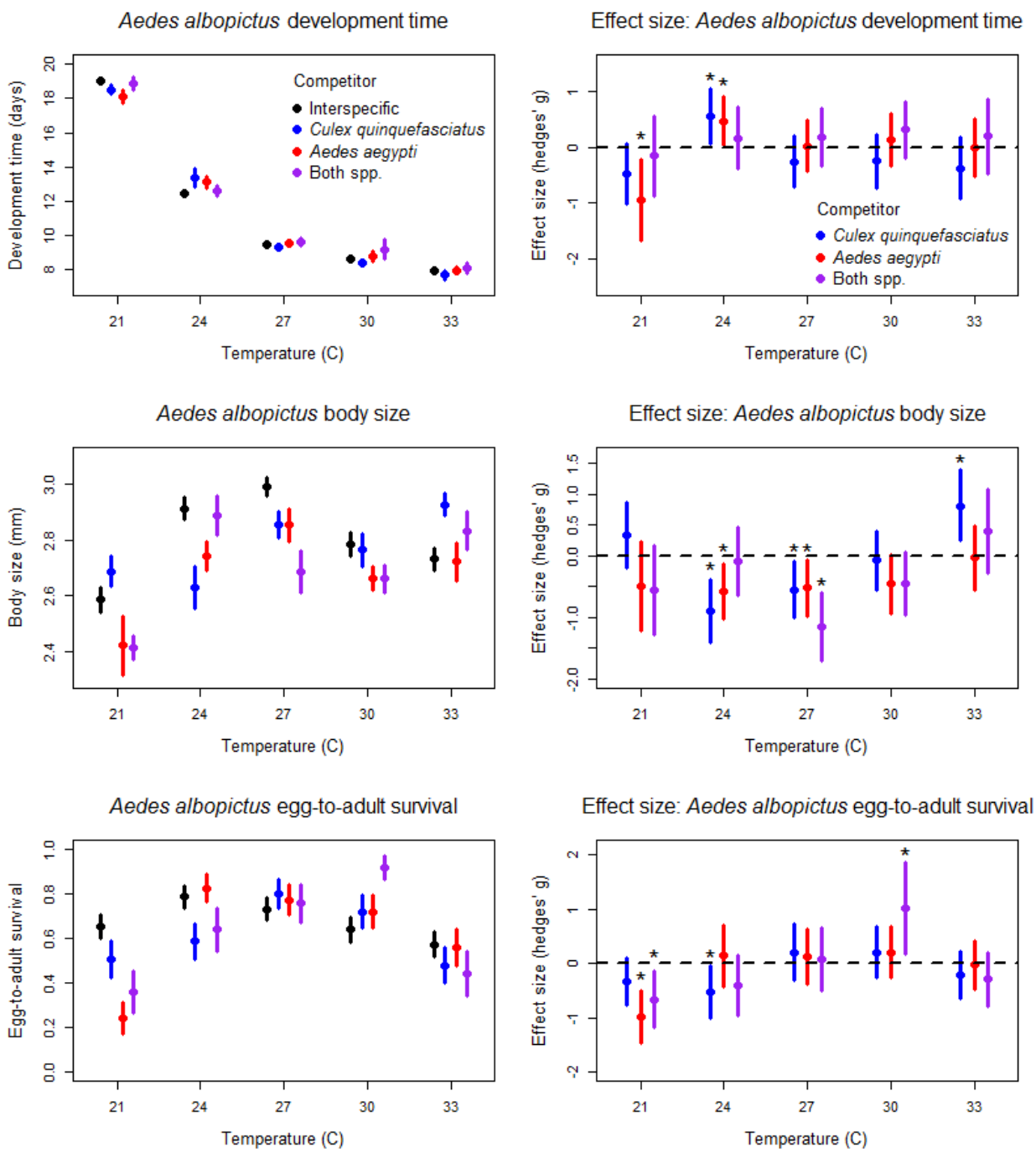

Figure 3.4: Mean and standard error (left) and effect size and standard error (right) of *Aedes albopictus* development time, body size, and egg-to-adult survival.

Table 3.4: Mean and standard error of *Ae. albopictus* vital rates (*days; **millimeters).

Competitor	Temp. (°C)	N	Mean development time* (± SE)	Mean body size** (± SE)	Mean egg-to-adult survival (± SE)	Mean intrinsic growth rate (± SE)
Intraspecific	21	49	19.04 (0.14)	2.586 (0.047)	0.653 (0.055)	0.115 (0.004)
	24	59	12.42 (0.15)	2.914 (0.039)	0.787 (0.047)	0.167 (0.004)
	27	55	9.49 (0.09)	2.994 (0.032)	0.733 (0.051)	0.186 (0.006)
	30	48	8.60 (0.16)	2.786 (0.042)	0.640 (0.055)	0.179 (0.005)
	33	43	7.95 (0.09)	2.732 (0.040)	0.573 (0.057)	0.176 (0.005)
Cx. quinquefasciatus	21	19	18.53 (0.29)	2.689 (0.055)	0.507 (0.082)	0.113 (0.006)
	24	22	13.36 (0.55)	2.629 (0.076)	0.587 (0.080)	0.135 (0.012)
	27	30	9.33 (0.10)	2.856 (0.049)	0.800 (0.065)	0.185 (0.007)
	30	27	8.37 (0.13)	2.765 (0.058)	0.720 (0.073)	0.184 (0.009)
	33	18	7.67 (0.24)	2.929 (0.039)	0.480 (0.082)	0.180 (0.007)
Ae. aegypti	21	9	18.11 (0.35)	2.422 (0.106)	0.240 (0.070)	0.070 (0.013)
	24	31	13.10 (0.31)	2.741 (0.052)	0.827 (0.062)	0.155 (0.009)
	27	29	9.52 (0.17)	2.855 (0.056)	0.773 (0.068)	0.183 (0.006)
	30	27	8.78 (0.29)	2.662 (0.043)	0.720 (0.073)	0.175 (0.006)
	33	21	7.95 (0.80)	2.723 (0.069)	0.560 (0.081)	0.171 (0.011)
Both spp.	21	9	18.89 (0.39)	2.415 (0.043)	0.360 (0.096)	0.093 (0.007)
	24	16	12.63 (0.30)	2.888 (0.070)	0.640 (0.096)	0.154 (0.008)
	27	19	9.63 (0.23)	2.687 (0.076)	0.760 (0.085)	0.167 (0.012)
	30	23	9.17 (0.56)	2.661 (0.050)	0.920 (0.054)	0.185 (0.008)
	33	11	8.09 (0.28)	2.834 (0.067)	0.440 (0.099)	0.164 (0.012)

Table 3.5: Effect sizes with 95% CIs and p-values for *Ae. albopictus* vital rates at each temperature treatment. Effect sizes were calculated as the standardized mean difference with pooled standard deviation (Hedges' *g*) between vital rates under intraspecific vs. interspecific competition.

Competitor	Temperature	Development time	Body size	Egg-to-adult survival	Intrinsic growth rate
Cx. quinqs.	21	-0.48 (-1.02, 0.06) p = 0.08	0.33 (-0.20, 0.87) p = 0.22	-0.33 (-0.77, 0.11) p = 0.14	-0.20 (-1.52, 1.13) p = 0.74
	24	0.56 (0.06, 1.06) p = 0.03	-0.90 (-1.41, -0.38) p < 0.01	-0.52 (-1.00, -0.05) p = 0.03	-1.43 (-2.93. 0.08) p = 0.06
	27	-0.25 (-0.70, 0.20) p = 0.27	-0.55 (-1.01, -0.10) p = 0.02	0.21 (-0.32, 0.73) p = 0.44	-0.11 (-1.43, 1.21) p = 0.85
	30	-0.24 (-0.72, 0.24) p = 0.32	-0.07 (-0.55, 0.40) p = 0.77	0.20 (-0.27, 0.68) p = 0.40	0.27 (-1.05, 1.59) p = 0.65
	33	-0.37 (-0.93, 0.19) p = 0.19	0.81 (0.24, 1.39) p = 0.01	-0.21 (-0.64, 0.23) p = 0.35	0.30 (-1.03, 1.62) p = 0.62
Ae. aegypti	21	-0.94 (-1.68, -0.21) p = 0.01	-0.50 (-1.22, 0.23) p = 0.17	-0.98 (-1.47, -0.49) p < 0.01	-1.85 (-3.48, -0.23) p = 0.03
	24	0.48 (0.04, 0.92) p = 0.03	-0.58 (-1.03, -0.13) p = 0.01	0.14 (-0.42, 0.70) p = 0.62	-0.72 (-2.09, 0.65) p = 0.26
	27	0.03 (-0.42, 0.49) p = 0.88	-0.52 (-0.98, -0.06) p = 0.03	0.12 (-0.39, 0.63) p = 0.65	-0.26 (-1.59, 1.06) p = 0.66
	30	0.14 (-0.34, 0.61) p = 0.57	-0.46 (-0.94, 0.02) p = 0.06	0.20 (-0.27, 0.68) p = 0.40	-0.27 (-1.59, 1.06) p = 0.65
	33	0.00 (-0.53, 0.53) p = 1.00	-0.03 (-0.56, 0.49) p = 0.90	-0.03 (-0.47, 0.41) p = 0.89	-0.24 (-1.56, 1.08) p = 0.69
Both spp.	21	-0.15 (-0.87, 0.57) p = 0.67	-0.55 (-1.28, 0.17) p = 0.13	-0.66 (-1.18, -0.14) p = 0.01	-1.74 (-3.46, -0.03) p = 0.05
	24	0.17 (-0.39, 0.73) p = 0.55	-0.09 (-0.64, 0.47) p = 0.75	-0.40 (-0.95, 0.15) p = 0.15	-0.88 (-2.28. 0.51) p = 0.18
	27	0.18 (-0.34, 0.71) p = 0.49	-1.15 (-1.71, -0.59) p < 0.01	0.08 (-0.50, 0.66) p = 0.79	-0.82 (-2.21, 0.56) p = 0.21
	30	0.32 (-0.19, 0.82) p = 0.21	-0.45 (-0.96, 0.05) p = 0.08	1.02 (0.18, 1.86) p = 0.02	0.39 (-0.95, 1.72) p = 0.52
	33	0.20 (-0.47, 0.87) p = 0.56	0.39 (-0.28, 1.07) p = 0.24	-0.29 (-0.80, 0.21) p = 0.25	-0.52 (-1.86, 0.83) p = 0.40

Mean *Ae. aegypti* development time ranged from 6.06 (± 0.06) days at 33°C to 16.75 (± 0.16) days at 21°C (Figure 5, top-left; Table 6). Competition with *Cx. quinquefasciatus* significantly decreased *Ae. aegypti* development time at 21°C (Hedges' g = -0.61, p = 0.01), but had no significant effect at higher temperatures (Figure 5, top-right; Table 7). Competition with *Ae. albopictus* significantly decreased *Ae. aegypti* development time at 21°C (Hedges' g = -1.11, p < 0.01) and 24°C (Hedges' g = -0.55, p = 0.01). Multi-species competition decreased *Ae. aegypti* development time at 21°C (Hedges' g = -0.93, p < 0.01), but had no significant effect at higher temperatures.

Mean *Ae. aegypti* body size ranged from 2.923 (± 0.044) mm at 21°C to 3.397 (± 0.028) mm at 27°C (Figure 5, middle-left; Table 6). Competition with *Cx. quinquefasciatus* significantly increased *Ae. aegypti* body size at 27°C (Hedges' g = 0.83, p < 0.01) and 30°C (Hedges' g = 0.83, p < 0.01). Competition with *Ae. albopictus* significantly increased *Ae. aegypti* body size at 24°C (Hedges' g = 0.91, p < 0.01), 27°C (Hedges' g = 0.51, p = 0.02), and 33°C (Hedges' g = 0.66, p = 0.01). Multi-species competition significantly increased *Ae. aegypti* body size at 27°C (Hedges' g = 0.88, p < 0.01), 30°C (Hedges' g = 0.87, p < 0.01), and 33°C (Hedges' g = 0.64, p = 0.02). Interspecific competition has no effect on *Ae. aegypti* body size at 21°C (Figure 5, middle-right; Table 7).

Mean *Ae. aegypti* egg-to-adult survival ranged from 0.613 (± 0.080) at 27°C to 100% survival in multiple treatments at both 24°C and 27°C (Figure 5, bottom-left; Table 6). Due to the lack of variation in survival at these temperatures, we were unable to calculate effect sizes for all treatments. Of the effect sizes we were able to calculate (21°C, 30°C, and 33°C), none were statistically significant (Figure 5, bottom-right; Table 7).

Mean *Ae. aegypti* intrinsic growth rate ranged from 0.152 (± 0.003) at 21°C to 0.246 (± 0.005) at 30°C (Figure 6, bottom-left; Table 6). Interspecific competition had no significant effect on *Ae. aegypti* intrinsic growth rate at any temperature (Figure 6, bottom-right; Table 7).

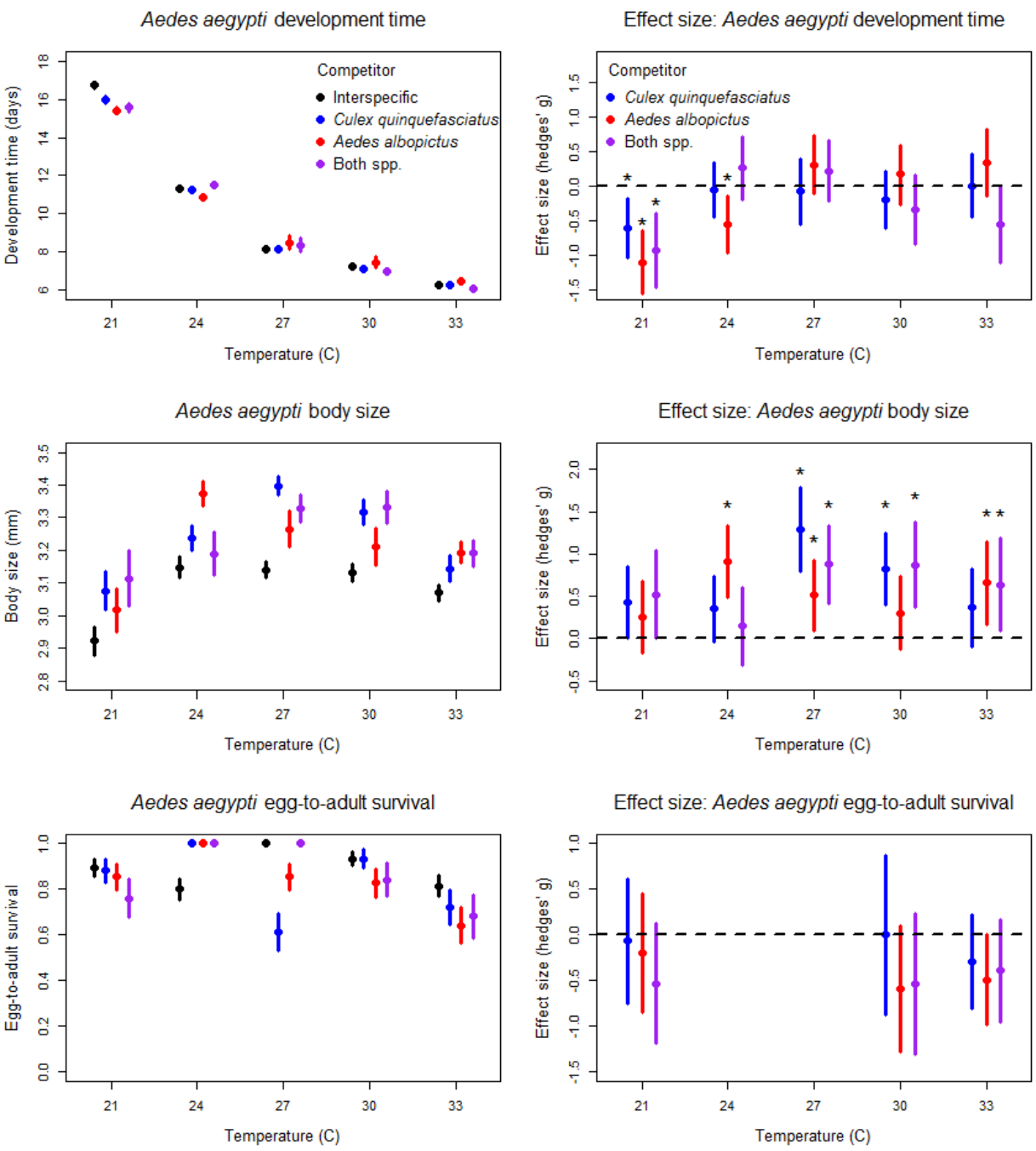

Figure 3.5: Mean and standard error (left) and effect size and standard error (right) of *Aedes aegypti* development time, body size, and egg-to-adult survival.

Table 3.6: Mean and standard error of *Ae. aegypti* vital rates (*days; **millimeters).

Competitor	Temp. (°C)	N	Mean development time* (± SE)	Mean body size** (± SE)	Mean egg-to-adult survival (± SE)	Mean intrinsic growth rate (± SE)
Intraspecific	21	67	16.75 (0.16)	2.923 (0.044)	0.893 (0.036)	0.152 (0.003)
	24	60	11.32 (0.10)	3.147 (0.033)	0.800 (0.046)	0.189 (0.005)
	27	75	8.16 (0.04)	3.140 (0.024)	1.00 (0.000)	0.230 (0.004)
	30	70	7.26 (0.10)	3.132 (0.027)	0.933 (0.029)	0.237 (0.005)
	33	61	6.30 (0.06)	3.070 (0.025)	0.813 (0.045)	0.239 (0.005)
Cx. quinquefasciatus	21	33	15.97 (0.20)	3.076 (0.060)	0.880 (0.053)	0.159 (0.006)
	24	44	11.27 (0.10)	3.237 (0.039)	1.00 (0.000)	0.188 (0.029)
	27	23	8.13 (0.07)	3.397 (0.028)	0.613 (0.080)	0.214 (0.006)
	30	35	7.11 (0.08)	3.317 (0.036)	0.933 (0.041)	0.246 (0.005)
	33	27	6.30 (0.12)	3.144 (0.039)	0.720 (0.073)	0.231 (0.013)
Ae. albopictus	21	32	15.41 (0.16)	3.018 (0.067)	0.853 (0.058)	0.159 (0.005)
	24	39	10.90 (0.11)	3.374 (0.038)	1.00 (0.000)	0.209 (0.009)
	27	32	8.50 (0.35)	3.266 (0.054)	0.853 (0.058)	0.216 (0.020)
	30	31	7.45 (0.30)	3.210 (0.057)	0.827 (0.062)	0.233 (0.005)
	33	24	6.46 (0.10)	3.193 (0.033)	0.640 (0.078)	0.228 (0.007)
Both spp.	21	19	15.58 (0.22)	3.114 (0.086)	0.760 (0.085)	0.156 (0.007)
	24	27	11.52 (0.15)	3.190 (0.067)	1.00 (0.000)	0.202 (0.007)
	27	27	8.37 (0.34)	3.328 (0.041)	1.00 (0.000)	0.235 (0.013)
	30	21	7.00 (0.07)	3.331 (0.049)	0.840 (0.073)	0.236 (0.013)
	33	17	6.06 (0.06)	3.191 (0.039)	0.680 (0.093)	0.236 (0.008)

Table 3.7: Effect sizes with 95% CIs and p-values for *Ae. aegypti* vital rates at each temperature treatment. Effect sizes were calculated as the standardized mean difference with pooled standard deviation (Hedges' *g*) between vital rates under intraspecific vs. interspecific competition.

Competitor	Temperature	Development time	Body size	Egg-to-adult survival	Intrinsic growth rate
Cx. quinqs.	21	-0.61 (-1.04, -0.18) p = 0.01	0.43 (0.00, 0.85) p = 0.05	-0.07 (-0.75, 0.61) p = 0.83	0.54 (-0.81, 1.88) p = 0.39
	24	-0.06 (-0.45, 0.33) p = 0.76	0.35 (-0.04, 0.74) p = 0.08	n/a	-0.03 (-1.35, 1.28) p = 0.96
	27	-0.08 (-0.55, 0.39) p = 0.73	1.29 (0.79, 1.79) p < 0.01	n/a	-1.27 (-1.74, 0.20) p = 0.08
	30	-0.19 (-0.60, 0.22) p = 0.36	0.83 (0.40, 1.25) p < 0.01	0.00 (-0.87, 0.87) p = 1.00	0.72 (-0.65, 2.09) p = 0.26
	33	0.00 (-0.45, 0.46) p = 0.99	0.37 (-0.09, 0.83) p = 0.11	-0.29 (-0.80, 0.22) p = 0.26	-0.31 (-1.64, 1.01) p = 0.60
Ae. albopictus	21	-1.11 (-1.56, -0.65) p < 0.01	0.26 (-0.17, 0.68) p = 0.24	-0.20 (-0.84, 0.45) p = 0.54	0.74 (-0.63, 2.11) p = 0.25
	24	-0.55 (-0.97, -0.14) p = 0.01	0.91 (0.49, 1.34) p < 0.01	n/a	1.16 (-0.29, 2.61) p = 0.10
	27	0.30 (-0.11, 0.72) p = 0.15	0.51 (0.09, 0.93) p = 0.02	n/a	-0.40 (-1.73, 0.93) p = 0.51
	30	0.17 (-0.26, 0.59) p = 0.44	0.30 (-0.13, 0.73) p = 0.17	-0.59 (-1.28, 0.10) p = 0.09	-0.32 (-1.64, 1.01) p = 0.60
	33	0.34 (-0.14, 0.82) p = 0.16	0.66 (0.17, 1.14) p = 0.01	-0.49 (-0.98, 0.00) p = 0.05	-0.67 (-2.04, 0.69) p = 0.29
Both spp.	21	-0.93 (-1.46, -0.39) p < 0.01	0.52 (0.00, 1.04) p = 0.05	-0.53 (-1.18, 0.12) p = 0.11	0.26 (-1.06, 1.59) p = 0.66
	24	0.26 (-0.20, 0.71) p = 0.27	0.15 (-0.31, 0.61) p = 0.52	n/a	0.85 (-0.54, 2.24) p = 0.20
	27	0.22 (-0.22, 0.66) p = 0.33	0.88 (0.42, 1.33) p < 0.01	n/a	0.21 (-1.12, 1.53) p = 0.73
	30	-0.34 (-0.83, 0.16) p = 0.18	0.87 (0.37, 1.38) p < 0.01	-0.54 (-1.31, 0.24) p = 0.17	-0.03 (-1.35, 1.29) p = 0.96
	33	-0.55 (-1.10, 0.00) p = 0.05	0.64 (0.09, 1.19) p = 0.02	-0.39 (-0.96, 0.17) p = 0.17	-0.17 (-1.49, 1.15) p = 0.77

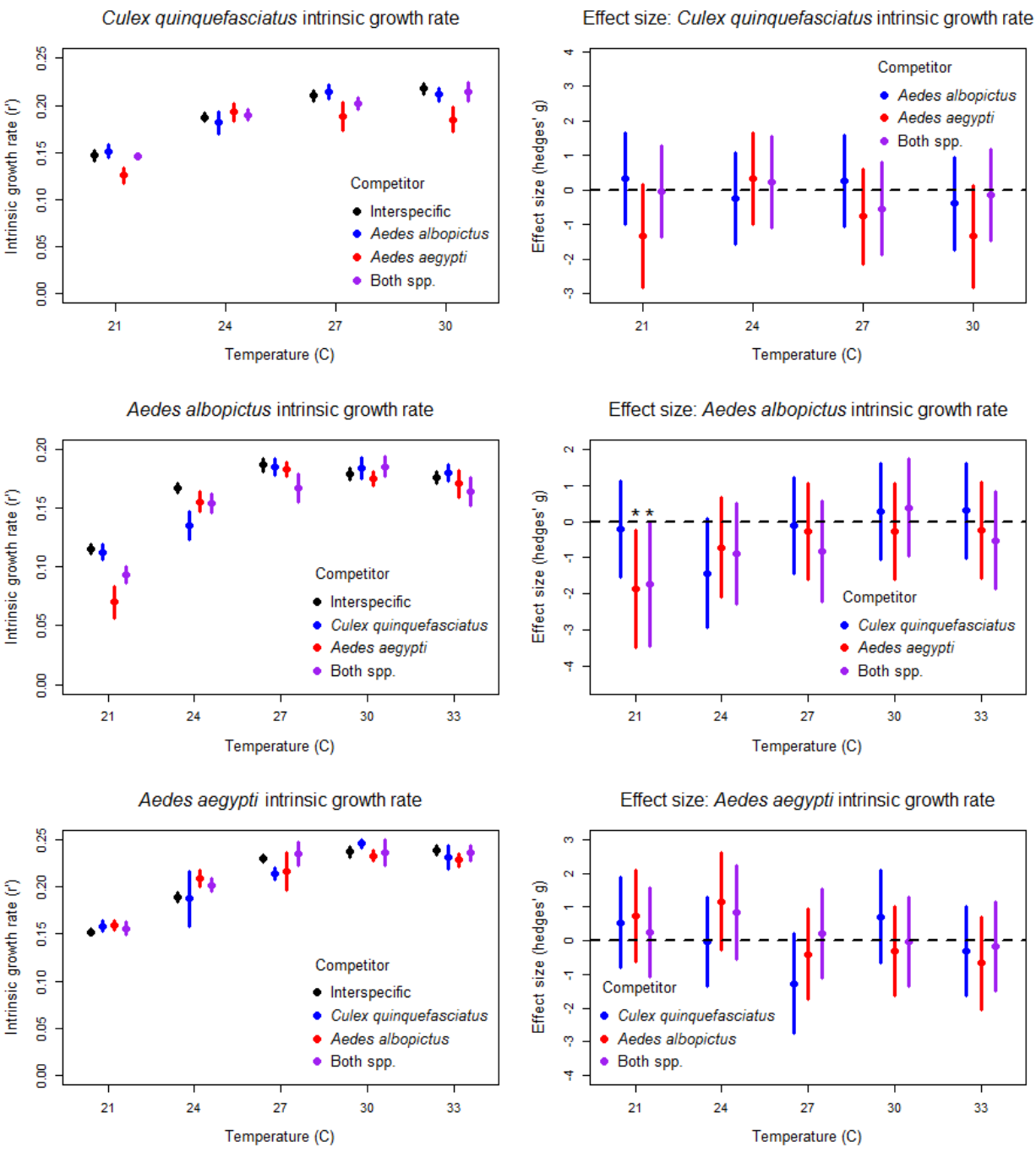

Figure 3.6: Mean and standard error (left) and effect size and standard error (right) of estimated intrinsic growth rates for each species.

The estimated intraspecific thermal niche of *Cx. quinquefasciatus* ranged from 11.26 (± 2.08) °C to 40.46 (± 2.11) °C, with a thermal optimum at 29.27 (± 0.007) °C (Table 8). Competition with *Ae. aegypti* significantly decreased the thermal maximum and thermal optimum, as well as increased the thermal minimum, of *Cx. quinquefasciatus'* thermal niche relative to intraspecific competition (Figure 7, Figure 8). The estimated intraspecific thermal niche of *Ae. albopictus* ranged from 12.75 (± 1.298) °C to 40.00 (± 0.913) °C, with a thermal optimum at 29.41 (± 0.003) °C (Table 9). Competition with *Cx. quinquefasciatus* significantly increased the thermal optimum of *Ae. albopictus'* thermal niche. Competition with *Ae. aegypti* significantly increase the thermal minimum and decreased the thermal maximum of *Ae. albopictus'* thermal niche relative to intraspecific competition (Figure 7, Figure 9). The estimated intraspecific thermal niche of *Ae. aegypti* ranged from 10.53 (± 1.440) °C to 43.47 (± 1.270) °C, with a thermal optimum of 31.12 (± 0.005) °C (Table 10). Competition with *Ae. albopictus*, as well as multi-species competition, significantly lowered the thermal optimum of *Ae. aegypti*'s thermal niche (Figure 7, Figure 10).

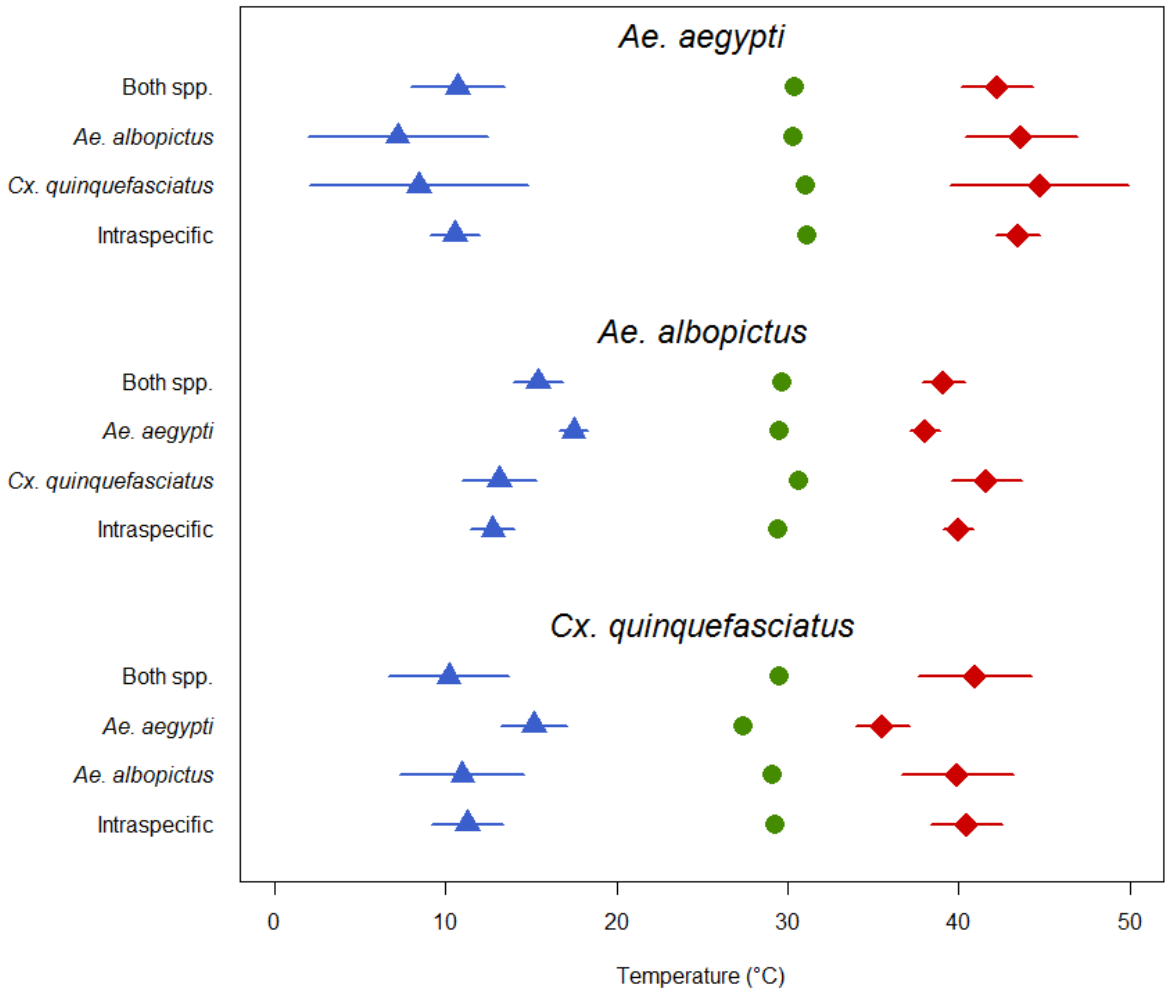

Figure 3.7: Estimated minimum (T_{min}, blue), optimum (T_{opt}, green), and maximum (T_{max}, red) temperature of each thermal niche with standard error bars.

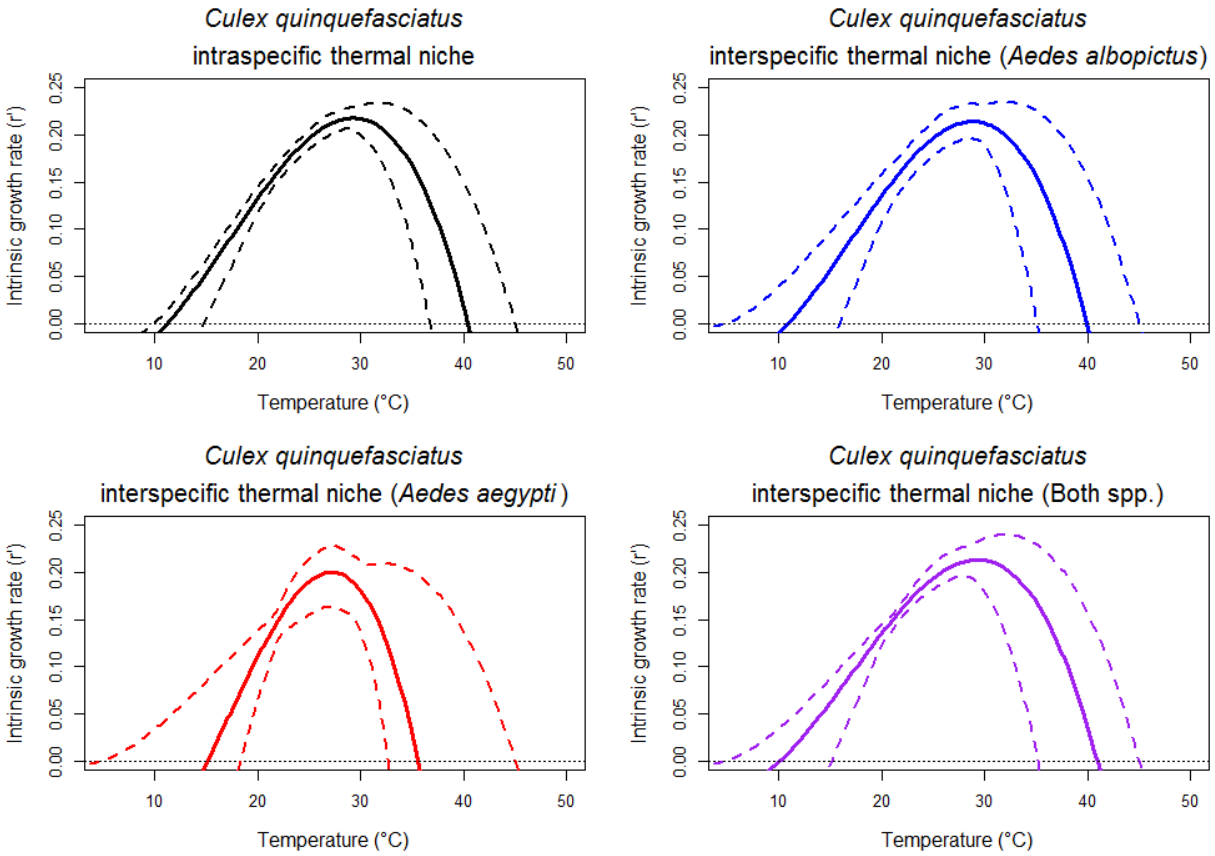

Figure 3.8: Estimated thermal niche of *Culex quinquefasciatus* under intraspecific and interspecific competition. Solid lines are Briére curves fitted to intrinsic growth rate estimates. Dashed lines are the minimum and maximum of curves fitted to 10,000 bootstrap samples of intrinsic growth rate estimates.

Table 3.8: Nonlinear least-squares parameter value estimates for each *Cx. quinquefasciatus* thermal niche, with standard errors for the scaling parameter (c), thermal minimum (T_{min}), and thermal maximum (T_{max}) in parentheses. The thermal optimum (T_{opt}) is the temperature at which estimated intrinsic growth rate is maximized. Mean T_{opt} and standard error were calculated from curves fit to 10,000 bootstrap samples of estimated intrinsic growth rates.

Competitor	c	T_{min}	T_{max}	T_{opt}
Intraspecific	7.395×10^{-5} ($\pm 2.195 \times 10^{-5}$)	11.26 ($\pm$ 2.08)	40.46 ($\pm$ 2.11)	29.27 ($\pm$ 0.007)
Ae. albopictus	7.503×10^{-5} ($\pm 3.726 \times 10^{-5}$)	10.98 ($\pm$ 3.65)	39.94 ($\pm$ 3.31)	29.13 ($\pm$ 0.012)
Ae. aegypti	1.464×10^{-4} ($\pm 5.416 \times 10^{-5}$)	15.16 ($\pm$ 1.94)	35.56 ($\pm$ 1.61)	27.39 ($\pm$ 0.008)
Both spp.	6.556×10^{-5} ($\pm 3.004 \times 10^{-5}$)	10.18 ($\pm$ 3.53)	40.93 ($\pm$ 3.31)	29.54 ($\pm$ 0.013)

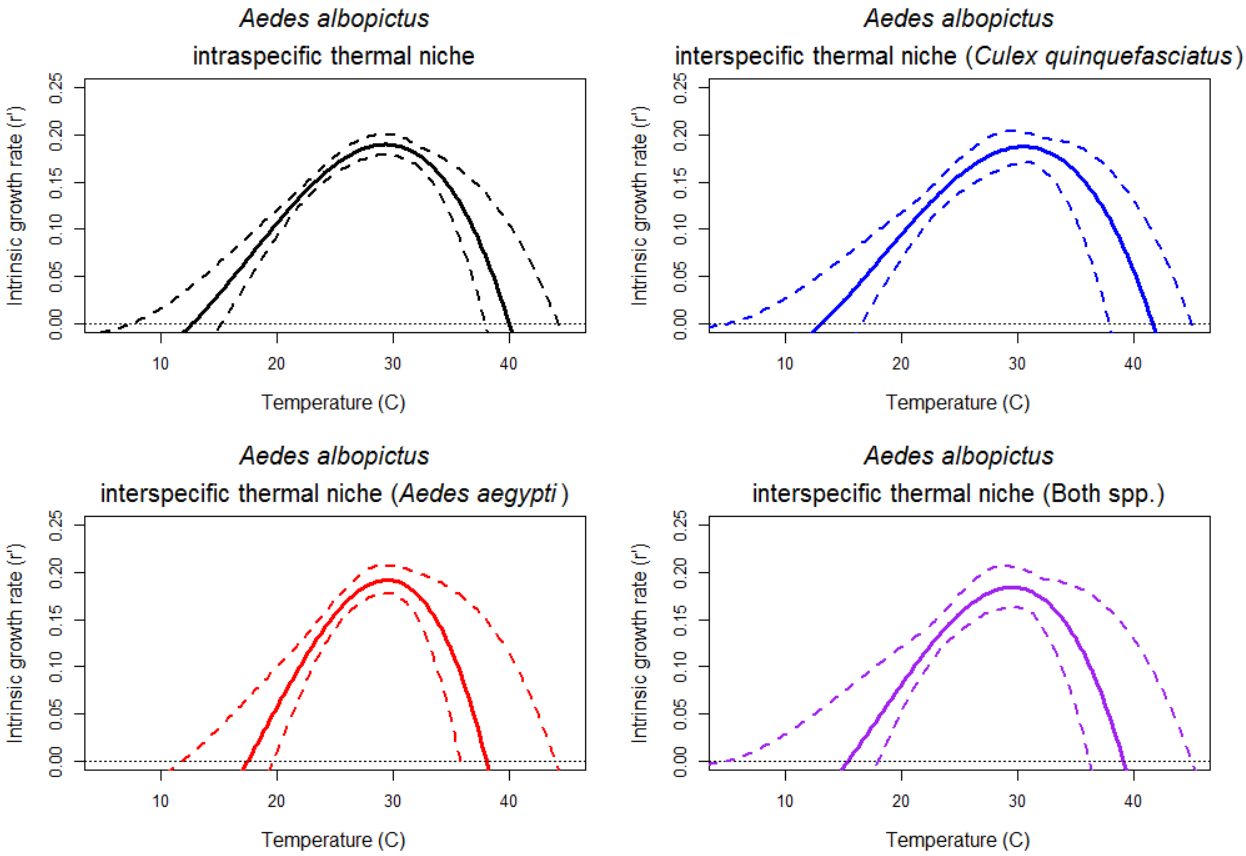

Figure 3.9: Estimated thermal niche of *Aedes albopictus* under intraspecific and interspecific competition. Solid lines are Briére curves fitted to intrinsic growth rate estimates. Dashed lines are the minimum and maximum of curves fitted to 10,000 bootstrap samples of intrinsic growth rate estimates.

Table 3.9: Nonlinear least-squares parameter value estimates for each *Ae. albopictus* thermal niche, with standard errors for the scaling parameter (c), thermal minimum (T_{min}), and thermal maximum (T_{max}) in parentheses. The thermal optimum (T_{opt}) is the temperature at which estimated intrinsic growth rate is maximized. Mean T_{opt} and standard error were calculated from curves fit to 10,000 bootstrap samples of estimated intrinsic growth rates.

Competitor	c	T_{min}	T_{max}	T_{opt}
Intraspecific	7.327×10^{-5} ($\pm 1.246 \times 10^{-5}$)	12.75 ($\pm$ 1.298)	40.00 ($\pm$ 0.913)	29.41 ($\pm$ 0.003)
Cx. quinquefasciatus	6.360×10^{-5} ($\pm 1.996 \times 10^{-5}$)	13.14 ($\pm$ 2.164)	41.64 ($\pm$ 2.092)	30.67 ($\pm$ 0.007)
Ae. aegypti	1.268×10^{-4} ($\pm 2.380 \times 10^{-5}$)	17.48 ($\pm$ 0.844)	38.04 ($\pm$ 0.926)	29.54 ($\pm$ 0.004)
Both spp.	9.204×10^{-5} ($\pm 2.279 \times 10^{-5}$)	15.40 ($\pm$ 1.480)	39.12 ($\pm$ 1.280)	29.66 ($\pm$ 0.006)

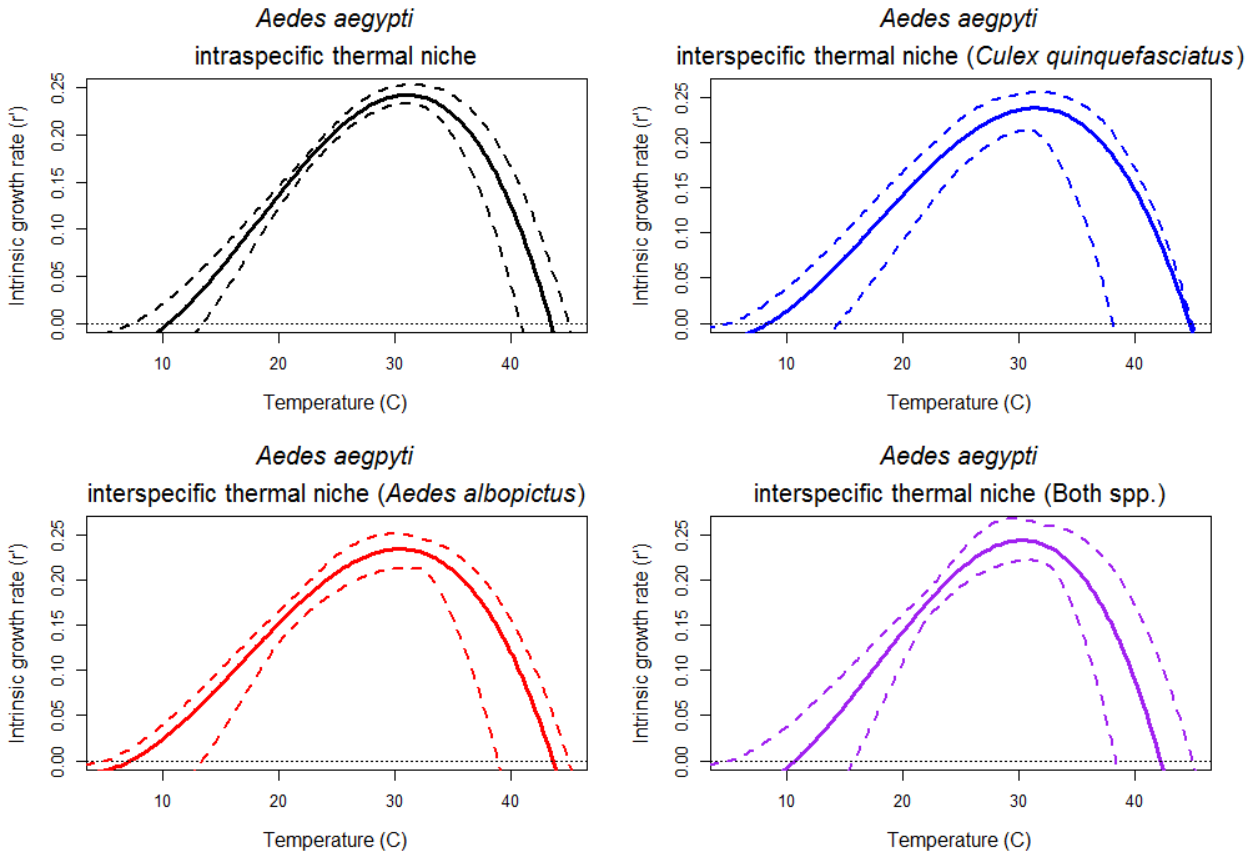

Figure 3.10: Estimated thermal niche of *Aedes aegypti* under intraspecific and interspecific competition. Solid lines are Briére curves fitted to intrinsic growth rate estimates. Dashed lines are the minimum and maximum of curves fitted to 10,000 bootstrap samples of intrinsic growth rate estimates.

Table 3.10: Nonlinear least-squares parameter value estimates for each *Ae. aegypti* thermal niche, with standard errors for the scaling parameter (c), thermal minimum (T_{min}), and thermal maximum (T_{max}) in parentheses. The thermal optimum (T_{opt}) is the temperature at which estimated intrinsic growth rate is maximized. Mean T_{opt} and standard error were calculated from curves fit to 10,000 bootstrap samples of estimated intrinsic growth rates.

Competitor	c	T_{min}	T_{max}	T_{opt}
Intraspecific	6.130×10^{-5} ($\pm 1.047 \times 10^{-5}$)	10.53 ($\pm$ 1.440)	43.47 ($\pm$ 1.270)	31.12 ($\pm$ 0.005)
Cx. quinquefasciatus	4.949×10^{-5} ($\pm 3.260 \times 10^{-5}$)	8.43 ($\pm$ 6.409)	44.73 ($\pm$ 5.200)	31.09 ($\pm$ 0.009)
Ae. albopictus	5.002×10^{-5} ($\pm 2.395 \times 10^{-5}$)	7.19 ($\pm$ 5.277)	43.66 ($\pm$ 3.296)	30.34 ($\pm$ 0.007)
Both spp.	6.875×10^{-5} ($\pm 2.201 \times 10^{-5}$)	10.70 ($\pm$ 2.763)	42.23 ($\pm$ 2.101)	30.39 ($\pm$ 0.006)

Discussion

Our results illustrate how interspecific competition can alter the thermal niche for population growth in mosquito vectors. Competition with *Ae. aegypti* significantly narrowed the thermal niche of *Cx. quinquefasciatus* and lowered the thermal optimum. Similarly, competition with *Ae. aegypti* significantly narrowed the thermal niche of *Ae. albopictus*, but did not alter the thermal optimum. Competition with *Cx. quinquefasciatus* significantly increased the thermal optimum of *Ae. albopictus*, but did not alter the thermal minimum or maximum. The thermal niche of *Ae. aegypti*, which was previously identified as the superior competitor in this group of species, was not significantly altered by interspecific competition. Interestingly, competition with *Ae. aegypti* altered the thermal niche of both *Ae. albopictus* and *Cx. quinquefasciatus* individually, but the thermal niche of both inferior competitors was not significantly affected by multi-species competition. This positive, indirect effect suggests that the addition of a third species can ease the burden of interspecific competition on the inferior competitor.

Additionally, our results show that effects of interspecific competition vary by temperature, focal/competitor species, and vital rate. For example, interspecific competition with *Ae. aegypti* significantly increased *Cx. quinquefasciatus* development time at low temperatures (21°C), while significantly decreasing survival at high temperatures (30°C). Interspecific competition with *Ae. aegypti* significantly decreased *Ae. albopictus* development time at 21°C, but significantly increased development time at 24°C. All cases of interspecific competition significantly decreased *Ae. aegypti* development time at low temperatures (21°C) and significantly increased *Ae. aegypti* body size at higher temperatures (27-33°C). Despite significant effects of interspecific competition on different vital rates, interspecific competition had no significant effect on the estimated intrinsic growth rate of *Ae. aegypti* or *Cx. quinquefasciatus*. Competition with *Ae. aegpyti*, as well as multi-

species competition, significantly decreased *Ae. albopictus* growth rate at low temperatures (21°C) only. However, the lack of detection of statistical differences in intrinsic growth rates between competition treatments may be due to the low sample size (n=5) for population-level metrics (e.g., growth rate) compared to individual-level metrics (e.g., development time, body size, egg-to-adult survival).

No *Cx. quinquefasciatus* females survived to emergence in our 33°C treatments. This could be due to (1) physiological thermal limits, (2) poor egg quality, or (3) strong competition at high temperatures. It is unlikely that this extremely high mortality rate was caused by competition because mortality was uniform across all treatments. As we obtained eggs for these experiments from another laboratory, it is possible that the source of blood meals was suboptimal for this species, which can influence fecundity (Olayemi et al. 2011, Farjana and Tuno 2012, 2013). Fecundity is also known to decrease with senescence in adult mosquitoes, so the eggs could have been produced by older females (Styer et al. 2007, McCann et al. 2009). Previous studies report egg-to-adult survival of *Cx. quinquefasciatus* at temperatures greater than 33°C (e.g., Rueda et al. 1990), but genetic variations between populations may contribute to the exact physiological thermal threshold for this species. If the high mortality observed in our experiments was due to poor egg quality and not a thermal threshold, the thermal maxima for *Ae. aegpyti* and *Ae. albopictus* may be overestimated due to lack of interspecific interactions with *Cx. quinquefasciatus* at 33°C. If interspecific competition with *Cx. quinquefasciatus* was strong at 33°C, then the absence of this species could result in an artificially high intrinsic growth rate estimate and over-estimated of the thermal maximum. Additionally, if *Cx. quinquefasciatus* larvae died early in the experiment (i.e., before consuming significant resources), this could have inflated intrinsic growth rate estimates and resulted in overestimation of the thermal maximum of *Ae. aegpyti* and/or *Ae.*

albopictus as well. We fitted thermal niche curves for *Cx. quinquefasciatus* data under the assumption of (2) above, so the thermal optimum of this species may also be overestimated if 33°C is the true physiological thermal threshold.

Given the results of this experiment, future empirical work on thermal responses of mosquito vectors should incorporate the outcome of species interactions, in addition to common vital rate measurements, across a wide temperature gradient. There is also a lack of empirical data on mosquito vital rates at thermal extremes (e.g., < 20°C and > 30°C); increased data on vital rates at these temperatures will allow for more accurate estimates of thermal niches for both mosquito population growth and vector-borne disease transmission. Given the dependence of important vector-borne disease transmission parameters (like vector competence) on temperature, a vital next step will be to determine if there are interacting effects of temperature and interspecific competition on those traits and what consequences those interactions may have for disease transmission.

References

Bara, J., Z. Rapti, C. E. Cáceres, and E. J. Muturi. 2015. Effect of Larval Competition on Extrinsic Incubation Period and Vectorial Capacity of Aedes albopictus for Dengue Virus. PLOS ONE 10:e0126703.

Briere, J.-F., P. Pracros, A.-Y. Le Roux, and J.-S. Pierre. 1999. A novel rate model of temperature-dependent development for arthropods. Environmental Entomology 28:22–29.

Carrington, L. B., S. N. Seifert, M. V. Armijos, L. Lambrechts, and T. W. Scott. 2013. Reduction of Aedes aegypti Vector Competence for Dengue Virus under Large Temperature Fluctuations. American Journal of Tropical Medicine and Hygiene 88:689–697.

Couret, J., E. Dotson, and M. Q. Benedict. 2014. Temperature, larval diet, and density effects on development rate and survival of Aedes aegypti (Diptera: Culicidae). PloS one 9:e87468.

Delatte, H., G. Gimonneau, A. Triboire, and D. Fontenille. 2009. Influence of Temperature on Immature Development, Survival, Longevity, Fecundity, and Gonotrophic Cycles of Aedes albopictus, Vector of Chikungunya and Dengue in the Indian Ocean. Journal of Medical Entomology 46:33–41.

Farjana, T., and N. Tuno. 2012. Effect of body size on multiple blood feeding and egg retention of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae). Medical Entomology and Zoology 63:123–131.

Farjana, T., and N. Tuno. 2013. Multiple Blood Feeding and Host-Seeking Behavior in Aedes aegypti and Aedes albopictus (Diptera: Culicidae). Journal of Medical Entomology 50:838–846.

Farjana, T., N. Tuno, and Y. Higa. 2012. Effects of temperature and diet on development and interspecies competition in Aedes aegypti and Aedes albopictus. Medical and veterinary entomology 26:210–217.

Harvell, D., S. Altizer, I. M. Cattadori, L. Harrington, and E. Weil. 2009. Climate change and wildlife diseases: When does the host matter the most? Ecology 90:912–920.

Kirby, M. J., and S. W. Lindsay. 2009. Effect of temperature and inter-specific competition on the development and survival of Anopheles gambiae sensu stricto and An. arabiensis larvae. Acta tropica 109:118–123.

Lafferty, K. D. 2009. The ecology of climate change and infectious diseases. Ecology 90:888–900.

Livdahl, T. P., and G. Sugihara. 1984. Non-Linear Interactions of Populations and the Importance of Estimating Per Capita Rates of Change. Journal of Animal Ecology 53:573–580.

Lounibos, L. P., S. Suárez, Z. Menéndez, N. Nishimura, R. L. Escher, S. M. O Connell, and J. R. Rey. 2002. Does temperature affect the outcome of larval competition between Aedes aegypti and Aedes albopictus? Journal of Vector Ecology 27:86–95.

McCann, S., J. F. Day, S. Allan, and C. C. Lord. 2009. Age modifies the effect of body size on fecundity in Culex quinquefasciatus Say (Diptera: Culicidae). Journal of Vector Ecology 34:174–181.

Mordecai, E. A., K. P. Paaijmans, L. R. Johnson, C. Balzer, T. Ben-Horin, E. de Moor, A. McNally, S. Pawar, S. J. Ryan, T. C. Smith, and K. D. Lafferty. 2013. Optimal temperature for malaria transmission is dramatically lower than previously predicted. Ecology Letters 16:22–30.

Muturi, E. J., and B. W. Alto. 2011. Larval environmental temperature and insecticide exposure alter Aedes aegypti competence for arboviruses. Vector-Borne and Zoonotic Diseases 11:1157–1163.

Muturi, E. J., M. Blackshear, and A. Montgomery. 2012. Temperature and density-dependent effects of larval environment on Aedes aegypti competence for an alphavirus. Journal of Vector Ecology 37:154–161.

Olayemi, I. K., A. T. Ande, G. Danlami, and U. Abdullahi. 2011. Influence of Blood Meal Type on Reproductive Performance of the Malaria Vector, Anopheles gambiae s.s. (Diptera: Culicidae). Journal of Entomology 8:459–467.

Richards, S. L., C. N. Mores, C. C. Lord, and W. J. Tabachnick. 2007. Impact of Extrinsic Incubation Temperature and Virus Exposure on Vector Competence of Culex pipiens quinquefasciatus Say (Diptera: Culicidae) for West Nile Virus. Vector-Borne and Zoonotic Diseases 7:629–636.

Rueda, L. M., K. J. Patel, R. C. Axtell, and R. E. Stinner. 1990. Temperature-Dependent Development and Survival Rates of Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae). Journal of Medical Entomology 27:892–898.

Styer, L. M., J. R. Carey, J.-L. Wang, and T. W. Scott. 2007. Mosquitoes Do Senesce: Departure from the Paradigm of Constant Mortality. The American Journal of Tropical Medicine and Hygiene 76:111–117.

CHAPTER 4

CONTRIBUTIONS OF TRAITS AT DIFFERENT DEVELOPMENTAL STAGES TO LARVAL COEXISTENCE OF MOSQUITO VECTORS[1]

[1]Bowden, Sarah E. and Drake, John M. To be submitted to *Ecology and Evolution*.

Abstract

Understanding the population dynamics of mosquito vectors is critical to effective control of arboviral disease. While many studies infer species coexistence and competitive exclusion from a small subset of mosquito traits (e.g., larval development time, egg-to-adult survival, fecundity, adult longevity), the contribution of traits at all developmental stages to coexistence and exclusion in mosquito communities have not been well-investigated. We created a stage-structured model – parameterized by a combination of empirical data, allometric functions, and literature-derived values – to explore the effects of differences in life history traits during the egg, pupal, and adult stages on population dynamics and resulting coexistence or exclusion of two competing mosquito species, *Culex quinquefasciatus* and *Aedes albopictus*. While results from laboratory experiments indicated that *Ae. albopictus* should competitively exclude *Cx. quinquefasciatus*, our results showed that coexistence between these species was possible when *Ae. albopictus* egg stage duration was 12 or more days and that of *Cx. quinquefasciatus* was 4-10 days shorter, or when *Ae. albopictus* adult mortality was higher than that of *Cx. quinquefasciatus*. These results underscore the need to consider the influence of all life stages on population and community dynamics of species with complex life cycles

Introduction

Understanding the population dynamics of mosquito vectors is critical to effective control of arboviral disease. Tracking these dynamics in natural populations is made difficult by the complex life cycle of mosquitoes and their ontogenetic niche shift from the aquatic to terrestrial ecosystem. Population growth rates of mosquitoes may be empirically estimated from field data using the intrinsic growth rate model developed by (Livdahl and Sugihara 1984):

$$r' = \frac{\ln\frac{1}{N_0}\sum_x A_x f(\bar{w}_x)}{D+\frac{\sum_x xA_x f(\bar{w}_x)}{\sum_x A_x f(\bar{w}_x)}} \quad \text{(Eqn. 1),}$$

This model allows for the estimation of per capita growth rates without the need to track daily larval or adult survival by substituting experimentally-determined functions in place of empirical measurements, and is used ubiquitously in mosquito and vector ecology research. Additionally, this model requires relatively few parameters for growth rate estimation: N_0 is the initial number of females in the population (assumed to be half of the total population size), A_x is the number of females eclosing on day x, and $f(\bar{w}_x)$ is an allometric scaling function that relates mean body size of females eclosing on day x to fecundity (Livdahl and Sugihara 1984). These growth rate estimates can be used to infer conditions for species coexistence or competitive exclusion (Murrell and Juliano 2008, Armistead et al. 2008) in the context of Lotka-Volterra species competition (Volterra 1926, Lotka 1932). However, non-competing life stages (e.g., egg, pupae, adults) of mosquitoes, which are not explicitly included in the Livdahl and Sugihara (1984) model, have been shown to be important in determining population dynamics of competing species (Costanzo et al. 2005a). Therefore, the contribution of vital rates and life-history traits at non-larval life stages to species coexistence and competitive exclusion warrants additional attention.

Previous laboratory microcosm experiments (detailed in Chapter 1) looking at density-dependent larval competition between two vector mosquito species (*Culex quinquefasciatus* and *Aedes albopictus*) showed that *Ae. albopictus* would competitively exclude *Cx. quinquefasciatus* under laboratory conditions (e.g., 27°C, animal-based food source). However, coexistence between these two species, or species in the same complex (e.g., *Cx. pipiens*), has been reported in some locations (Carrieri et al. 2003, Juliano and Philip Lounibos 2005, Costanzo et al. 2005b, LaDeau et al. 2013). We sought to explain this discrepancy between field and laboratory studies

by considering a more complex model of mosquito population dynamics that incorporates traits from all life history stages to account for differences between laboratory experiments and field observations. Specifically, we investigated whether differences in traits at non-larval life stages between these two species allow for coexistence in the larval environment. For example, we hypothesized that higher adult mortality of the superior competitor (e.g., *Ae. albopictus*) might mitigate the effect of larval competition on population dynamics by reducing the number of new individuals (eggs) that are produced from the adult population.

To test these hypotheses, we created a stage-structured population model parameterized from empirical data to explore the effects of trait differences between species at non-larval life stages on their ability to coexist; specifically (1) duration of egg stage, (2) duration of pupal stage, (3) daily adult mortality, and (4) length of gonotrophic cycle. Our results predict that *Cx. quinquefasciatus* is able to coexist with, and sometimes exclude, *Ae. albopictus* when daily adult mortality is higher in the latter species. Additionally, this analysis predicts coexistence to be possible when the egg stage duration is short (< 6 days) in *Cx. quinquefasciatus* and much longer (12-14 days) in *Ae. albopictus* than in *Cx. quinquefasciatus* . These results underscore the need to consider the influence of all life stages on population and community dynamics of species with complex life cycles, even if competitors do not necessarily interact during all stages.

Methods

To represent the contribution of all life stages to dynamics of mosquito populations, we created a stage-structured population model with five distinct life history stages (Figure 1), represented by a system of delay differential equations. To obtain density-dependent functions for empirically-derived parameters, we fit linear (development time) and exponential decay (body size, egg-to-adult survival) models to data from previous laboratory experiments (detailed in

Chapter 1). Larval density measurements from laboratory experiments were recorded as number of individuals per 200 mL DI water. Estimates for all other parameter values were obtained from the primary literature (Table 1).

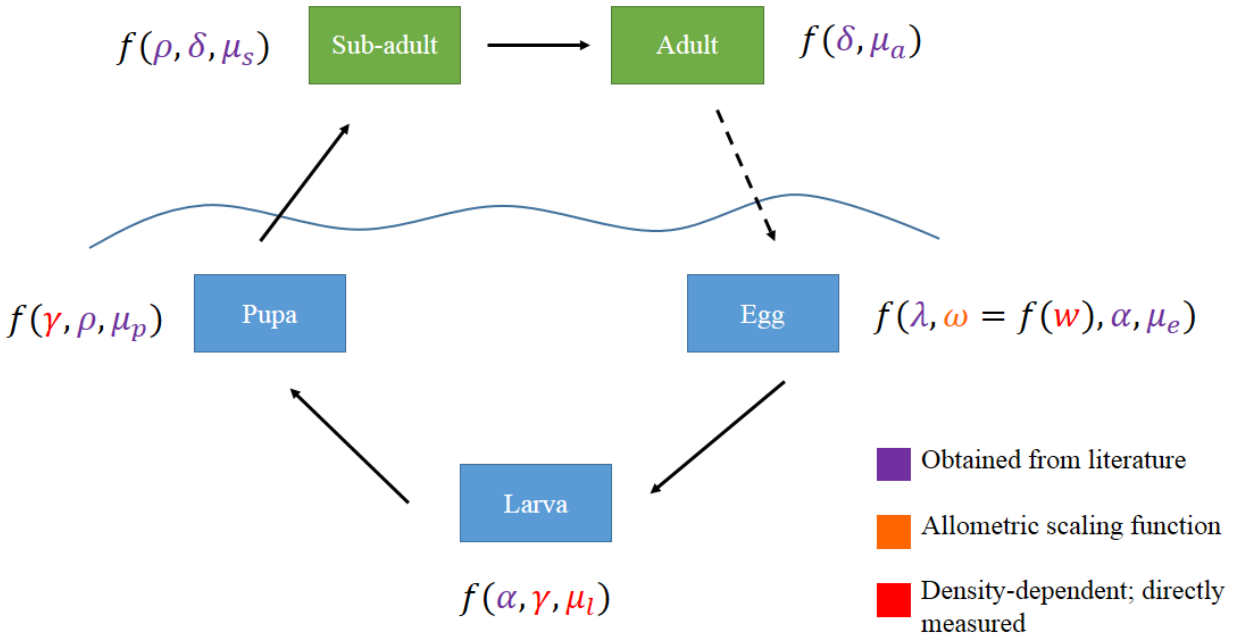

Figure 4.1: Conceptual diagram of the stage-structured population model with five developmental stages: egg, larval, pupal, sub-adult, and adult. Stages in blue take place in the aquatic environment, and those in green take place in the terrestrial environment. Parameters in the model (Table 1) are a combination of literature-derived (purple), allometric scaling functions (orange), or density-dependent functions from directly measured data (red).

Table 4.1: Parameters and selected values for stage-structured population model simulations.

Parameter	Symbol	Value (Range)	Units	References
Gonotrophic cycle length	λ	7 (1-30)	Days	(Elizondo-Quiroga et al. 2006, Ulloa et al. 2006, García-Rejón et al. 2008, Wong et al. 2011)
Fecundity	ω	Density-dependent function	Eggs per female	Derived from empirical data (chapter 1)
Egg death	μ_e	0.15	Day^{-1}	(Vitek and Livdahl 2006, Olayemi et al. 2011, Aida et al. 2011, Phasomkusolsil et al. 2013)
Larval death	μ_l	Density-dependent function	Day^{-1}	Derived from empirical data (chapter 1)
Pupal death	μ_p	0.01	Day^{-1}	(Monteiro et al. 2007, Aida et al. 2011, Li et al. 2014)
Sub-adult/adult death	$\mu_s = \mu_a$	0.05 (0-0.5)	Day^{-1}	(Elizondo-Quiroga et al. 2006, Ulloa et al. 2006, García-Rejón et al. 2008, Moller-Jacobs et al. 2014)
Egg development time/ stage duration	α	1.5 (1-14)	Days	(Olayemi et al. 2011)
Larval development time/ stage duration	γ	Density-dependent function	Days	Derived from empirical data (chapter 1)
Pupal development time/ stage duration	ρ	2 (1-10)	Days	(Monteiro et al. 2007, Li et al. 2014)
Time to first reproduction/ sub-adult stage duration	δ	7	Days	(Elizondo-Quiroga et al. 2006, Ulloa et al. 2006, García-Rejón et al. 2008, Wong et al. 2011)

Egg stage dynamics are represented by

$$\frac{dE_i(t)}{dt} = \frac{1}{\lambda}\omega(t)A_i(t) - E_i(t-\alpha) - \mu_e E_i(t) \quad \text{(Eqn. 2),}$$

where λ is the length of the adult reproductive (gonotrophic) cycle in days; ω(t) is the average adult fecundity in number of eggs per individual at time t; α is the duration of the egg stage in days; and μ_e is the daily egg stage mortality rate. Fecundity (ω) was represented by a species-specific allometric function that relates adult body size (w) to number of eggs produced for *Cx. quinquefasciatus* (McCann et al. 2009)

$$\omega(t) = f(\mathrm{w(t)}) = 90.31\mathrm{w(t)} - 123.88 \quad \text{(Eqn. 3)}$$

and *Ae. albopictus* (Farjana and Tuno 2012)

$$\omega(t) = f\big(w(t)\big) = 104.8\mathrm{w(t)} - 201.37 \quad \text{(Eqn. 4).}$$

Size (w) at time t is a density-dependent function

$$w(t) = f(L_1(t-\lambda), L_2(t-\lambda)) \quad \text{(Eqn. 5),}$$

where L_i (i = {1,2}) is the number of larvae of species i at time t – λ, and λ is the length of the adult gonotrophic cycle. Larval stage dynamics are represented by

$$\frac{dL_i(t)}{dt} = E_i(t-\alpha) - \mu_l(t)L_i(t) - L_i(t-\gamma(t)) \quad \text{(Eqn. 6),}$$

where $\mu_l(t)$ is the daily larval mortality rate, represented by the density-dependent function

$$\mu(t) = f(L_1(t), L_2(t)) \quad \text{(Eqn. 7),}$$

and γ is the larval development period in days, represented by the density-dependent function

$$\gamma(t) = \frac{1}{f(L_1(t-\gamma(t)), L_2(t-\gamma(t)))} \quad \text{(Eqn. 8).}$$

Pupal stage dynamics are represented by

$$\frac{dP_i(t)}{dt} = L_i\big(t-\gamma(t)\big) - \mu_p P_i(t) - P_i(t-\rho) \quad \text{(Eqn. 9),}$$

where ρ is the duration of the pupal stage in days and μ_p is the daily pupal mortality rate. Sub-adult stage (i.e., pre-reproductive adult) dynamics are represented by

$$\frac{dS_i(t)}{dt} = P_i(t-\rho) - \mu_s S_i(t) - S_i(t-\delta) \quad \text{(Eqn. 10)},$$

where μ_s is the daily sub-adult mortality rate and δ is the time to first reproduction in days. Finally, reproducing adult dynamics are represented by

$$\frac{dA_i(t)}{dt} = S_i(t-\delta) - \mu_a A_i(t) \quad \text{(Eqn. 11)},$$

where μ_a is the daily adult mortality rate.

We solved this model numerically in the R computing environment (R Core Team 2016)using delay differential equations ('stagePop' package (Kettle 2015)) which allowed us to mimic the average time an individual would spend in each life stage. Time delays for the first four stages were represented as the development time or stage duration (e.g., parameters α, γ, ρ, and δ). After obtaining numerical solutions for each species in both the single-species and two-species models at estimated parameter values, we explored a large parameter space for egg stage duration, pupal stage duration, daily adult mortality, and gonotrophic cycle length to determine if differences in these parameters between the two species resulted in coexistence or exclusion. We elected to focus on these parameters because they are known to differ intrinsically among different species and genera, and can be sensitive to environmental factors and therefore potentially variable in nature. Pairs of parameter values for each simulation were selected via a grid search on each parameter: egg stage duration from 1-14 days in increments of 0.5 days (729 unique pairs of parameter values), pupal stage duration from 1-10 days in increments of 0.5 days (361 parameter value pairs), daily adult mortality from 0-0.5 in increments of 0.01 (2,601 parameter pairs), and gonotrophic cycle length from 1-30 days in increments of 1 day (900 parameter pairs). Model

simulations began with 10 individuals of each species in the egg stage, with initial population sizes of all other stages set to zero. Each parameter set was solved for 200 days, and larval population sizes at the final time step were used to determine coexistence or exclusion of each species. If the final larval population size of both species was ≥ 1 individual, the species were presumed to coexist under that set of parameter values.

Results

Body size and egg-to-adult survival consistently decreased with increasing density for each species under both intraspecific and interspecific competition (Figures 2 & 3). Fitted parameters with standard error for each vital rate model – body size (exponential decay; $y = a(1-b)^x$), development time (linear; $y = mx + b$), and egg-to-adult survival (exponential decay; $y = a(1-b)^x$) – are reported in Table 2.

In the single-species population model for *Cx. quinquefasciatus*, all life-history stages reached equilibrium within 200 time steps. Equilibrium population sizes are reported per unit area (e.g., per 200 mL DI water based on empirical data from which density-dependent functions were derived). The equilibrium population sizes for each stage were approximately 60 eggs, 56.5 larvae, <1 pupa (0.35), 1.05 sub-adults, and 2.5 adults. All stages in the single-species *Ae. albopictus* model also reached equilibrium within 200 time steps. The equilibrium population sizes for each stage were approximately 49.6 eggs, 65.5 larvae, <1 pupa (0.67), 2 sub-adults, and 4.7 adults. *Cx. quinquefasciatus* population dynamics fluctuated at a higher magnitude than *Ae. albopictus* prior to reaching equilibrium (Figure 4). While the equilibrium population sizes for *Cx. quinquefasciatus* eggs was slightly higher (~3.5 individuals) than that of the larval stage class, the larval equilibrium for *Ae. albopictus* was ~16 individuals higher than the egg stage class. In the two-species model, competition with *Ae. albopictus* at the larval stage drives *Cx. quinquefasciatus*

to extinction by 150 time steps. After the eradication of *Cx. quinquefasciatus*, all stages of *Ae. albopictus* enter limit cycles (Figure 5).

A portion of the parameter space in which the *Ae. albopictus* egg stage duration is >11.5 days allowed for coexistence between *Ae. albopictus* and *Cx. quinquefasciatus*, but only when the egg stage duration of the latter species was several days shorter than the former (Figure 6, top-left); otherwise, *Ae. albopictus* excluded *Cx. quinquefasciatus*. No subset of the parameter space for pupal stage duration allowed for coexistence between these two species; *Ae. albopictus* always excluded *Cx. quinquefasciatus* (Figure 6, top-right). *Cx. quinquefasciatus* was able to coexist with or exclude *Ae. albopictus* when daily adult mortality was higher in the latter species (Figure 6, bottom-left). A very small portion of the parameter space where *Ae. albopictus* gonotrophic cycle length was drastically higher than that *Cx. quinquefasciatus* allowed for coexistence between these species; otherwise, *Ae. albopictus* excluded *Cx. quinquefasciatus* (Figure 6, bottom-right).

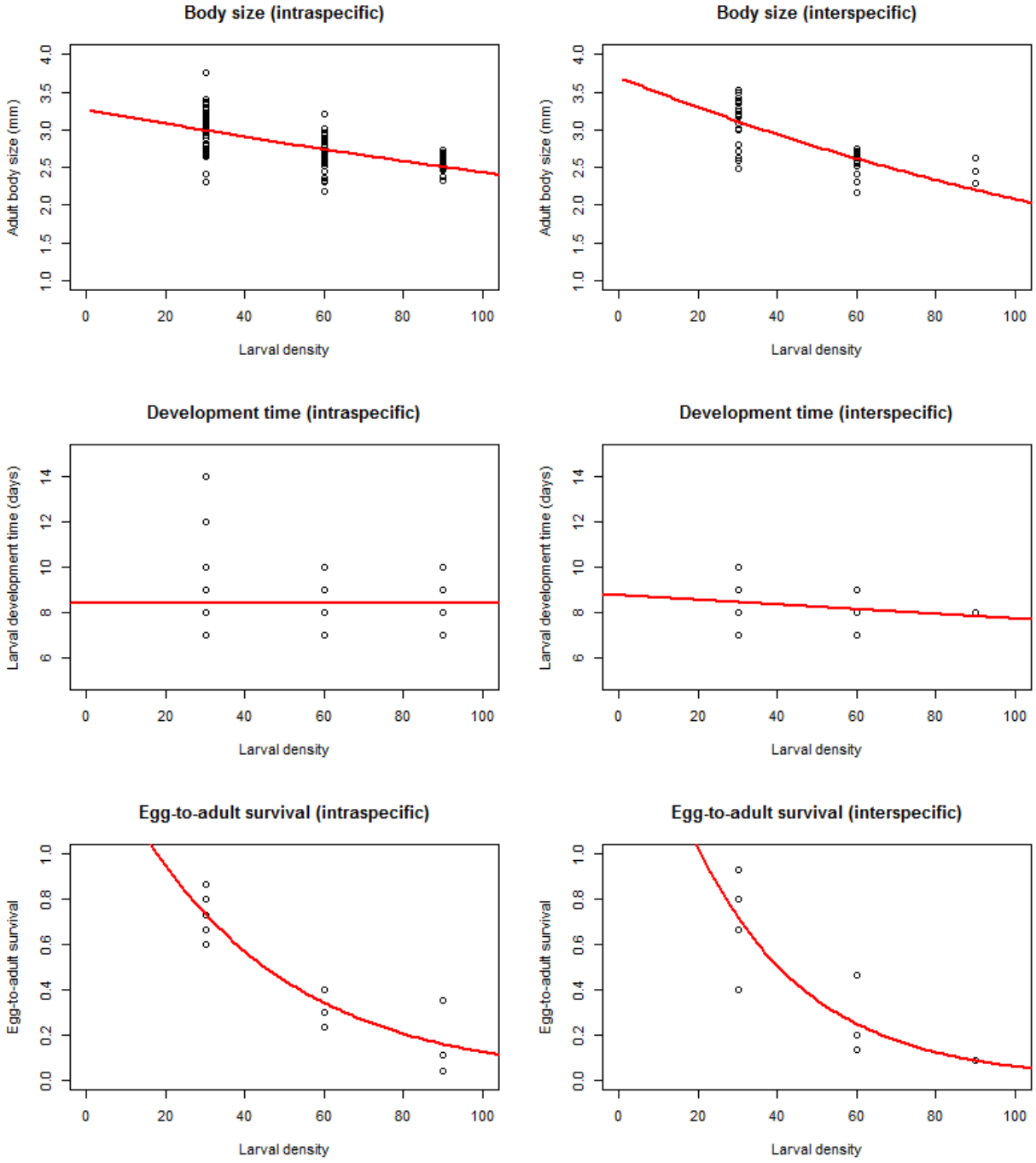

Figure 4.2: Plots of empirical data and fitted linear (development time) and non-linear (body size, egg-to-adult survival) density-dependent functions for *Cx. quinquefasciatus*.

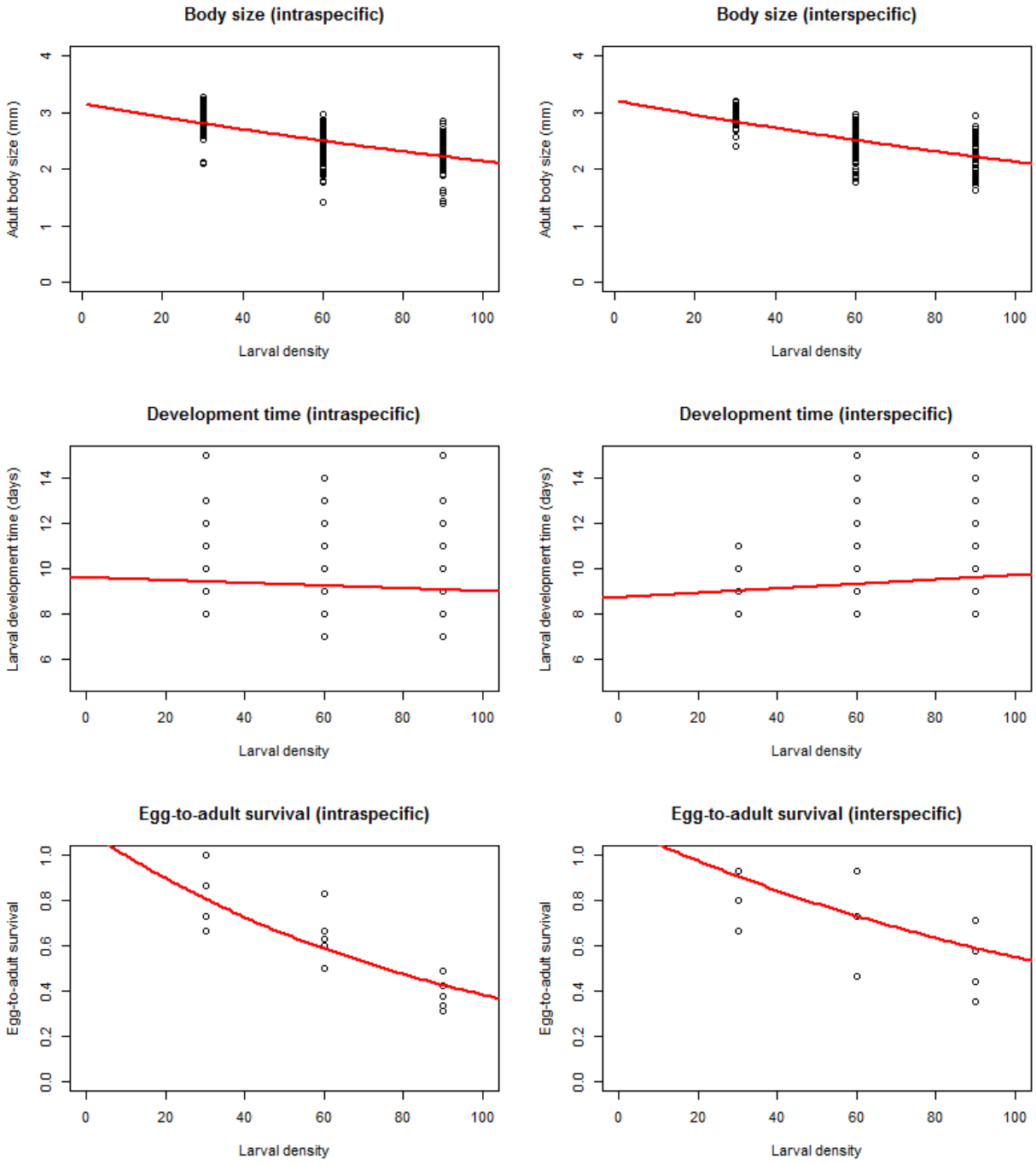

Figure 4.3: Plots of empirical data and fitted linear (development time) and non-linear (body size, egg-to-adult survival) density-dependent functions for *Ae. albopictus*.

Table 4.2: Regression coefficients for linear and non-linear models fit to empirical data on body size, development time, and egg-to-adult survival of each species.

Species	Competition	Body size		Development time		Egg-to-adult survival	
		a	b	b (intercept)	m (slope)	a	b
Cx. quinquefasciatus	Intraspecific	3.2635 (0.0537)	0.0029 (0.0003)	8.444 (0.186)	3.53e-17 (3.17e-03)	1.5710 (0.2356)	0.0250 (0.0037)
	Interspecific	3.6986 (0.1302)	0.0057 (0.0008)	8.764 (0.249)	-0.010 (0.005)	2.0707 (0.7849)	0.0346 (0.0107)
Ae. albopictus	Intraspecific	3.1519 (0.0630)	0.0039 (0.0003)	9.610 (0.267)	-0.006 (0.004)	1.1114 (0.1320)	0.0106 (0.0022)
	Interspecific	3.2116 (0.0812)	0.0041 (0.0004)	8.751 (0.423)	0.010 (0.006)	1.1256 (0.2011)	0.0071 (0.0031)

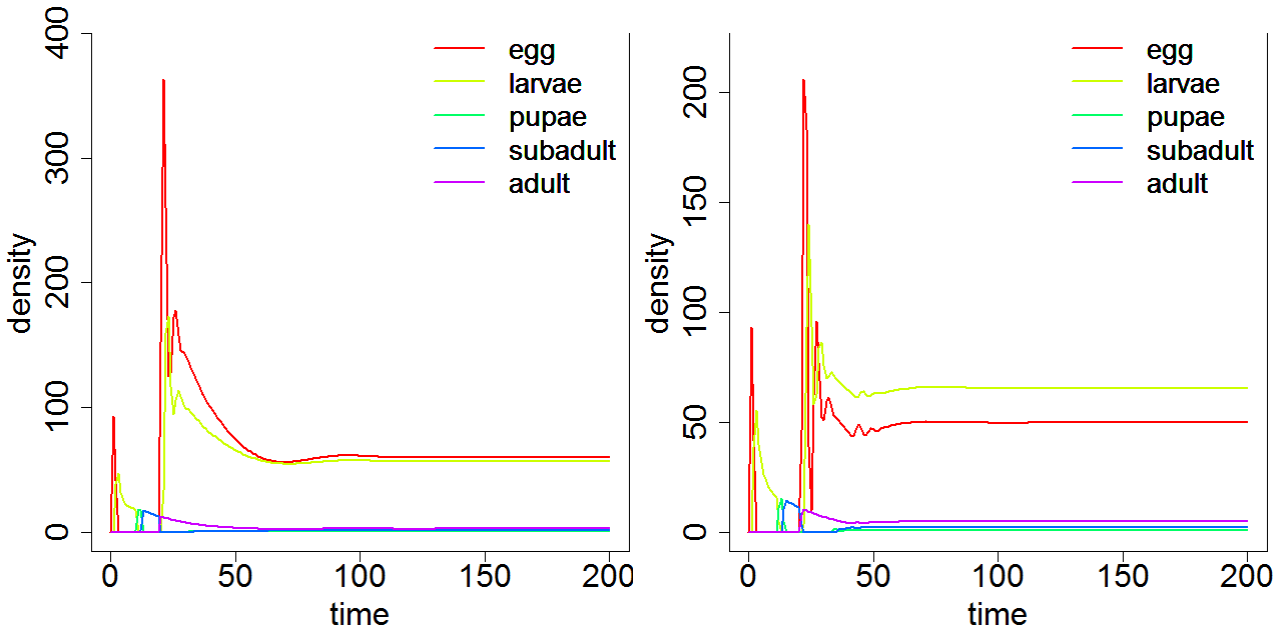

Figure 4.4: Numerical solutions for single-species stage-structured population dynamics model.

Stage-specific density is reported as the number of individuals per 200 mL DI water.

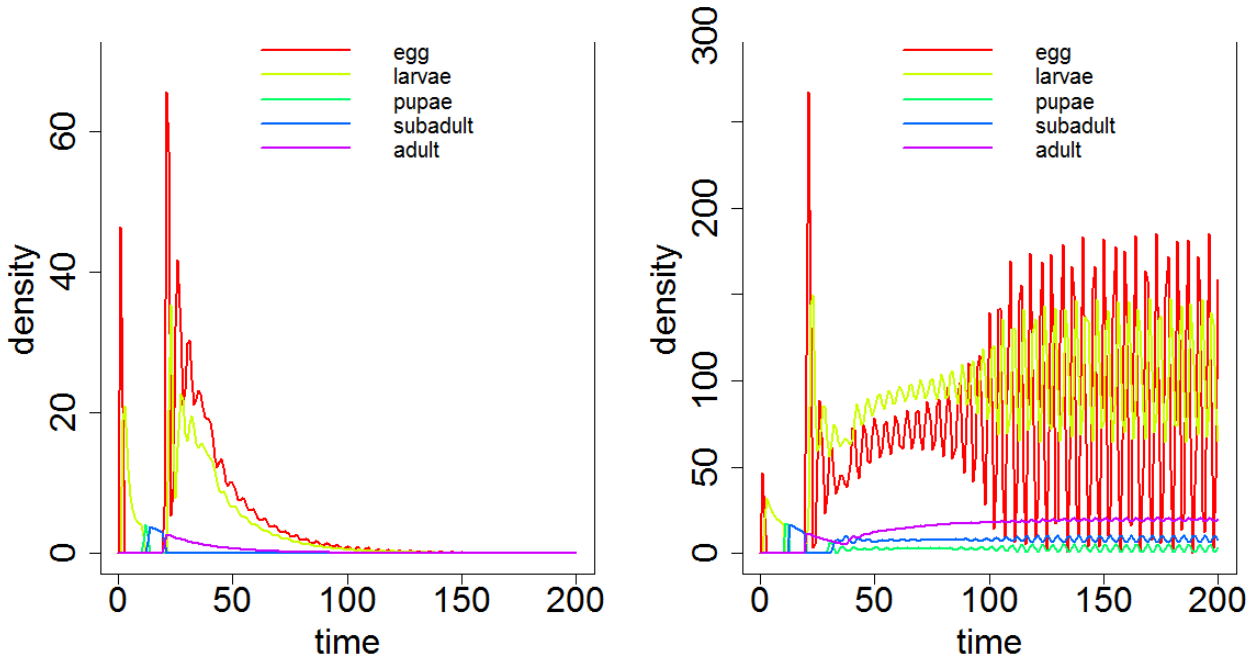

Figure 4.5: Numerical solutions for two-species stage-structured population dynamics model. Moving from the one-species to the two-species form of the model, species interact through density-dependent functions for larval development time, larval survival, and adult body size, which are dependent on the total larval density of both species. Stage-specific density is reported as the number of individuals per 200 mL DI water.

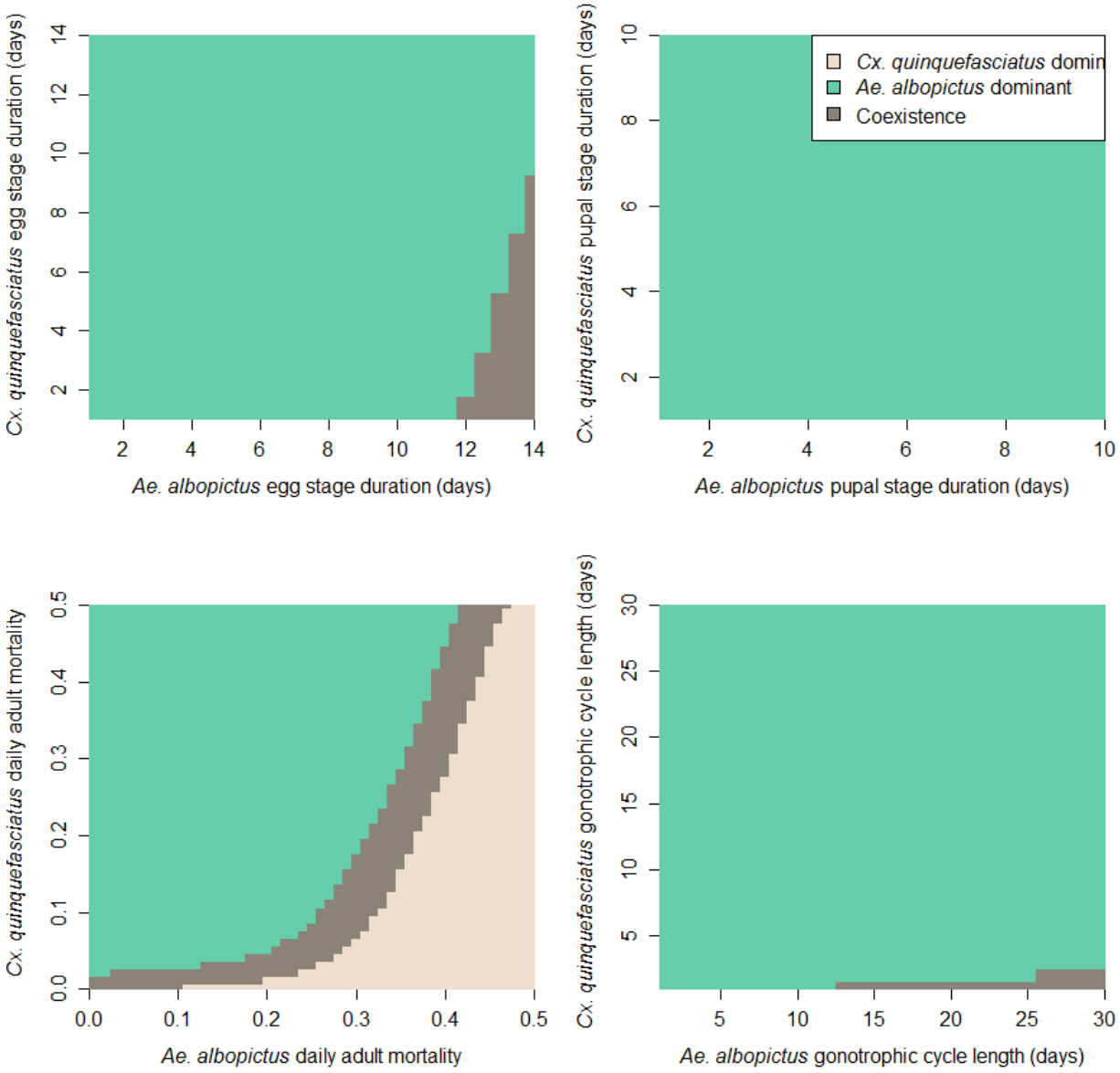

Figure 4.6: Coexistence-exclusion plots for simulations with varying egg stage duration (top-left), pupal stage duration (top-right), daily adult mortality (bottom-left), and gonotrophic cycle length (bottom-right) based on simulations of the two-species model form. All other non-density-dependent parameters besides those listed in each plot's axis labels (egg stage duration, top-left; pupal stage duration, top-right; daily adult mortality, bottom-left; and gonotrophic cycle length, bottom-right) were held constant during simulations.

Discussion

These results show how larval coexistence between *Cx. quinquefasciatus* and *Ae. albopictus* is possible when the notionally superior competitor (*Ae. albopictus*) has a higher daily adult mortality rate than *Cx. quinquefasciatus*, or has a much longer egg stage duration than *Cx. quinquefasciatus*, despite strong asymmetric competition (e.g., competitive dominance by *Ae. albopictus*) in the larval stage. As hypothesized, we found that higher adult mortality in the dominant larval competitor may promote coexistence either by reducing the total abundance of reproducing adults or by reducing average adult longevity, which would result in fewer reproductive events over an individual's lifespan. Interestingly, the influence of egg stage duration on coexistence corresponds to innate differences in the eggs of these genera. *Aedes* spp. eggs are generally desiccation-resistant (Juliano and Lounibos 2005); they can withstand drying of their larval habitat and begin to hatch upon rehydration. Conversely, *Culex* spp. eggs are not desiccation resistant and usually hatch within a few days of being laid. These intrinsic differences in the egg stage of each species provides an additional mechanism for coexistence despite asymmetrical larval competition.

An additional mechanism which was not explored in this model that could promote coexistence is difference in demographic frailty, which is essentially non-static death rates as adults senesce. Adult mortality tends be hump-shaped, with lower mortality in young and old individuals and higher mortality in middle-aged individuals (Styer et al. 2007, Harrington et al. 2008). However, the effects of adult senescence can be much more nuanced than just changes in mortality rates, potentially including changes in host-seeking behavior, host-feeding preference, frequency of blood meals, length of gonotrophic cycles, and fecundity, among other factors. As these traits are all important components of population dynamics and/or vector-borne disease

transmission, this area warrants further empirical research into the effects of senescence on adult traits and behavior.

While we only measured and included density-dependence in three rates (larval development time, egg-to-adult survival, and adult body size), interspecific competition might plausibly affect other parameters included in this model. For example, egg stage duration could be affected by chemical cues that signal high larval densities (Livdahl and Edgerly 1987); pupal stage duration could be prolonged due to undernourishment during larval stage; adult mortality could be increased and longevity could be reduced due to nutritional stress during the larval stage; and length of the gonotrophic cycle could be decreased if more frequent blood meals are required due to nutrient deficiencies resulted in smaller adult body size (Briegel 1990). A study by Edgerly and Livdahl (1992) determined that *Ae. albopictus* egg hatch rate was not dependent on larval density, suggesting static parameters for egg stage duration and mortality are adequate. Beyond pupal weight (which is correlated with adult body mass/size), few studies have investigated density-dependent effects in the pupal stage. Lack of research in this area is likely due to both the short duration of the stage and the fact that is it a non-feeding life stage. Evidence for the effect of larval density on adult longevity is mixed, with some studies reporting negative effects of high larval density on adult longevity in some species due to reduced nutrient reserves (Agnew et al. 2000), while others report that dietary restriction during the larval stage can significantly increase adult longevity (Joy et al. 2010). The lack of data or consensus in this area highlights the need for empirical measurements of traits at all life history stages across a gradient of larval densities.

One limitation of the model developed in this paper is the exclusion of external, abiotic factors and their effects on parameters from different life stages. For example, microclimate (e.g., temperature, precipitation, humidity) and larval nutrient quality are well-studied and known to

have a variety of effects on different vital rates and life-history traits. The empirically-derived, density-dependent parameters in our model represent the outcome of larval competition at a single static temperature (27°C). However, previous laboratory experiments (Chapter 2) show that the effects of larval competition on some vital rates (e.g., larval development time, egg-to-adult survival, adult body size) depend significantly on both temperature and the species identity of competitors. A problem for future study is the development of temperature- and density-dependent empirical estimates of these vital rates and extension of the model to assess the conditions for species coexistence and competitive exclusion across a temperature gradient. Such an understanding of how mosquito population dynamics are dependent upon both biotic (e.g., density, competition) and abiotic (e.g., microclimate) factors could significantly improve our ability to manage mosquito populations as both pests and vectors of disease.

References

Agnew, P., C. Haussy, and Y. Michalakis. 2000. Effects of Density and Larval Competition on Selected Life History Traits of Culex pipiens quinquefasciatus (Diptera: Culicidae). Journal of Medical Entomology 37:732–735.

Aida, H. N., H. Dieng, A. H. Ahmad, T. Satho, A. Nurita, M. C. Salmah, F. Miake, and B. Norasmah. 2011. The biology and demographic parameters of Aedes albopictus in northern peninsular Malaysia. Asian Pacific Journal of Tropical Biomedicine 1:472–477.

Armistead, J. S., J. R. Arias, N. Nishimura, and L. P. Lounibos. 2008. Interspecific larval competition between Aedes albopictus and Aedes japonicus (Diptera : Culicidae) in northern Virginia. Journal of Medical Entomology 45:629–637.

Briegel, H. 1990. Metabolic relationship between female body size, reserves, and fecundity of Aedes aegypti. Journal of Insect Physiology 36:165–172.

Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003. On the Competition Occurring Between Aedes albopictus and Culex pipiens (Diptera: Culicidae) in Italy. Environmental Entomology 32:1313–1321.

Costanzo, K. S., B. Kesavaraju, and S. A. Juliano. 2005a. Condition-Specific Competition in Container Mosquitoes: The Role of Noncompeting Life-History Stages. Ecology 86:3289–3295.

Costanzo, K. S., K. MORMANN, and S. A. JULIANO. 2005b. Asymmetrical Competition and Patterns of Abundance of Aedes albopictus and Culex pipiens (Diptera: Culicidae). Journal of medical entomology 42:559–570.

Edgerly, J. S., and T. P. Livdahl. 1992. Density-Dependent Interactions Within a Complex Life Cycle: The Roles of Cohort Structure and Mode of Recruitment. Journal of Animal Ecology 61:139–150.

Elizondo-Quiroga, A., A. Flores-Suarez, D. Elizondo-Quiroga, G. Ponce-Garcia, B. J. Blitvich, J. F. Contreras-Cordero, J. I. Gonzalez-Rojas, R. Mercado-Hernandez, B. J. Beaty, and I. Fernandez-Salas. 2006. Gonotrophic cycle and survivorship of Culex quinquefasciatus (Diptera: Culicidae) using sticky ovitraps in Monterrey, northeastern Mexico. Journal Of The American Mosquito Control Association 22:10–14.

Farjana, T., and N. Tuno. 2012. Effect of body size on multiple blood feeding and egg retention of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) (Diptera: Culicidae). Medical Entomology and Zoology 63:123–131.

García-Rejón, J. E., J. A. Farfan-Ale, A. Ulloa, L. F. Flores-Flores, E. Rosado-Paredes, C. Baak-Baak, M. A. Loroño-Pino, I. Fernández-Salas, and B. J. Beaty. 2008. Gonotrophic cycle estimate for Culex quinquefasciatus in Mérida, Yucatán, México. Journal Of The American Mosquito Control Association 24:344–348.

Harrington, L. C., J. J. Jones, S. Kitthawee, R. Sithiprasasna, J. D. Edman, T. W. Scott, and others. 2008. Age-dependent survival of the dengue vector Aedes aegypti (Diptera: Culicidae) demonstrated by simultaneous release–recapture of different age cohorts. Journal of medical entomology 45:307–313.

Joy, T. K., A. J. Arik, V. Corby-Harris, A. A. Johnson, and M. A. Riehle. 2010. The impact of larval and adult dietary restriction on lifespan, reproduction and growth in the mosquito Aedes aegypti. Experimental Gerontology 45:685–690.

Juliano, S. A., and L. Philip Lounibos. 2005. Ecology of invasive mosquitoes: effects on resident species and on human health. Ecology Letters 8:558–574.

Kettle, H. 2015. stagePop: Modelling the Population Dynamics of a Stage-Structured Species in Continuous Time.

LaDeau, S. L., P. T. Leisnham, D. Biehler, and D. Bodner. 2013. Higher Mosquito Production in Low-Income Neighborhoods of Baltimore and Washington, DC: Understanding Ecological Drivers and Mosquito-Borne Disease Risk in Temperate Cities. International Journal of Environmental Research and Public Health 10:1505–1526.

Li, Y., F. Kamara, G. Zhou, S. Puthiyakunnon, C. Li, Y. Liu, Y. Zhou, L. Yao, G. Yan, and X.-G. Chen. 2014. Urbanization Increases Aedes albopictus Larval Habitats and Accelerates Mosquito Development and Survivorship. PLOS Negl Trop Dis 8:e3301.

Livdahl, T. P., and J. S. Edgerly. 1987. Egg hatching inhibition: field evidence for population regulation in a treehole mosquito. Ecological Entomology 12:395–399.

Livdahl, T. P., and G. Sugihara. 1984. Non-Linear Interactions of Populations and the Importance of Estimating Per Capita Rates of Change. Journal of Animal Ecology 53:573–580.

Lotka, A. J. 1932. The growth of mixed populations: Two species competing for a common food supply. Jour Washington Acad Sci 22:461–469.

McCann, S., J. F. Day, S. Allan, and C. C. Lord. 2009. Age modifies the effect of body size on fecundity in Culex quinquefasciatus Say (Diptera: Culicidae). Journal of Vector Ecology 34:174–181.

Moller-Jacobs, L. L., C. C. Murdock, and M. B. Thomas. 2014. Capacity of mosquitoes to transmit malaria depends on larval environment. Parasites & Vectors 7:593.

Monteiro, L. C. C., J. R. B. de Souza, and C. M. R. de Albuquerque. 2007. Eclosion rate, development and survivorship of Aedes albopictus (Skuse)(Diptera: Culicidae) under different water temperatures. Neotropical Entomology 36:966–971.

Murrell, E. G., and S. A. Juliano. 2008. Detritus Type Alters the Outcome of Interspecific Competition Between Aedes aegypti and Aedes albopictus (Diptera: Culicidae). Journal of Medical Entomology 45:375–383.

Olayemi, I. K., A. T. Ande, G. Danlami, and U. Abdullahi. 2011. Influence of Blood Meal Type on Reproductive Performance of the Malaria Vector, Anopheles gambiae s.s. (Diptera: Culicidae). Journal of Entomology 8:459–467.

Phasomkusolsil, S., J. Tawong, N. Monkanna, K. Pantuwatana, N. Damdangdee, W. Khongtak, Y. Kertmanee, B. P. Evans, and A. L. Schuster. 2013. Maintenance of mosquito vectors: effects of blood source on feeding, survival, fecundity, and egg hatching rates. Journal Of Vector Ecology: Journal Of The Society For Vector Ecology 38:38–45.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Styer, L. M., J. R. Carey, J.-L. Wang, and T. W. Scott. 2007. Mosquitoes Do Senesce: Departure from the Paradigm of Constant Mortality. The American Journal of Tropical Medicine and Hygiene 76:111–117.

Ulloa, A., L. Gonzalez-Cerón, and M. H. Rodríguez. 2006. Host selection and gonotrophic cycle length of Anopheles punctimacula in southern Mexico. Journal Of The American Mosquito Control Association 22:648–653.

Vitek, C. J., and T. P. Livdahl. 2006. Field and laboratory comparison of hatch rates in Aedes albopictus (Skuse). Journal Of The American Mosquito Control Association 22:609–614.

Volterra, V. 1926. Variations and fluctuations in the number of individuals among animals living together. Mem R Accad Naz Lincei Cl Fis Mat E Nat 2:31–112.

Wong, J., H. Astete, A. C. Morrison, and T. W. Scott. 2011. Sampling Considerations for Designing Aedes aegypti (Diptera: Culicidae) Oviposition Studies in Iquitos, Peru: Substrate Preference, Diurnal Periodicity, and Gonotrophic Cycle Length. Journal of Medical Entomology 48:45–52.

CHAPTER 5

CAN INTERSPECIFIC COMPETITION BETWEEN MOSQUITOES ALTER VECTOR-BORNE DISEASE TRANSMISSION? [1]

[1]Bowden, Sarah E. and Drake, John M. To be submitted to *Journal of Vector Ecology*.

Abstract

Most studies on the effects of biodiversity on disease systems have been explored primarily with respect to the host community. However, many multi-host vector-borne pathogens, like West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Chikungunya virus (CHIKV), are also transmitted by diverse vector communities. These vector species may also interact via competition for space and resources, potentially resulting in changes to the richness and evenness of the vector community. To identify the conditions under which competition between vector species may influence vector-borne disease transmission, we studied a deterministic SEIR (host) – SEI (vector) model of disease transmission that can be generalized to include more than one vector species, then solved this model across a gradient of vector competence under multiple interspecific competition scenarios (e.g., intraspecific competition > interspecific competition, intraspecific competition = interspecific competition, intraspecific competition < interspecific competition). These results suggest that disease transmission may be diluted in vector-borne disease systems when vectors compete more strongly with individuals of a different species. Potential extensions of this model that include additional elements of host and vector biology, as well as host and pathogen epidemiology, are discussed. Our results emphasize the importance of incorporating vector community interactions into models of vector-borne disease transmission to obtain a more complete picture of vector-borne disease dynamics in multi-species systems.

Introduction

Understanding how infectious disease systems respond to changes in biodiversity can be important in managing disease transmission in both wildlife and human populations. Biodiversity provides key ecosystem services; promotes ecosystem, human, and wildlife health; and can provide a buffer to the introduction and spread of infectious diseases (Hooper et al. 2005). Specifically, high host diversity has been shown to decrease pathogen transmission of vector-borne pathogens in some systems – through a variety of mechanisms (Keesing et al. 2006), including differences in host competence (ability to harbor and transmit a pathogen) and abundance among different species, as well as competition between host species – a phenomenon known as the *dilution effect* (Ostfeld and Keesing 2000a, 2000b, Schmidt and Ostfeld 2001). While the extent of this phenomenon is debated (Randolph and Dobson 2012, Salkeld et al. 2013), the dilution effect has been empirically observed in a broad range of natural systems (Civitello et al. 2015), including Lyme disease (Ostfeld and Keesing 2000a), West Nile virus (Swaddle and Calos 2008), Sin Nombre virus (Dizney et al. 2009), and the amphibian fungal pathogen *Batrachochytrium dendrobatitis* (Searle et al. 2011). In recent studies, the effects of biodiversity on disease systems have been explored primarily with respect to the host community (LoGiudice et al. 2003, Swaddle and Calos 2008, Dizney et al. 2009). However, many multi-host vector-borne pathogens, like West Nile virus (WNV), St. Louis encephalitis virus (SLEV), and Chikungunya virus (CHIKV), are also transmitted by diverse vector communities (Monath and Tsai 1987, Turell et al. 2001, 2005, Sardelis et al. 2002, Pialoux et al. 2007). The effects of vector community composition and interactions between vector species on pathogen transmission have yet to be explored. As mosquitoes are the most abundant vector of arboviruses in the world, a deeper understanding of the ecology and transmission dynamics of vector communities will serve as a model for

understanding the ecology of multi-host pathogens in general, as well as important zoonoses like Dengue virus and Zika virus.

Research focusing on the effects of biodiversity and the dilution effect is primarily concentrated on Lyme disease, with a small number of studies on West Nile virus. Most mathematical models of vector-borne disease transmission (for mosquitoes) are of the Ross-Macdonald style and pertain specifically to malaria (Aron and May 1982, Koella 1991, Smith and Ellis McKenzie 2004, Ruan et al. 2008, Smith et al. 2012) which has a large impact on the human population, but rarely affects wildlife populations (exceptions being avian malaria and primate malaria, which are caused by different *Plasmodium* species (LaPointe et al. 2005, Zsolt Garamszegi 2009, Ewen et al. 2012)). Similar to host communities, vector species vary in their relative abundance and competence for different pathogens. Additionally, vectors also differ in their host preferences (Takken and Verhulst 2013) and biting rates on different host species, leading to differential contact rates and chances for transmission. Finally, vectors may compete for resources during the larval life stage, which can alter life history traits and population growth rates (Barrera 1996, Agnew et al. 2000, Carrieri et al. 2003, Braks et al. 2004, Costanzo et al. 2005, Armistead et al. 2008, Kirby and Lindsay 2009, Juliano 2010, Alto 2011, Noden et al. 2015), as well as key vector traits (e.g., competence (Bevins 2008, Alto and Lounibos 2013)). Therefore, the model assumption of a homogeneous vector community is often invalid. Commonly studied vector-borne pathogens transmitted by more than one vector species include human malaria (Sinka et al. 2010), West Nile virus (WNV; (Turell et al. 2001, 2005)), Zika virus (ZIKV; (Ayres 2016)), Dengue virus (DENV; (Failloux et al. 2002, Gratz 2004)), Chikungunya virus (CHIKV; (Pialoux et al. 2007)), and avian malaria (Kimura et al. 2009). Thus, while multi-vector pathogens are abundant, there is relatively little theory developed to study multi-vector pathogens. A review of

seven vector-borne disease models by (Wonham et al. 2006) identified only one study (Choi et al. 2002) that included more than one vector species in modeling vector-borne disease transmission. While more recent vector-borne disease models have incorporated increasing biological complexity of vectors (e.g., (Dobson and Auld 2016)), there remains a gap in theoretical studies of disease transmission in systems with diverse vector communities.

To identify the conditions under which competition between vector species may influence transmission in vector-borne disease models, we studied a deterministic SEIR (host) – SEI (vector) model of disease transmission that can be generalized to include more than one vector species. We then solved this model across a gradient of vector competence and competition coefficients, then compared final host outbreak sizes to determine if and when competition between vectors dilutes or amplifies disease transmission. Model simulations of different competition scenarios indicate that disease transmission may be diluted in vector-borne disease systems when vectors compete more strongly with individuals of a different species than with conspecific individuals. These results emphasize the importance of incorporating vector community interactions into models of vector-borne disease transmission to obtain a more complete picture of vector-borne disease dynamics in multi-species systems.

Methods

To explore the conditions under which competition between vector species may influence transmission in vector-borne disease models, we created a compartmental SEI (vector) – SEIR (host) model that can be easily generalized to include multiple hosts and/or vectors (Figure 1). Given the relatively short lifespan of mosquito vectors compared to their hosts, we included demographic parameters (birth, death) for the vector only (Table 1).

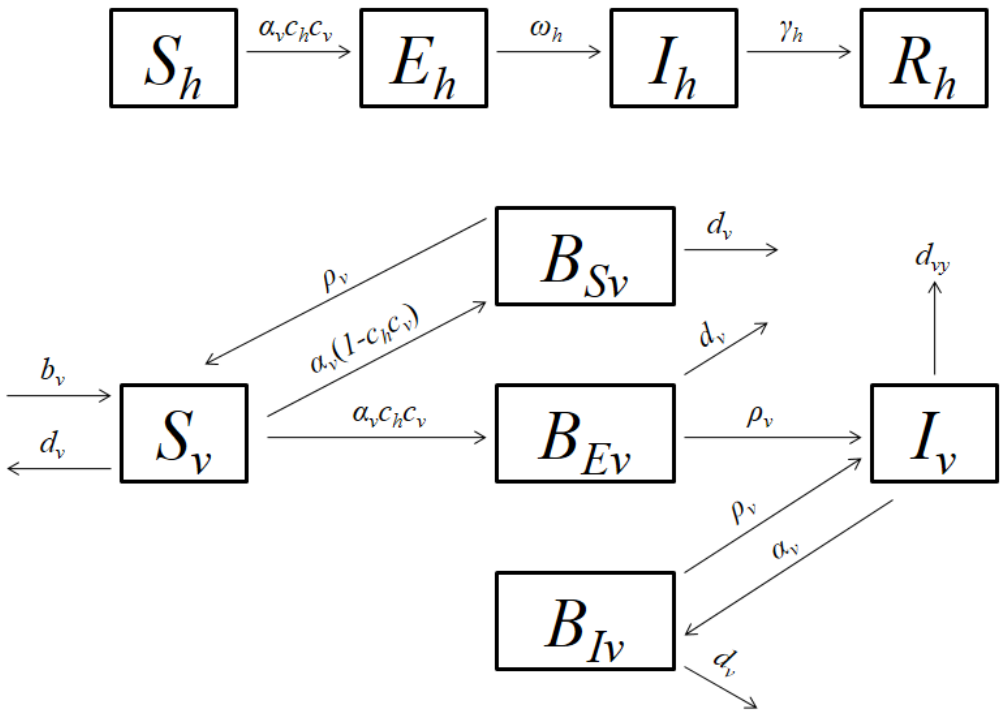

Figure 5.1: Conceptual diagram of single-vector model. Hosts move through compartments from susceptible (S_h) to exposed (E_h), infected (I_h), and recovered (R_h). Vectors (assumed to be mosquitoes) begin as susceptible (S_v), become infected (I_v) by feeding on an infected host, and move to one of three blood-fed compartments once per reproductive (gonotrophic) cycle. Susceptible vectors feeding on susceptible hosts move to the blood-fed susceptible compartment (B_{Sv}) before moving back to the susceptible compartment (S_v); susceptible vectors feeding on infected hosts move to the blood-fed exposed compartment (B_{Ev}) before moving to the infected compartment (I_v); and infected vectors move to the blood-fed infected compartment (B_{Iv}) before moving back to the infected compartment (I_v).

Table 5.1: Model parameters, their symbolic representation in the conceptual diagram and equations, values, and units (where applicable).

Parameter	Symbol	Value (range)	Units
Host preference (biting rate)	α_v	0.5	—
Host competence	c_h	0.5	—
Vector competence	c_v	0-1	—
Incubation period (within host)	ω_h	0.1	day^{-1}
Host recovery	γ_h	0.05	day^{-1}
Vector birth	b_v	10	day^{-1}
Vector death	d_v	0.01	day^{-1}
Gonotrophic cycle length	ρ_v	0.1	day^{-1}
Competition coefficient	α_{ij}	0.8-1.2	—
Carrying capacity	K	1000	mosquitoes

The following set of differential equations describes the transmission dynamics of a multi-vector system. All hosts start in the susceptible compartment and become exposed as they are fed on by infected vectors:

$$\frac{dS_h}{dt} = -\sum_{i=1}^{n} \frac{(c_h \cdot c_{vi} \cdot \alpha_{vi}) S_h I_{vi}}{N_{vi}}$$

(Eqn. 1),

where n is the total number of vectors in the system (n=2 in our simulations) and i indexes different vector species; c_h and c_{vi} are host and vector competence, respectively; α_{vi} is the biting rate of the vector on a specific host species, which incorporates host-feeding preferences of the vector into model dynamics; and N_{vi} is the total vector population among all compartments. After a host is bitten by an infected vector and transmission occurs, the host moves to the exposed compartment for the duration of the pathogen incubation period (e.g., the time it take for the host to become infectious):

$$\frac{dE_h}{dt} = \sum_{i=1}^{n} \frac{(c_h \cdot c_{vi} \cdot \alpha_{vi}) S_h I_{vi}}{N_{vi}} - \omega E_h$$

(Eqn. 2),

where ω is the incubation rate in days^{-1}. Once a host becomes infectious, it moves into the infected compartment until it recovers from infection:

$$\frac{dI_h}{dt} = \omega E_h - \gamma I_h$$

(Eqn. 3),

where γ is the recovery period in days^{-1}. Host recovery from infection confers permanent immunity, so recovered hosts remain in the recovered compartment until death (though not explicitly included in this model):

$$\frac{dR_h}{dt} = \gamma I_h$$

(Eqn. 4).

Vectors are born into the susceptible compartment and can leave the compartment through blood-feeding or natural death. Susceptible vectors that blood-feed on non-infected hosts move back into the susceptible compartment after they have assimilated their blood meal:

$$\frac{dS_{vi}}{dt} = b\left(1 - \frac{N_i + \alpha_{ij} N_j}{K}\right)\rho(B_{Svi} + B_{Evi} + B_{Ivi}) + \rho B_{Svi} - \frac{(c_h \cdot c_{vi} \cdot \alpha_{vi}) S_{vi} I_h}{N_{vi}}$$

$$- \frac{\alpha S_{vi}(1 - c_h \cdot c_{vi})(S_h + E_h + R_h)}{N_{vi}} \quad - dS_{vi}$$

(Eqn. 5),

$$\frac{dB_{Svi}}{dt} = \frac{\alpha S_{vi}(1 - c_h \cdot c_{vi})(S_h + E_h + R_h)}{N_{vi}} - \rho B_{Svi} - dB_{Svi}$$

(Eqn. 6),

where *b* is the vector birth rate in number of offspring produced per individual per day; N_i is the population size of vector species *i*; N_j is the population size of vector species *j*; α_{ij} is the competition coefficient which describes the relative strength of intraspecific versus interspecific competition (e.g., the effect of species *j* on species *i*); K is the carrying capacity for the vector; ρ is the duration of the gonotrophic cycle in days^{-1} (e.g., the period of time between blood meals); and *d* is the vector death rate. Susceptible vectors that blood-feed on infected hosts where transmission occurs move to the blood-fed exposed compartment until they assimilate their blood-meal, then move to the infected vector compartment:

$$\frac{dB_{Evi}}{dt} = \frac{(c_h \cdot c_{vi} \cdot \alpha_{vi})S_{vi}I_h}{N_{vi}} - \rho B_{Evi} - dB_{Evi}$$

(Eqn. 7).

Infected vectors that take a blood-meal (on hosts in any compartment) move to the blood-fed infected compartment until they assimilate their blood-meal and reproduce, then move back to infected vector compartment:

$$\frac{dB_{Ivi}}{dt} = \frac{\alpha I_{vi}N_h}{N_{vi}} - \rho B_{Ivi} - dB_{Ivi}$$

(Eqn. 8),

$$\frac{dI_{vi}}{dt} = \rho(B_{Evi} + B_{Ivi}) - \frac{\alpha I_{vi}N_h}{N_{vi}} - dI_{vi}$$

(Eqn. 9).

We solved this model numerically using the ordinary differential equations (ODE) solver 'lsoda' (Soetaert et al. 2010) in the R computing environment (R Core Team 2016). We explored the effect of vector competence on final host outbreak size while holding host competence and biting rate (host preference) constant. Two-vector model simulations started with 100 susceptible

hosts, 225 susceptible vectors of each species, and 25 infected vectors of each species. We repeated simulations of the two-vector model across a range of competition coefficients (i.e., $\alpha_{ij} = \{0.8, 1.0, 1.2\}$) to represent vector community dynamics when intraspecific competition is stronger than interspecific competition ($\alpha_{ij} = 0.8$), intraspecific competition is equal to interspecific competition ($\alpha_{ij} = 1.0$), and intraspecific competition is weaker than interspecific competition ($\alpha_{ij} = 1.2$).

Results

Simulations showed that when vector competence for both species was low (e.g., < 30%), relatively few hosts were infected (Figure 2). When interspecific was weaker than or equal to intraspecific competition in both species, the number of infected hosts increased quickly with increasing host competence. In these scenarios (intraspecific >= interspecific competition), it was possible for all hosts to become infected even when one vector species had a competence of 0% (e.g., one species was unable to acquire or transmit the pathogen). However when interspecific competition is stronger than intraspecific competition in at least one species, infection of all hosts only occurred when the competence of both species was high (e.g., > 75%).

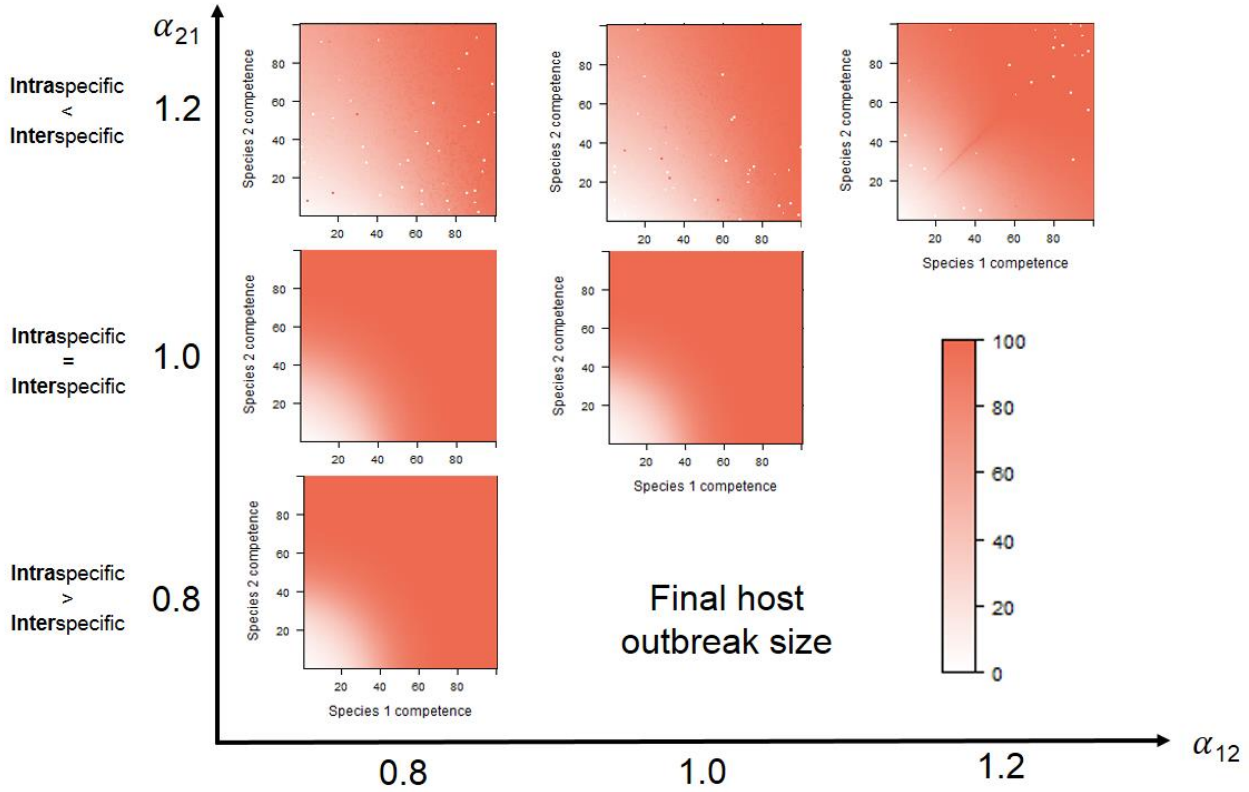

Figure 2: Final host outbreak size (out of 100 total hosts) for six competition scenarios. The competition coefficient for each species was specified as either 0.8 (intraspecific competition > interspecific competition), 1.0 (intraspecific competition = interspecific competition), or 1.2 (intraspecific competition < interspecific competition). The white pixels in the top row of plots indicate numerically unstable parameter combinations.

To visualize the effect of asymmetrical competition on final host outbreak size, we subtracted final host outbreak size of the baseline competition scenario ($\alpha_{12} = \alpha_{21} = 1.0$) from each of the other five scenarios (Figure 3). There was no difference in final host outbreak size (compared to the baseline competition scenario) when intraspecific competition was stronger than interspecific competition for both vector species. When intraspecific competition was stronger than interspecific competition for one species and intraspecific competition was equal to interspecific competition in the other species, total host outbreak size increased at very low values of vector competence (e.g., < 20 %). At slightly higher competence values (e.g., ~ 40 %), total host outbreak size slightly decreased relative to the baseline scenario). When intraspecific competition was weaker than interspecific competition (α_{12} and/or $\alpha_{21} = 1.2$) in at least one vector species, final host outbreak size decreased by up to 60 individuals relative to the baseline scenario. This pattern of decreased host outbreak size occurred across a wide range of vector competence values (e.g., 30% - 90%).

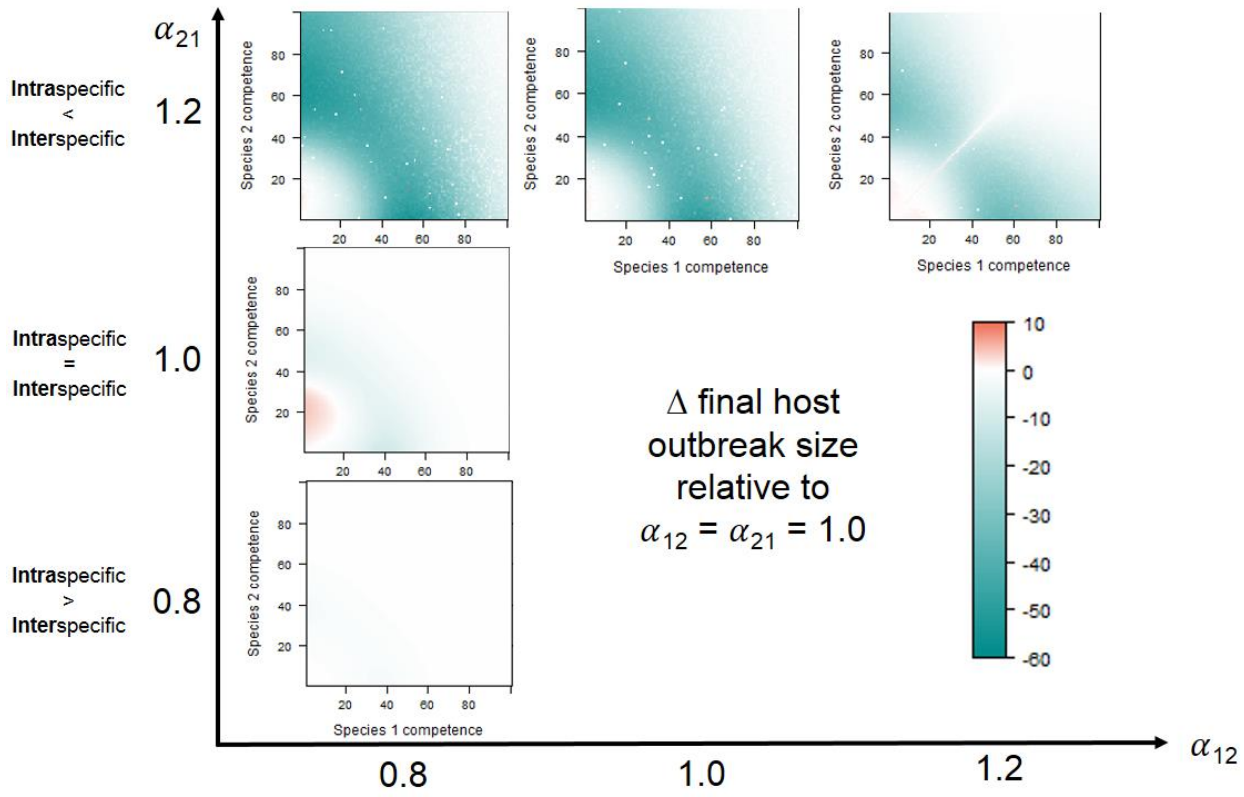

Figure 3: Change in final host outbreak size relative to the baseline scenario ($\alpha_{12} = \alpha_{21} = 1.0$) for each other competition scenario. Areas shaded in red represent parameter space where final host outbreak size increased (e.g., disease amplification), and areas shaded in red represent parameter space where final host outbreak size decreased (e.g., disease dilution) relative to the baseline scenario.

Discussion

Our simulations showed that when intraspecific competition was weaker than interspecific competition for at least one vector species, disease transmission was diluted across a broad range of vector competence values (e.g., 30% - 90%). These results suggest that disease transmission may be diluted in vector-borne disease systems when vectors compete more strongly with individuals of a different species. Exceptions to this pattern occurred when competence of both vectors was very low (< 20%) or very high (> 90%); in this parameter space, there was no change to final host outbreak size relative to the baseline competition scenario. Of the parameter space that we explored, amplification of disease transmission only occurred when intraspecific competition was weaker than or equal to interspecific competition in both vector species *and* vector competence was very low (< 20%). Researchers have previously proposed several mechanisms which may drive disease dilution in host communities, including interspecific competition between host species (Keesing et al. 2006). The results presented here lend weight to the inclusion of vector community interactions as an additional potential mechanism for dilution of disease transmission.

Future extensions of this model could include the incorporation of multiple host species and more than two vector species; additional parameters describing vector biology, such as temperature-dependence of vital rates; and elements vector community ecology, such as competition or predation. Though human malaria is a prime example of a vector-borne disease with a single host species and multiple vector species, many other multi-vector diseases are transmitted within a diverse community of host species. For example, West Nile virus (WNV) can be transmitted by roughly 60 mosquito species to nearly 300 avian species worldwide (Kramer et al. 2008). Scaling this model up to include more than one host species could provide information

on the relative importance of host community versus vector community diversity in the amplification or dilution of disease transmission. Extending this model to include additional components of vector biology (in a similar fashion to a recently developed model by (Dobson and Auld 2016)) would allow for fine-tuning the output of the model to systems transmitted by different groups of vectors (e.g., mosquitoes, ticks, or flies). Additionally, components of arthropod vector biology, such as survival and development rates, are highly temperature-dependent (RUEDA et al. 1990, Lyimo et al. 1992, Tun-Lin et al. 2000, Alto and Juliano 2001, Kirby and Lindsay 2009, Delatte et al. 2009, Muturi et al. 2012, Beck-Johnson et al. 2013, Couret et al. 2014, Simoy et al. 2015). Including temperature-dependence of traits of vectors in this model may help researchers to better understand how vector-borne disease transmission varies with temperature, as well as to determine the role that temperature plays in disease dilution and amplification. There is also the potential for predation on vectors at different life stages (Edgerly et al. 1999, Sunahara et al. 2002, Munga et al. 2006, DuRant and Hopkins 2008).

Future forms of this model could also include host epidemiology, such as pathogen-induced mortality (virulence) and waning immunity; pathogen epidemiology, such as extrinsic incubation period; and seasonality or multi-year dynamics. Pathogen-induced mortality and waning or partial immunity are common components of models of human infectious diseases (Nowak and May 1994), but have the potential to affect non-human host communities as well (Berger et al. 2005, Lively 2006). Infectious disease transmission dynamics are also influence by elements of pathogen epidemiology, especially extrinsic incubation period of vector-borne pathogens (Richards et al. 2007, Bellan 2010, Carpenter et al. 2011). Incorporating pathogen epidemiology into this model could aid in understanding the relative importance of vector, host, and pathogen traits in shaping disease dilution and amplification in vector-borne disease systems. Additionally, this model does

not currently account for seasonality or multi-year dynamics, which can be common in tropical vector-borne disease dynamics where transmission can occur (to some extent) year-round. Anticipating how multi-vector transmission dynamics will change in both the short term (i.e., seasonal) and long term (i.e., between years) will allow researchers to better forecast vector-borne disease transmission in tropical areas where the burden of these pathogens is often high (Hay et al. 2005, Hotez et al. 2008). Our results demonstrate the potential for both dilution and amplification of vector-borne disease transmission due to asymmetric competition between mosquito species. The model presented here is easily generalizable, allowing for future studies of the effects of vector community composition on disease transmission in an array of vector-borne disease systems.

References

Agnew, P., C. Haussy, and Y. Michalakis. 2000. Effects of Density and Larval Competition on Selected Life History Traits of Culex pipiens quinquefasciatus (Diptera: Culicidae). Journal of Medical Entomology 37:732–735.

Alto, B. W. 2011. Interspecific Larval Competition Between Invasive Aedes japonicus and Native Aedes triseriatus (Diptera: Culicidae) and Adult Longevity. Journal of Medical Entomology 48:232–242.

Alto, B. W., and S. A. Juliano. 2001. Precipitation and temperature effects on populations of Aedes albopictus (Diptera: Culicidae): implications for range expansion. Journal of medical entomology 38:646–656.

Alto, B. W., and L. P. Lounibos. 2013. Vector competence for arboviruses in relation to the larval environment of mosquitoes. Pages 81–101Ecology of parasite-vector interactions. Springer.

Armistead, J. S., J. R. Arias, N. Nishimura, and L. P. Lounibos. 2008. Interspecific larval competition between Aedes albopictus and Aedes japonicus (Diptera : Culicidae) in northern Virginia. Journal of Medical Entomology 45:629–637.

Aron, J. L., and R. M. May. 1982. The population dynamics of malaria. Pages 139–179The population dynamics of infectious diseases: theory and applications. Springer.

Ayres, C. F. J. 2016. Identification of Zika virus vectors and implications for control. The Lancet Infectious Diseases 16:278–279.

Barrera, R. 1996. Competition and resistance to starvation in larvae of container-inhabiting Aedes mosquitoes. Ecological Entomology 21:117–127.

Beck-Johnson, L. M., W. A. Nelson, K. P. Paaijmans, A. F. Read, M. B. Thomas, and O. N. Bjørnstad. 2013. The effect of temperature on Anopheles mosquito population dynamics and the potential for malaria transmission. PloS one 8:e79276.

Bellan, S. E. 2010. The importance of age dependent mortality and the extrinsic incubation period in models of mosquito-borne disease transmission and control. PLoS One 5:e10165.

Berger, L., G. Marantelli, L. F. Skerratt, and R. Speare. 2005. Virulence of the amphibian chytrid fungus Batrachochytrium dendrobatidis varies with the strain. Diseases of aquatic organisms 68:47–50.

Bevins, S. 2008. Invasive mosquitoes, larval competition, and indirect effects on the vector competence of native mosquito species (Diptera: Culicidae). Biological Invasions 10:1109–1117.

Braks, M. a H., N. A. Honório, L. P. Lounibos, R. Lourenço-De-Oliveira, and S. A. Juliano. 2004. Interspecific Competition Between Two Invasive Species of Container Mosquitoes, Aedes aegypti and Aedes albopictus (Diptera: Culicidae), in Brazil. Annals of the Entomological Society of America 97:130–139.

Carpenter, S., A. Wilson, J. Barber, E. Veronesi, P. Mellor, G. Venter, and S. Gubbins. 2011. Temperature Dependence of the Extrinsic Incubation Period of Orbiviruses in Culicoides Biting Midges. Plos One 6:e27987.

Carrieri, M., M. Bacchi, R. Bellini, and S. Maini. 2003. On the Competition Occurring Between Aedes albopictus and Culex pipiens (Diptera: Culicidae) in Italy. Environmental Entomology 32:1313–1321.

Choi, Y. H., C. Comiskey, M. D. A. Lindsay, J. A. Cross, and M. Anderson. 2002. Modelling the transmission dynamics of Ross River virus in Southwestern Australia. Ima Journal of Mathematics Applied in Medicine and Biology 19:61–74.

Civitello, D. J., J. Cohen, H. Fatima, N. T. Halstead, J. Liriano, T. A. McMahon, C. N. Ortega, E. L. Sauer, T. Sehgal, S. Young, and J. R. Rohr. 2015. Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proceedings of the National Academy of Sciences 112:8667–8671.

Costanzo, K. S., K. MORMANN, and S. A. JULIANO. 2005. Asymmetrical Competition and Patterns of Abundance of Aedes albopictus and Culex pipiens (Diptera: Culicidae). Journal of medical entomology 42:559–570.

Couret, J., E. Dotson, and M. Q. Benedict. 2014. Temperature, larval diet, and density effects on development rate and survival of Aedes aegypti (Diptera: Culicidae). PloS one 9:e87468.

Delatte, H., G. Gimonneau, A. Triboire, and D. Fontenille. 2009. Influence of Temperature on Immature Development, Survival, Longevity, Fecundity, and Gonotrophic Cycles of Aedes albopictus, Vector of Chikungunya and Dengue in the Indian Ocean. Journal of Medical Entomology 46:33–41.

Dizney, L. J., L. A. Ruedas, and others. 2009. Increased host species diversity and decreased prevalence of Sin Nombre virus. Emerging infectious diseases 15:1012–1018.

Dobson, A. D. M., and S. K. J. R. Auld. 2016. Epidemiological Implications of Host Biodiversity and Vector Biology: Key Insights from Simple Models. The American Naturalist 187:405–422.

DuRant, S. E., and W. A. Hopkins. 2008. Amphibian predation on larval mosquitoes. Canadian Journal of Zoology 86:1159–1164.

Edgerly, J. S., M. S. Willey, and T. Livdahl. 1999. Intraguild Predation Among Larval Treehole Mosquitoes, Aedes albopictus, Ae. aegypti, and Ae. triseriatus (Diptera: Culicidae), in Laboratory Microcosms. Journal of Medical Entomology 36:394–399.

Ewen, J. G., S. Bensch, T. M. Blackburn, C. Bonneaud, R. Brown, P. Cassey, R. H. Clarke, and J. Perez-Tris. 2012. Establishment of exotic parasites: the origins and characteristics of an avian malaria community in an isolated island avifauna. Ecology Letters 15:1112–1119.

Failloux, A.-B., M. Vazeille, and F. Rodhain. 2002. Geographic genetic variation in populations of the dengue virus vector Aedes aegypti. Journal of Molecular Evolution 55:653–663.

Gratz, N. G. 2004. Critical review of the vector status of Aedes albopictus. Medical and Veterinary Entomology 18:215–227.

Hay, S. I., C. A. Guerra, A. J. Tatem, P. M. Atkinson, and R. W. Snow. 2005. Tropical infectious diseases: Urbanization, malaria transmission and disease burden in Africa. Nature Reviews Microbiology 3:81–90.

Hooper, D. U., F. S. Chapin Iii, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, and others. 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological monographs 75:3–35.

Hotez, P. J., M. E. Bottazzi, C. Franco-Paredes, S. K. Ault, and M. R. Periago. 2008. The neglected tropical diseases of Latin America and the Caribbean: a review of disease burden and distribution and a roadmap for control and elimination. PLoS Negl Trop Dis 2:e300.

Juliano, S. A. 2010. Coexistence, Exclusion, or Neutrality? A Meta-Analysis of Competition between Aedes Albopictus and Resident Mosquitoes. Israel Journal of Ecology & Evolution 56:325–351.

Keesing, F., R. D. Holt, and R. S. Ostfeld. 2006. Effects of species diversity on disease risk. Ecology Letters 9:485–498.

Kimura, M., J. M. Darbro, and L. C. Harrington. 2009. Avian Malaria Parasites Share Congeneric Mosquito Vectors. Journal of Parasitology 96:144–151.

Kirby, M. J., and S. W. Lindsay. 2009. Effect of temperature and inter-specific competition on the development and survival of Anopheles gambiae sensu stricto and An. arabiensis larvae. Acta tropica 109:118–123.

Koella, J. C. 1991. On the use of mathematical models of malaria transmission. Acta tropica 49:1–25.

Kramer, L. D., L. M. Styer, and G. D. Ebel. 2008. A Global Perspective on the Epidemiology of West Nile Virus. Annual Review of Entomology 53:61–81.

LaPointe, D. A., M. L. Goff, and C. T. Atkinson. 2005. Comparative susceptibility of introduced forest dwelling mosquitoes in Hawai'i to avian malaria, Plasmodium relictum. Journal of Parasitology 91:843–849.

Lively, C. M. 2006. The ecology of virulence. Ecology Letters 9:1089–1095.

LoGiudice, K., R. S. Ostfeld, K. A. Schmidt, and F. Keesing. 2003. The ecology of infectious disease: effects of host diversity and community composition on Lyme disease risk. Proceedings of the National Academy of Sciences 100:567–571.

Lyimo, E. O., W. Takken, and J. C. Koella. 1992. Effect of rearing temperature and larval density on larval survival, age at pupation and adult size of Anopheles gambiae. Entomologia experimentalis et applicata 63:265–271.

Monath, T. P., and T. F. Tsai. 1987. St. Louis encephalitis: lessons from the last decade. The American journal of tropical medicine and hygiene 37:40S–59S.

Munga, S., N. Minakawa, G. Zhou, O.-O. J. Barrack, A. K. Githeko, and G. Yan. 2006. Effects of larval competitors and predators on oviposition site selection of Anopheles gambiae sensu stricto. Journal of medical entomology 43:221–224.

Muturi, E. J., M. Blackshear, and A. Montgomery. 2012. Temperature and density-dependent effects of larval environment on Aedes aegypti competence for an alphavirus. Journal of Vector Ecology 37:154–161.

Noden, B. H., P. A. O'neal, J. E. Fader, and S. A. Juliano. 2015. Impact of inter- and intra-specific competition among larvae on larval, adult, and life-table traits of Aedes aegypti and Aedes albopictus females. Ecological Entomology:n/a-n/a.

Nowak, M. A., and R. M. May. 1994. Superinfection and the Evolution of Parasite Virulence. Proceedings of the Royal Society of London B: Biological Sciences 255:81–89.

Ostfeld, R. S., and F. Keesing. 2000a. Biodiversity and disease risk: the case of Lyme disease. Conservation Biology 14:722–728.

Ostfeld, R. S., and F. Keesing. 2000b. Biodiversity series: the function of biodiversity in the ecology of vector-borne zoonotic diseases. Canadian Journal of Zoology 78:2061–2078.

Pialoux, G., B.-A. Gaüzère, S. Jauréguiberry, and M. Strobel. 2007. Chikungunya, an epidemic arbovirosis. The Lancet infectious diseases 7:319–327.

R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Randolph, S. E., and A. D. M. Dobson. 2012. Pangloss revisited: a critique of the dilution effect and the biodiversity-buffers-disease paradigm. Parasitology 139:847–863.

Richards, S. L., C. N. Mores, C. C. Lord, and W. J. Tabachnick. 2007. Impact of Extrinsic Incubation Temperature and Virus Exposure on Vector Competence of Culex pipiens

quinquefasciatus Say (Diptera: Culicidae) for West Nile Virus. Vector-Borne and Zoonotic Diseases 7:629–636.

Ruan, S., D. Xiao, and J. C. Beier. 2008. On the delayed Ross–Macdonald model for malaria transmission. Bulletin of mathematical biology 70:1098–1114.

RUEDA, L. M., K. J. PATEL, R. C. AXTELL, and R. E. STINNER. 1990. Temperature-Dependent Development and Survival Rates of Culex quinquefasciatus and Aedes aegypti (Diptera: Culicidae). Journal of Medical Entomology 27:892–898.

Salkeld, D. J., K. A. Padgett, and J. H. Jones. 2013. A meta-analysis suggesting that the relationship between biodiversity and risk of zoonotic pathogen transmission is idiosyncratic. Ecology Letters 16:679–686.

Sardelis, M. R., M. J. Turell, M. L. O'Guinn, R. G. Andre, and D. R. Roberts. 2002. Vector competence of three North American strains of Aedes albopictus for West Nile virus. Journal of the American Mosquito Control Association 18:284–289.

Schmidt, K. A., and R. S. Ostfeld. 2001. Biodiversity and the dilution effect in disease ecology. Ecology 82:609–619.

Searle, C. L., L. M. Biga, J. W. Spatafora, and A. R. Blaustein. 2011. A dilution effect in the emerging amphibian pathogen Batrachochytrium dendrobatidis. Proceedings of the National Academy of Sciences 108:16322–16326.

Simoy, M. I., M. V. Simoy, and G. A. Canziani. 2015. The effect of temperature on the population dynamics of Aedes aegypti. Ecological Modelling 314:100–110.

Sinka, M. E., Y. Rubio-Palis, S. Manguin, A. P. Patil, W. H. Temperley, P. W. Gething, T. Van Boeckel, C. W. Kabaria, R. E. Harbach, and S. I. Hay. 2010. The dominant Anopheles

vectors of human malaria in the Americas: occurrence data, distribution maps and bionomic précis. Parasites & Vectors 3:72.

Smith, D. L., K. E. Battle, S. I. Hay, C. M. Barker, T. W. Scott, and F. E. McKenzie. 2012. Ross, Macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. PLoS pathog 8:e1002588.

Smith, D. L., and F. Ellis McKenzie. 2004. Statics and dynamics of malaria infection in Anopheles mosquitoes. Malaria Journal 3:13.

Soetaert, K., T. Petzoldt, and R. W. Setzer. 2010. Solving Differential Equations in R: Package deSolve 33:1--25.

Sunahara, T., K. Ishizaka, and M. Mogi. 2002. Habitat size: a factor determining the opportunity for encounters between mosquito larvae and aquatic predators. Journal of Vector Ecology 27:8–20.

Swaddle, J. P., and S. E. Calos. 2008. Increased avian diversity is associated with lower incidence of human West Nile infection: observation of the dilution effect. PloS one 3:e2488.

Takken, W., and N. O. Verhulst. 2013. Host preferences of blood-feeding mosquitoes. Annual review of entomology 58:433–453.

Tun-Lin, W., T. R. Burkot, and B. H. Kay. 2000. Effects of temperature and larval diet on development rates and survival of the dengue vector Aedes aegypti in north Queensland, Australia. Medical and veterinary entomology 14:31–37.

Turell, M. J., D. J. Dohm, M. R. Sardelis, M. L. O'guinn, T. G. Andreadis, and J. A. Blow. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. Journal of medical entomology 42:57–62.

Turell, M. J., M. L. O'Guinn, D. J. Dohm, and J. W. Jones. 2001. Vector Competence of North American Mosquitoes (Diptera: Culicidae) for West Nile Virus. Journal of Medical Entomology 38:130–134.

Wonham, M. J., M. A. Lewis, J. Rencławowicz, and P. Van Den Driessche. 2006. Transmission assumptions generate conflicting predictions in host–vector disease models: a case study in West Nile virus. Ecology Letters 9:706–725.

Zsolt Garamszegi, L. 2009. Patterns of co-speciation and host switching in primate malaria parasites. Malaria Journal 8:110.