UNIVERSITY OF CENTRAL OKLAHOMA

Edmond, Oklahoma

Jackson College of Graduate Studies

PREFERRED PREY OF *TOXORHYNCHITES RUTILUS* BETWEEN TWO

COMMON VECTORS AND IMPLICATIONS FOR ITS FUTURE USE AS A

BIOCONTROL

A THESIS

SUBMITTED TO THE GRADUATE FACULTY

In partial fulfillment of the requirements

For the degree of

MASTER OF SCIENCE IN BIOLOGY

By

Daniel S. Marshall

Edmond, Oklahoma

2020

PREFERRED PREY OF *TOXORHYNCHITES RUTILUS* BETWEEN TWO COMMON VECTORS AND IMPLICATIONS FOR ITS FUTURE USE AS A BIOCONTROL

DANIEL S MARSHALL

APRIL 2020

JACKSON COLLEGE OF GRADUATE STUDIES AT THE UNIVERSITY OF

CENTRAL OKLAHOMA

A THESIS APPROVED FOR THE DEPARTMENT OF BIOLOGY

By *Wayne Lord*
Wayne Lord (Apr 16, 2020)
_____

Committee Chairperson

Elizabeth Allan
_____

Committee Member

Harold Cleveland
Harold Cleveland (Apr 16, 2020)
_____

Committee Member

Rebecca Williams
Rebecca Williams (Apr 15, 2020)
_____

Committee Member

ACKNOWLEDGMENTS

TABLE OF CONTENTS

II. CHAPTER 2: SURVIVAL OF *AEDES AEGYPTI* AND *CULEX*

QUINQUEFASCIATUS IN THE PRESENCE OF *TOXORHYNCHITES RUTILUS*

SEPTENTRIONALIS

III. CHAPTER 3: POTENTIAL DISTRIBUTION OF THE BIOCONTROL AGENT

TOXORHYNCHITES RUTILUS BY 2070

Appendices

LIST OF FIGURES

LIST OF TABLES

CHAPTER 2

CHAPTER 3

LIST OF APPENDICES

ABSTRACT OF THESIS

University of Central Oklahoma

Edmond, Oklahoma

NAME: Daniel S. Marshall

TITLE OF THESIS: Preferred Prey of *Toxorhynchites rutilus* Between Two Common

Vectors and Implications for its Future Use as a Biocontrol

DIRECTOR OF THESIS: Wayne D. Lord, Ph.D.

NUMBER OF PAGES: 125

ABSTRACT: In recent years, the incidence of mosquito-borne disease has increased on

a global scale. Currently, the most effective way to reduce the incidence of these diseases

is through the control of mosquitoes. Given the development of insecticide resistance and

public aversion to pesticides, natural mosquito controls are more necessary than ever as a

means of vector abatement. One natural control technique used in the United States is

inundative releases of the predatory mosquito, *Toxorhynchites rutilus*. The predatory

larvae colonize aquatic habitats preferred by mosquitoes where they feed on immature

vectors. It is unclear if *Tx. rutilus* preferentially feeds on one vector species over another

and what implications such a preference may have for mosquito control. Additionally,

biological control efficiency is expected to diminish in response to climate change. The

efficacy of continued use of *Tx. rutilus* in its southern range and potential for its use in

northern latitudes in the coming decades needs to be addressed. Here I measured the

predation of *Tx. rutilus* on *Aedes aegypti* and *Culex quinquefasciatus* and created species

distribution models of the predator's suitable habitat by the year 2070 under four climate change scenarios. Using quantitative analyses, I answered four hypotheses. First, the predation rate of T*x. rutilus* on one prey species will be greater than the other. Second, one prey species will have significantly greater survival probability than the other when in the presence of *Tx. rutilus.* Third, the projected southern habitat of *Tx. rutilus* will be retained under the four climate change models by the year 2070. Fourth, the projected range of *Tx. rutilus* will expand northward to include previously unoccupied areas.

Using a Mann-Whitney U test, Kaplan-Meier survivorship analysis, and a log-rank test, my analyses revealed no significant difference in predation rate or survivorship between prey species. The mean predation rate for *Ae. aegypti* was 0.363 and 0.381 for *Cx. quinquefasciatus*. The cumulative probability of survival at 12 hours for *Ae. aegypti* was 55.6% and 54.3% for *Cx. quinquefasciatus*. My results indicate that *Tx. rutilus* does not preferentially feed on one of the two vectors presented. I used a maximum entropy approach to create species distribution models for *Tx. rutilus* under four climate change scenarios by 2070. Mean temperature of warmest quarter, annual precipitation, and precipitation seasonality were the most important bioclimatic variables for suitable habitat. The centroid of the current possible habitat distribution of *Tx. rutilus* was in central Tennessee. Centroids shifted north-northeast as much as 280.1 km by 2070. The extreme change in area of greater than 50% suitable habitat probability was 141.1% with 99.4% area retained.

Given these results, I reject my hypotheses for predation rate and survivorship. Inundative mass releases for the purpose of general mosquito control will exert equal pressure on both *Ae. aegypti* and *Cx. quinquefasciatus*. I accept my habitat distribution

hypotheses. My models show limited change in current habitat as well as creation of northern habitats. Taken together, these studies indicate mosquito control utilizing mass releases of *Tx. rutilus* will be effective in controlling vectors broadly in the southern United States for the next 50 years. However, targeted releases intended for control of epidemic zoonoses will be ineffectual due to weakened predation pressure in response to the presence of heterospecific prey found in natural habitats. Additionally, suitable habitat will likely occur in the northern U.S. and southern Canada by that time making it possible to control northern vector populations using the predator. These results are promising for North American mosquito control programs for the continued and potential combat of vector mosquitoes using *Tx. rutilus*. Future research should include refined ecological niche modeling utilizing biotic factors such as conspecific co-occurrence and cannibalism in addition to the abiotic factors used here. Future predation studies should take place within microcosms with multiple trophic levels. In these microcosms, non-consumptive effects such as mosquito longevity and morphometrics should be measured in addition to direct consumption.

THESIS INTRODUCTION

Mosquito-borne illness continues to be a major public health concern despite more than a century of work to mitigate their incidence (Patterson 2016). Mosquito-borne diseases infected an estimated 347 million people in 2017 (WHO 2018). The incidence of these illnesses will rise due to increasing globalization, urbanization (Kilpatrick 2011), and climate change (Bouzid 2014). Much work has focused on prevention of these diseases by means of vaccines, but thus far, a cure-all has eluded researchers (Manning et al 2018). The most successful means of controlling mosquito-borne disease is by limiting human-mosquito interactions. Changing human behaviors and environment and by the deleterious reduction of mosquito populations are effective ways to reduce human contact with vectors (Becker et al 2010). A number of techniques are used to reduce mosquito populations including chemical controls (Faraji and Unlu 2016), the release of genetically modified adults and sterile males (Alphey et al 2010, Benelli et al 2016), mosquito-specific viruses, fungi, and bacteria (Atyame et al 2011), and the use of natural enemies such as predacious fish, plants, and arthropods (Becker et al 2010, Benelli et al 2016).

Mosquitoes of the genus *Toxorhynchites* Theobald are natural enemies of vector and pestiferous mosquitoes. *Toxorhynchites* larvae are predators of aquatic invertebrates and readily feed on mosquito larvae (Focks 2007). Adults feed on nectar and never take blood meals and are thus incapable of transmitting human or animal pathogens (Collins and Blackwell 2000). Because of their unique feeding adaptations and ability to locate hard to reach breeding habitats, these predacious mosquitoes are ideal for use as a biocontrol (Focks 2007). The majority of *Toxorhynchites* species live in the tropics where they breed in natural and artificial containers (Collins and Blackwell 2000). Two species

are native to the United States, *Tx. rutilus* (Coq.) and *Tx. moctezuma* (Dyar and Knab).

Toxorhynchites moctezuma ranges through Central America and Mexico with a small

population found in southern Arizona (Zavortink and Chaverri 2009). *Toxorhynchites*

rutilus is widely distributed across the eastern half of the United States (Darsie and Ward

2005).

The literature contains many records concerning the ability of *Tx. rutilus* to

control mosquitoes (Crans and Slaff 1977, Focks et al 1980, Focks et al 1982, Bailey et al

1983, Russo 1986, Campos and Lounibos 2000b). Mosquito control professionals take

advantage of this ability on large scales, breeding laboratory lineages of the predator *en*

masse and releasing them into areas at risk for mosquito-borne disease (Focks 2007,

Anita Schiller, personal communication). The efficacy of these mass releases under

climate change is in question as is the ability of *Tx. rutilus* to reduce populations of

specific vectors in the field. Here I present a review of the literature relevant to mosquito

control and the use of *Toxorhynchites* spp. as a biocontrol in addition to two original

studies inferring prey preference of this predator and the potential change in its habitat

under four different climate change scenarios.

REVIEW OF LITERATURE

Arboviruses

Zoonoses are diseases transmissible between vertebrate animals to humans by

direct or indirect means (WHO 1959). One such indirect means is by transmission

through a vector. A vector is an organism, often a hematophagous arthropod, capable of

transmitting or transporting a pathogen between hosts (Go 2014). Vector arthropods are

among the most significant causes of human disease globally and are responsible for many millions of deaths each year. The most concerning arthropod vectors are mosquitoes (Diptera: Culicidae), biting midges (Diptera: Ceratopogonidae), sandflies (Diptera: Psychodidae: Phlebotominae), tsetse flies (*Glossina* spp.), kissing bugs (Hemiptera: Reduviidae: Triatominae), fleas (Siphonaptera: multiple families) and hard ticks (Acari: Ixodidae). Of these arthropods, mosquitoes are the most significant vectors of human disease (Go 2014). Mosquitoes transmit protozoan (e.g. malaria), helminthic (e.g. lymphatic filariasis), bacterial (e.g. tularemia), and viral (arboviruses) pathogens to humans (Petersen et al 2009, Rodhain 2015).

Arbovirus transmission occurs between mosquitoes and reservoir hosts such as birds or rodents. Reservoir hosts serve as propagation sites where the virus replicates. At sufficient viremia, the host can infect mosquitoes with the virus during blood feeding. The virus incubates within the mosquito, increases viremia, and invades tissues (Go 2014). The virus may eventually invade the salivary glands, where it passes to either an incidental host or a reservoir host during blood feeding (Go 2014, Kuno 2005). In many cases, humans and other mammals are incidental hosts (Weaver and Reisen 2010). In an incidental host, the virus is incapable of building sufficient viremia to contribute to the natural transmission cycle (Mantke 2011). Dengue fever virus and Venezuelan equine encephalitis virus are both exceptions as human infections are capable of transmitting the virus back to mosquitoes during blood meals (Davis 2008). Once infected, the host may develop febrile illness with muscle, joint and eye pain, rash (Fauci 2016), fatigue, headache (Weaver 2015), hepatitis, pancreatitis, encephalitis, and meningitis (Simon 2017). Infections can be symptomatic or asymptomatic with symptomatic infections

divided into neuroinvasive and non-neuroinvasive (Simon 2017). Neuroinvasive

infections can present with meningitis, encephalitis, and paralysis (Simon 2017).

Mosquito-borne viruses capable of neuroinvasive disease include alphaviruses

(Togaviridae: *Alphavirus*) such as Eastern equine encephalitis virus, Western equine

encephalitis virus, and Venezuelan equine encephalitis virus, flaviviruses (Flaviviridae:

Flavivirus) such as Japanese encephalitis virus, St. Louis encephalitis virus, dengue fever

virus, yellow fever virus, Zika virus, and West Nile virus, and bunyaviruses

(Bunyaviridae: *Bunyavirus*) such as La Crosse virus (Davis 2008). North American

mosquitoes vector La Crosse virus, St. Louis encephalitis virus, Eastern equine

encephalitis virus, and West Nile virus, which cause sporadic outbreaks in the United

States (Burakoff et al 2018). Due to abiotic drivers of mosquito populations and disease,

the threat of arboviral outbreaks is increasing in the United States and abroad (Bouzid et

al 2014, Kraemer et al 2019).

Vectors

Mosquitoes are blood-sucking flies present on all continents except Antarctica

(Harbach 2007). They evolved their hematophagous habit from either entomophagous or

phytophagous dipteran ancestors (Waage 1979). Borkent and Grimaldi (2004) identified

the earliest mosquito fossil in 100 – 90-million-year-old amber from the mid-Cretaceous.

The authors suspect that they diverged from their sister clade in the Jurassic period,

forming the modern family Culicidae. Culicidae contains approximately 3,600 species of

which only several hundred are of human or animal concern. The family consists of three

subfamilies: Anophilinae, Culicinae, and Toxorhynchitinae (Fang 2010, Rodhain 2015).

The Culicinae are the primary arboviral vectors with more than 200 arboviruses

transmitted to humans and animals. The genera *Aedes* Meigen and *Culex* Linnaeus,

vector some of the most severe and pervasive arboviruses, both belong to this subfamily

(Lehane 2005). Males never blood feed, acquiring all necessary energy from nectar.

Females feed on nectar as a source of energy for locomotion and metabolic processes and

take blood meals only for the purpose of egg development (Peach and Gries 2019).

Because of this, only female mosquitoes vector pathogens.

The ability of mosquitoes to transmit pathogens depends on many factors

including their vectorial capacity, or the efficiency of pathogen transmission (Kean

2015), their feeding habits and host phenology (Kilpatrick et al 2006), capacity for

vertical transmission (Dohm et al 2002), population density, overwintering strategies

(Farajollahi et al 2011), and vector competence (Kilpatrick et al 2010). Arboviral vector

competence is the ability of a virus to infect and propagate in mosquito tissues resulting

in virulent saliva that can infect hosts during blood feeding (Kean 2015). Vector

competence changes between strains of pathogen, species of mosquito, vector population,

and with individuals of a population. This is because competence is dependent on vector-

pathogen genotype interactions (Hardy 1983, Lambrechts 2011), gut microbiota (Short et

al 2017), larval diet (Gunathilaka et al 2019), con- and heterospecific competition (Alto

et al 2008, Bellamy and Alto 2018), both consumptive and non-consumptive predation

effects (Meadows et al 2017), environmental factors (Muturi et al 2011), and mosquito

immune response (Mellor 2000, Tabachnick 2013). In competent vectors, the virus

overcomes four physiological barriers after ingestion of an infective blood meal. First, the

virus attaches to and reproduces in the midgut epithelium. Second, it escapes from the

midgut and propagates in tissues throughout the mosquito. Third, the virus invades and

reproduces within the salivary glands. Lastly, the virus enters the saliva where it must be able to survive until the mosquito feeds. Only after these barriers are overcome is the virus capable of entering a host during blood feeding (Tabachnick 2013, Kean 2015, Mills 2017). The ability of viruses to escape these four barriers is poorly understood, but it certainly depends on the viral strain, inoculating dose, the individual mosquito's immunity and microbiota, and physiology (Tabachnick 2013, Franz et al 2015, Mills et al 2017). *Aedes aegypti* (Linnaeus) and *Culex quinquefasciatus* Say are of particular concern for their complicity in the transmission of some of the most devastating arboviruses.

  Aedes aegypti, the Yellow Fever Mosquito, is a domesticated vector frequently found around dwellings. Its commensal tendency and adaptability of this mosquito has allowed it to invade nearly every area of human habitation on the planet (Powell 2013). In a study aimed at determining the preferred host of *Ae. aegypti,* Ponlawat and Harrington (2005) found that 99% of gravid females captured in Thailand had fed solely on human blood. The other 1% had blood meals taken from peridomestic animals such as dogs, pigs, chickens, cows, and goats. Artificial containers such as tires are the preferred oviposition sites around human dwellings. In sylvan habitats, tree holes and other naturally occurring containers are preferred (de Abreu 2015). *Aedes aegypti* is a vector of yellow fever virus, dengue virus, Zika virus, and chikungunya virus globally, although autochthonous transmission of these diseases is rare within the United States (Hahn 2017). In the United States, *Ae. aegypti* vectors Venezuelan equine encephalitis virus, Eastern equine encephalitis virus, West Nile virus, and La Crosse virus (Shope 1980, Turell 2005, Davis 2008). Because of its competence for a wide range of viruses and its

propensity for urban areas and success within peridomestic habitats, *Ae. aegypti* is a significant vector of arboviruses globally (Powell 2013).

Culex quinquefasciatus, the southern house mosquito, is the major West Nile virus vector through much of the United States and is part of the *Cx. pipiens* complex (Andreadis 2012, Turell 2012). *Culex pipiens* (L.) and *Cx. quinquefasciatus* and their hybrids are common members of the complex throughout temperate regions of the globe (Farajollahi 2011, Kothera 2012). In North America, the species complex varies regionally resulting in different rates of viral infections across the continent. The complex in the southern United States is comprised primarily of *Cx. quinquefasciatus* and *Cx. pipiens* dominates in the north (Andreadis 2012). The change in composition of *Cx. pipiens* complex has a drastic effect on the persistence of West Nile virus throughout the seasons. In the north, the virus overwinters in diapausing *Cx. pipiens* and reemerges in spring with the adult mosquitoes as well as their larvae via vertical transmission (Fonseca 2004, Farajollahi 2011). In southern regions, *Cx. quinquefasciatus* enters a state of quiescence and seeks out blood meals and mates at times of higher temperature throughout the year, allowing infections to occur year-round (Andreadis 2012, Nelms 2013).

Culex quinquefasciatus vectors West Nile virus, St. Louis encephalitis virus, Eastern equine encephalitis virus, Western equine encephalitis virus, and Venezuelan equine encephalitis virus in North America (Reisen 1992, Bogh 1998, Turell 2001, Davis 2008). In the United States it is a major vector of West Nile virus due to its overwintering strategies (Farajollahi 2005), high vectorial capacity (Sardelis 2001, Turell 2005), feeding behavior (Hamer 2008), urban habitation (Kilpatrick 2005, Ruiz 2010, Savage 2006), and

ability to transmit the virus vertically (Dohm 2002). Although considered an ornithophilic mosquito species, the *Cx. quinquefasciatus* will opportunistically feed on reptilian, amphibian, and mammalian hosts (Farajollahi 2011). These feeding habits change geographically as the species complex changes in composition (Nelms 2013) and seasonally as prey change their habits through the year (Lehane 2005). *Aedes aegypti* and *Cx. quinquefasciatus* commonly share oviposition sites in urban environments (Yee et al 2004). Species partition habitats based on differences in phenology and foraging behavior. *Culex* spp. begin development earlier in the year and dominate habitats until *Aedes* spp. begin to eclose (Vezzani 2009). *Culex* larvae graze on bacteria within the water column, but *Aedes* larvae feed on bacteria on submerged surfaces (Yee et al 2004). Larval co-occurrence makes controls simpler as a single treatment affects both vectors simultaneously (Riviere et al 1987).

Vector Control

To limit the transmission of mosquito-borne illness, vector control professionals employ various mosquito control methods. These methods include precautions taken by individuals such as bed nets, window screens, and source reduction (Healy et al 2014, Wilson et al 2014), application of insecticides (WHO 2019), release of genetically modified or sterilized adults, and biological controls such as mosquito-specific pathogens or predators (Becker et al 2010, Benelli et al 2016). Vector control professionals survey mosquitoes to determine necessary control measures. Surveillance utilizes light and CO_2 traps, dip nets, gravid female traps, and aspirators (Pezzin 2016, Holderman 2018). Mosquitocidal treatments of organophosphates, pyrethroids, neonicotinoids, or carbamates are commonplace in areas of concern (WHO 2019) and sometimes with

multiple control methods (Kroeger 2013). At times difficult to administer, pesticides can be detrimental to non-target arthropods and vertebrates when not applied correctly (Kroeger 2013, Ginsberg 2017). Another drawback to mosquitocidal controls is the development of insecticide resistance in mosquitoes upon exposure to sublethal doses (Richards 2018). Insecticides become ineffective as mosquito populations develop resistance and both *Ae. aegypti* and the *Cx. quinquefasciatus* have shown resistance to various mosquitocides (Dusfour 2011). Mosquitoes exposed to biological controls do not develop resistance and most do not have deleterious effects on non-target organisms (Huang et al 2017). Biological control methods include entomopathogenic bacteria, fungi, and viruses (Atyame 2011, Kean 2015), nematodes (Petersen 1981), predators such as flatworms (Tranchida 2014), fish (Chandra 2008), crustaceans (Hintz 1951, Kroeger 2013) mosquitoes (Steffan 1975), odonates (Akram 2016), hemipterans (Sivagnaname 2009), and crustaceans (Hintz 1951, Kroeger 2013), and genetically modified and sterilized adults (Lacroix 2012, Benelli 2016).

The symbiotic bacterium *Wolbachia* (Rickettsiales) occurs in many mosquito populations and its high prevalence in wild mosquitoes is due to its ability for vertical transmission (Atyame 2011). *Wolbachia* infections result in reproductive failure between adults with different strains of the bacterium and between infected and naive individuals in a phenomenon called cytoplasmic incompatibility (Atyame 2011). Entomopathogenic fungi of classes Hyphomycetes and Zygomycetes cause host death by production of toxins or by tissue inundation of fungal cells (Shah 2003). The fungus *Culicinomyces clavisporus* Couch (Hypocreales: Clavicipitaceae) is pathogenic only to flies within the suborder Nematocera making it an excellent choice for the biological control of

mosquitoes while minimally affecting other invertebrates (Couch 1935; 1974). Insect-specific flaviviruses occur in high concentrations in some mosquito populations (Hobson-Peters 2013). These viruses appear to replicate only in invertebrate cells and have been isolated from *Aedes* spp. and *Culex* spp. (Bolling 2011). Hobson-Peters (2013) found that they compete with other flaviviruses, such as West Nile virus in a phenomenon called superinfection exclusion whereby one virus inhibits the replication of the other. In some mosquito systems, this reduces the possibility of human infection as the insect-specific viruses can regulate pathogenic viruses (Hall-Mendelin et al 2016).

The Sterile Insect Technique (SIT) involves the release of sterilized male mosquitoes into a wild population (Knipling 1955, Lacroix 2012). Sterile males mate with wild females, thereby decreasing the local mosquito population by reducing the number of fertile eggs oviposited (Lacroix 2012). To sterilize SIT mosquitoes, laboratory type adult males are irradiated (Knipling 1955). A more advanced technique, release of insects carrying a dominant lethal (RIDL), genetically modifies male mosquitoes. Modified RIDL males carry the dominant lethal transgene insertion, OX513A (Lacroix 2012). Males mate with wild females passing down a copy of OX513A. Offspring of RIDL mosquitoes experience OX513A-induced mortality at a predetermined developmental stage, typically in the fourth instar or pupal stage (Lacroix 2012).

Freshwater planaria (Platyhelminthes: Turbellaria) are carnivorous and seek out small aquatic arthropods as prey (Legner 1975). Planaria often co-occur in habitats with immature mosquitoes and are extremely effective at reducing the number of emerging adults (Legner 1975). Although they readily feed on larvae, planaria do not prey on eggs or pupae (Kar 2003). Mermithid nematodes (Mermithida: Mermithidae) parasitize

mosquitoes (Platzer 1980). In laboratory studies, nematodes are effective at controlling

mosquitoes. Field tests of mermithid effects on mosquitoes yield mixed results due to

nematodes falling prey to other aquatic invertebrates (Platzer 1980, Petersen 1981).

Further, these nematodes are intolerant of low oxygen and high salt environments

rendering them ineffective in some mosquito habitats (Platzer 1980). Nematode control is

better suited for integrated management methods rather than a stand-alone control

(Petersen 1981). The copepods *Macrocyclops albidus* and *Mesocyclops* spp. (Cyclopoida:

Cyclopidae) attack and kill 100% of *Aedes albopictus* larvae in laboratory experiments

(Marten 1989). Because of their ability to survive desiccation, broad global distribution,

high fecundity, and easy application in field conditions, cyclopoid copepods are excellent

candidates for the biological control (Marten 1989). Larvivorous fish such as *Gambusia*

spp. (Cyprinodontiformes: Poeciliidae) feed on mosquito larvae and have been used

extensively as biocontrol agents since the early twentieth century (Chandra et al 2008a).

They efficiently lower mosquito populations, but when introduced into non-native waters

they frequently become invasive (Pyke 2005). Another biocontrol method of great

potential is the release of predatory mosquitoes. Members of the genus *Toxorhynchites*

are predators in their larval stadiums. Adults feed exclusively on nectar and never blood

feed (Collins and Blackwell 2000). The control of vector and pestiferous mosquitoes

using the predator, *Tx. rutilus septentrionalis* Dyar and Knab, is an effective and

environmentally safe means of reducing incidence of arboviral disease vectored by North

America tree hole mosquitoes (Focks 2007).

Toxorhynchites

Adults of this genus are diurnal, large, and brilliantly colored (Trimble 1974). Dyar and Knab (1907) provided early descriptions of both male and female *Tx. r. septentrionalis* (as *Megarhinus septentrionalis*). The male thorax is blue and violet with a yellow median stripe, the abdomen is blue dorsally and yellow ventrally with a violet median stripe, fourth tarsal segments are white; females are similar in coloration, but with the abdomen green turning blue distally and each tarsal segment has white markings (Dyar and Knab 1907). Both sexes are similar in size, growing to 7 mm long with a 12 mm wingspan and a pronounced bend in the proboscis (Jones and Schreiber 1994, Lounibos 1996). Eggs are large and ovoid with a granular texture upon close inspection (Focks 2007, Marshall, personal observation). Upon oviposition, ova are white and darken with age (Anita Schiller, personal communication). Larvae are red brown in color, robust bodied, and large with dense setae (Focks 2007). Fourth instar larvae grow to nearly 2 cm (Jones and Schreiber 1994).

Distribution

Toxorhynchites spp. prefer sylvan habitats in both the New and Old Worlds. Of the 70 species within the genus, most are located in the tropics. Some species range as far north as 45°N in Canada and 54°N in Russia (Focks 2007). Two species, *Tx. moctezuma* and *Tx. rutilus,* represent the genus in the United States (Darsie and Ward 2005). *Tx. rutilus* consists of two subspecies, *Tx. r. rutilus* and *Tx. r. septentrionalis.* The former inhabits the eastern coast of Florida through Georgia and South Carolina (Frank 1984) while the latter ranges from northern Mexico to southern Canada between the Great Plains and the Atlantic coast (Focks 2007, Darsie and Ward 2005).

Life Cycle

Females oviposit singly into containers such as tree holes, leaf axils, bamboo shoots, and artificial containers (Bailey 1983, Steffan 1985). Females hover in an elliptical pattern above the mouth of a container releasing eggs at the apex of the ellipse flinging the ova into the container (Breland 1949, Marshall unpublished data). They do not appear to have a preference in water quality, as females do not touch the water during oviposition (Yap 1984). Focks (2007) described females ovipositing on black surfaces such as a telephone and a pair of sunglasses suggesting that visual and not chemosensory cues trigger oviposition. Ova are hydrophobic and remain at the surface of the water until eclosion (Linley 1993). Eggs deposited in suitable containers develop 24 to 48 hours after oviposition (Holzapfel 1976).

Toxorhynchites spp. develop more quickly in natural containers compared to artificial containers. The relatively greater prey abundance found in natural containers contributes to this success (Campos and Lounibos 2000). Male *Tx. rutilus* develop more quickly than females. Larval instars in males require less time than in females, but pupal development requires the same amount of time for both sexes (Lounibos 1996). Each successive instar takes longer to complete for both sexes (Trpis, 1972). The average development time from hatch to adult emergence is 18.9 days for males and 19.6 days for females at 27°C (Lounibos 1996). Attempts made to rear *Tx. r. septentrionalis* in the laboratory have been largely unsuccessful. Trimble (1974), Holzapfel (1976), and Crans (1977) all were able to maintain colonies, but the use of induced copulation was required, as the mosquitoes would not readily mate in the lab setting. The Harris County Precinct 4 Biocontrol Initiative (Texas, USA) has successfully reared *Tx. r. septentrionalis* using

rearing cages that facilitate mating pair coupling (Schiller 2015; 2019). Prior to copulation, pairs harmonize wingbeats in the upper part of their cage then couple and fall to the bottom of the cage where they release (Schiller personal communication, Marshall unpublished data). Taller rearing cages are required for mating success (Schiller 2015; 2019). Other mosquito control agencies have had similar success with walk-in rearing cages (Schiller personal communication).

Feeding Behavior

Toxorhynchites larvae are ambush predators that typically lie motionless on the substrate waiting for prey (Russo 1986). One study found that *Tx. splendens* relocate and swim toward prey that move vigorously (Zuharah 2015). Researchers have proposed that larvae detect prey movement via deflection of setae (Linley 1990). Lounibos et al (1987) noted that *Tx. haemorrhoidalis* (Fabricius) attack probes when vibrated at a certain frequency. Larvae respond to visual and tactile cues, often slowly bending towards prey or entirely repositioning their body before striking (Linley 1990). Strikes are rapid, only taking 0.05 seconds (Linley 1990). During this time, the head extends forward, the palatal brushes open, grasp prey, and then the head retracts (Russo 1986, Linley 1990). Palatal brushes are recurved with terminal hooks and have overlapping arcs of motion which aid in grasping prey. Head extension appears to be due to hydrostatic pressure induced by contractions of muscles in abdominal segments I - III (Linley 1990). When a strike fails, *Toxorhynchites* often reposition and strike again. Typically, subsequent strikes result in successful capture of prey (Linley 1990, Marshall unpublished data). After consuming prey, larvae contort their abdomen into an "S" shape and hold this position for several minutes. Posturing likely facilitates movement of prey within the gut as larvae that had

taken multiple prey often defecated immediately after assuming this position (Rubio and Ayesta 1984, Russo 1986, Marshall unpublished data).

Campos and Lounibos (2000) performed midgut dissections of wild *Tx. rutilus* found in natural and artificial containers. Mosquitoes accounted for 6% of their diet in tree holes and 5% in tires. Smaller invertebrates such as rotifers and microcrustaceans were the dominant prey items. Terrestrial arthropods including mites and spiders consisted of 8% of tree hole prey and 9% of tire prey. The study showed that prey is dependent on habitat and that *Tx. rutilus* is a generalist predator. Perhaps most importantly, these results demonstrate that in field settings, *Tx. rutilus* does not exert a strong consumptive pressure on mosquitoes.

The literature reports mixed results on the control of mosquitoes by *Toxorhynchites* spp. in laboratory and field settings. In the laboratory, *Toxorhynchites* larvae are capable of consuming large numbers of mosquitoes. Trpis (1972) observed *Tx. brevipalpis* killing on average 154 – 358 *Ae. aegypti* depending on temperature. Crans and Slaff (1977) found that female *Tx. r. septentrionalis* kill on average nearly 238 *Ae. aegypti* during their immature stages while males kill an average of 227 larvae at 27°C. *Toxorhynchites* larvae are polyphagous and they experience reduced efficacy for mosquito control in the presence of other prey (Lounibos et al 1987, Campos and Lounibos 2000). In the field, *Toxorhynchites* spp. will perform with less efficiency than in the laboratory. Indeed, predation by *Tx. rutilus* on *Ochlerotatus triseriatus* in Florida tree holes did not cause local extinctions of prey species (Lounibos et al 1997, Nannini and Juliano 1997). However, Bradshaw and Holzapfel (1983) did find that *Tx. rutilus* exerted greater predation pressure on *Oc. triseriatus* than two other vectors in the shared

habitat. In 1980, Focks et al (1982) released *Tx. r. rutilus* in New Orleans and observed a 74% reduction in the populations of *Aedes* and *Culex* species. The same predator reduced *Ae. aegypti* in a Florida tire dump by 50% (Bailey et al 1983). The 1955 introduction of *Tx. amboinensis* and *Tx. brevipalpis* into American Samoa resulted in the successful control of *Ae. polynesiensis* (Peterson 1956, Engber et al 1978). Focks et al (1980) observed seasonal eradication of *Ae. aegypti* on Seahorse Key, Florida due to *Tx. rutilus* predation. Other releases were less successful, however. *Tx. amboinensis* released on the Hawaiian Islands in 1955 was unable to control its target organism, *Ae. albopictus* (Hu 1955, Nakagawa 1963). In New Orleans, released *Tx. r. rutilus* adults ignored their intended oviposition sites and instead moved into nearby forest resulting in no control of *Ae. aegypti* (Focks et al 1983). Diminished efficacy and outright failures of field studies are attributed to complex aquatic communities where the predator selects from a diverse prey community (Campos and Lounibos 2000). Additionally, low *Toxorhynchites* population growth rate as a result of slow development time (Trpis 1981, Bailey 1983), *Toxorhynchites* adults and immatures falling prey to other predators (Lounibos 1987), and predator-prey asynchrony in oviposition sites and emergence times (Lounibos 1979, Focks et al 1983, Annis et al 1990) reduce efficacy in the field. Repeat mass releases of *Toxorhynchites* spp. just before and throughout the mosquito season can mitigate some of these problems, but differences in oviposition site selection remain an issue (Bailey 1983, Jones 1993).

Prepupal Surplus Killing

Besides seeking out habitats in which vector and pest mosquitoes breed, *Toxorhynchites* spp. demonstrate an additional behavior that is beneficial for mosquito

control. In a phenomenon called surplus killing the predators kill but do not consume other organisms in excess. A number of aquatic invertebrate predators such as odonates and the midge *Corethrella appendiculata* exhibit this behavior (Johnson et al. 1975, Lounibos 2008). In fourth instar *Toxorhynchites* larvae, the onset of surplus killing is 3 – 4 days prior to pupation and peaks 24 to 48 hours before pupation (Crans and Slaff 1977, Lounibos 2008). Interestingly, only *Tx. splendens* performs surplus killing outside of the terminal instar (Chan 1968). Additionally, *Tx. rutilus* behaves in the same manner as it begins to undergo diapause in its fourth instar (Lounibos 1998). Male *Toxorhynchites* larvae exhibit surplus killing to a greater degree than females and at times eliminate 100% of prey offered (Crans and Slaff 1977). There are two hypotheses proposed to explain surplus killing in *Toxorhynchites* larvae. First, the behavior removes potential predators before the individual becomes a vulnerable pupa (Corbet and Griffiths 1963). Second, by reducing the availability of prey, younger unrelated conspecifics will be less successful leaving less competition for future progeny (Russo 1986). Still, it is unclear how and why *Toxorhynchites* spp. exhibit surplus killing. Future research could work to develop genetically modified larvae that begin surplus killing earlier to enhance the impact of the behavior.

Cannibalism

Cannibalism is a major source of mortality of immature *Toxorhynchites* spp. Mortality due to cannibalism of eggs and first instar larvae is significantly greater than that of later life stages (Linley and Duzak 1989, Amalraj and Das 1992). Early instar larvae more frequently consume conspecifics than later instars (Annis et al 1990b). In the absence of prey larvae, *Toxorhynchites* larvae actively seek out and impartially consume

17

conspecific and heterospecific eggs (Linley and Duzak 1989, Linley 1993). When

deprived of heterospecific prey, *Tx. splendens* consumes 40 – 60% of first instar

conspecifics and nearly all eggs present (Amalraj and Das 1992). Some *Toxorhynchites*

spp. rarely participate in cannibalism while others will take conspecifics despite the

availability of other prey (Annis et al 1990b). Cannibalism is common in temporary

aquatic habitats where crowding, desiccation, and lack of prey availability are frequent

(Yasuda and Hashimoto 1995). In general, two factors control cannibalism in these

systems. First, predators more frequently encounter conspecifics at low prey density such

that prey density is inversely proportional to cannibalism intensity (Annis et al 1990b,

Yasuda and Hashimoto 1995). Second, age structure diversity of the conspecific

population enables later instars to exert greater predation pressure on earlier, smaller

instars (Yasuda and Hashimoto 1995). Same instar cannibalism is largely size dependent

with the larger larvae overpowering the smaller (Campos and Lounibos 2000). Size-

specific predation regulates the body mass of *Toxorhynchites* larvae in the wild. Lounibos

(1996) reported the body mass of co-occurring fourth instars differs by 4.3 mg on average

while similar cohorts occurring alone differ by 13.9 mg on average. Cannibalism is

advantageous for *Toxorhynchites* spp. because it lends to the survival of individuals

through drought and prey-depletion that would otherwise cause local extinctions (Yasuda

and Hashimoto 1995).

Non-consumptive Predation Effects

Changes in prey behavior in response to predation risk alters prey fitness (Relyea

2000, Peacor and Werner 2001). The ability to acquire food, longevity, fecundity, and

development time change in response to behavioral changes and are major trophic drivers

in aquatic communities (Preisser et al 2005). In mosquitoes, these factors contribute to vector competence (Meadows et al 2017). Some researchers observed shorter development time was due to more active feeding and increased prey density (Grill and Juliano 1996, Zuharah 2015). While this behavior exposes individuals to predators, it does allow them to emerge sooner and eliminate the risk of aquatic predation (Kesavaraju and Juliano 2004). Alternatively, other species opt for slower development time and less food in exchange for lower predation risk (Peacor and Werner 2001). The results of which are reduced adult mass and longevity both of which lower vector competence (Fischer et al 2012, Meadows et al 2017, Bellamy and Alto 2018). Prey responses vary with predator community dynamics. In complex systems of multiple predators, prey alter their behavior based on the perceived greatest threat (Meadows et al 2017). The change in behavior is commensurate with risk of predation, food availability, and prey density. In high density, low food availability systems riskier behaviors prevail despite predator presence (Turner 2004). Some predator-prey systems suggest increased fitness for mosquitoes in response to predation of con- and heterospecifics. Daugherty et al (2000) found that the availability of prey carcasses contributed to increased mass, survivorship, and shortened development time of *Ae. aegypti* and *Ae. albopictus*. Grazing larvae are more successful as a result of increased bacterial growth due to the presence of carcasses (Albeny-Simoes et al 2015). Although the larvae observed by Albeny-Simoes et al are the same shown reducing their feeding behavior in other studies (Grill and Juliano 1996, Kesavaraju and Juliano 2004, Juliano et al 2019). Many of the above studies focused on the non-consumptive effects of *Tx. rutilus*. The complex interactions observed were often in simple laboratory or semi-field systems. Future research into more complex

Toxorhynchites systems will provide useful information on the ramifications of non-consumptive effects on mosquito populations and vector competence.

The Future of Mosquito Control

In addition to the consideration of non-consumptive effects, future research should consider climate change as the incidence of mosquito-borne disease will broadly increase in response to a warmer, wetter climate (Epstein 1998, Hongoh et al 2012, Ryan et al 2019). Models predicting changes in mosquito population through time under climate change scenarios are useful in explaining mosquito-borne disease dynamics and predicting geographic areas at risk of future disease outbreaks (Epstein et al. 1998, Hongoh et al 2012, Brown et al 2015). Poleward latitudes and higher elevations are increasingly at risk of emerging diseases due to warmer and wetter conditions for longer periods each year (Ryan et al 2019). The extent to which changing climatic conditions will affect *Toxorhynchites* spp. and other biocontrols is currently unclear (Lord 2007). Thurman et al (2017) predict that native natural enemies may be at risk of extinction due to climate change. Ecological niche modeling of *Tx. rutilus* using the Maxent approach indicates that the species, like most mosquitoes, will expand its native range but the model does not support the hypothesis that extinction events will occur within its native range (Marshall and Butler, unpublished manuscript). Additionally, researchers predict poor performance of previously successful biocontrols in response to climate change (Guzman et al 2016). Mosquito predation increased in response to warmer habitats. However, development time decreases in these warmer conditions resulting in greater numbers of mosquitoes emerging despite increased predation (Culler et al 2015). Predicted increase in mosquito-favorable conditions along with insecticide resistance

mean that vector control professionals will need to utilize more rigorous and novel

abatement methods to enact control in the future (Benelli et al 2016, Thomas 2018).

REFERENCES CITED

Akram W, Ali-Khan HA. 2016. Odonate nymphs: generalist predators and their potential

in the management of dengue mosquito, *Aedes aegypti* (Diptera: Culicidae). *J*

Arthropod-Borne Dis, 10(2):252-257.

Albeny-Simoes D, Murrell EG, Vilela EF, Juliano SA. 2015. A multifaceted trophic

cascade in a detritus-based system: density-, trait-, or processing-chain-mediated

effects? *Ecosphere*, 6(3).

Alphey L, Benedict M, Bellini R, Clark GC, Dame DA, *et al*. 2010. Sterile-insect

methods for control of mosquito-borne diseases: an analysis. *Vector-Borne*

Zoonot, 10(3):295-311.

Alto BW, Lounibos LP, Mores CN, Reiskind MH. 2008. Larval competition alters

susceptibility of adult *Aedes* mosquitoes to dengue infection. *Proc R Soc*, *B*

275:463-471.

Amalraj DD, Das PK. 1992. Cannibalism and carnivory in *Toxorhynchites splendens*

(Diptera: Culicidae). *SE Asian J Trop Med*, 23(3):450-452.

Andreadis TG. 2012. The contribution of *Culex pipiens* complex mosquitoes to

transmission and persistence of West Nile virus in North America. *J Am Mosquito*

Contr, 28(4):137-151.

Annis B, Nalim S, Hadisuwasono, Widiarti, Boewono DT. 1990a. *Toxorhynchites*

amboinensis larvae released in domestic containers fail to control dengue vectors

in a rural village in central Java. *J Am Mosquito Contr*, 6(1):75-78.

Annis B, Sarojo UTIB, Hamzah N, Trenggono B. 1990b. Laboratory studies of larval
cannibalism in *Toxorhynchites amboinensis* (Diptera: Culicidae). *J Med Entomol*,
27(5):777-783.

Atyame CM, Pasteur N, Dumas E, Tortosa P, Tantely ML, *et al*. 2011. Cytoplasmic
incompatibility as a means of controlling *Culex pipiens quinquefasciatus*
mosquito in the islands of the South-Western Indian Ocean. *PLoS Negl Trop Dis*,
5(12):e1440.

Bailey DL, Jones RG, Simmonds PR. 1983. Effects of indigenous *Toxorhynchites rutilus
rutilus* on *Aedes aegypti* breeding in tire dumps. *Mosquito News*, 43(1):33-37.

Becker N, Petric D, Zgomba M, Boase C, Madon M, *et al*. 2010. *Mosquitoes and Their
Control*, 2nd Edition. Springer-Verlag Berlin Heidelberg.

Bellamy SK, Alto BW. 2018. Mosquito responses to trait- and density-mediated
interactions of predation. *Oecologia*, 187:233-243.

Benelli G, Jeffries CL, Walker T. 2016. Biological control of mosquito vectors: past,
present, and future. *Insects*, 7(52).

Bogh C, Pedersen EM, Mukoko DA, Ouma JH. 1998. Permethrin-impregnated bednet
effects on resting and feeding behaviour of lymphatic filariasis vector mosquitoes
in Kenya. *Med Vet Entomol*, 12:52-59.

Bolling BG, Eisen L, Moore CG, Blair CD. 2011. Insect-specific flaviviruses from *Culex*
mosquitoes in Colorado, with evidence of vertical transmission. *Am J Trop Med
Hyg*, 85(1):169-177.

Borkent A, Grimaldi DA. 2004. The earliest fossil mosquito (Diptera: Culicidae), in mid-
cretaceous Burmese amber. *Ann Entomol Soc Am*, 97(5):882-888.

Bouzid M, Colon-Gonzalez FJ, Lung T, Lake IR, Hunter PR. 2014. Climate change and

the emergence of vector-borne diseases in Europe: case study of dengue fever.

BMC Public Health, 14:781.

Bradshaw WE, Holzapfel CM. 1983. Predator-mediated, non-equilibrium coexistence of

tree-hole mosquitoes in southeastern North America. *Oecologia*, 57:239-256.

Breland OP. 1949. The biology and the immature stages of the mosquito, *Megarhinus*

septentrionalis Dyar & Knab. *Ann Entomol Soc Am*, 42(1):38-47.

Brown HE, Young A, Lega J, Andreadis TG, Schurich J, Comrie A. 2015. Projection of

climate change influences on U.S. West Nile virus vectors. *Earth Interact,* 19(18).

Burakoff A, Lehman J, Fischer M, Staples E, Lindsey NP. 2018. West Nile virus and

other nationally notifiable arboviral diseases – United States, 2016. *MMWR-*

Morbid Mortal W, 67(1):13-17.

Campos RE, Lounibos LP. 2000. Natural prey and digestion times of *Toxorhynchites*

rutilus (Diptera: Culicidae) in southern Florida. *Ann Entomol Soc Am*,

93(6):1280-1287.

Chan KL. 1968. Observations on *Toxorhynchites splendens* (Wiedemann) (Diptera:

Culicidae) in Singapore. *Mosquito News*, 28(1):91-95.

Chandra G, Bhattacharjee I, Chatterjee SN, Ghosh A. 2008. Mosquito control by

larvivorous fish. *Ind J Med Res*, 127:13-27.

Collins LE, Blackwell A. 2000. The biology of *Toxorhynchites* mosquitoes and their

potential as biocontrol agents. *BioControl*, 21(4):105N-116N.

Corbet PS, Griffiths A. 1963. Observations on the aquatic stages of two species of *Toxorhynchites* (Diptera: Culicidae) in Uganda. *Proc R Entomol Soc*, 38(7-9):125-135.

Couch JN. 1935. A new saprophytic species of *Lagenidium* with notes on other forms. *Mycologia*, 27(4):376-387.

Couch JN, Romney SV, Rao B. 1974. A new fungus which attacks mosquitoes and related diptera. *Mycologia*, 66(2):374-379.

Crans WJ, Slaff ME. 1977. Growth and behavior of colonized *Toxorhynchites rutilus septentrionalis*. *Mosquito News*, 37(2):207-211.

Culler LE, Ayres MP, Virginia RA. 2015. In a warmer Arctic, mosquitoes avoid increased mortality from predators by growing faster. *Proceedings of the Royal Society B*, 282:20151549.

Darsie RF, Ward RA. 2005. *Identification and Geographical Distribution of the Mosquitoes of North America, North of Mexico*. 2nd Edition. University Press of Florida, Gainesville, Florida.

Daugherty MP, Alto BW, Juliano SA. 2000. Invertebrate carcasses as a resource for competing *Aedes albopictus* and *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol*, 37(3):364-372.

Davis LE, Beckham JD, Tyler KL. 2008. North American encephalitic arboviruses. *Neurol Clin*, 26(3):727-ix.

de Abreu FVS, Morais MM, Ribeiro SP, Eiras AE. 2015. Influence of breeding site availability on the oviposition behavior of *Aedes aegypti*. *Mem Inst Oswaldo Cruz*, 110(3):669-676.

Dohm DJ, Sardelis MR, Turell MJ. 2002. Experimental vertical transmission of West

 Nile virus by *Culex pipiens* (Diptera: Culicidae). *J Med Entomol*, 39(4):640-644.

Dusfour I, Thalmensy V, Gaborit P, Issaly J, Carinci R, Girod R. 2011. Multiple

 insecticide resistance in *Aedes aegypti* (Diptera: Culicidae) populations

 compromises the effectiveness of dengue vector control in French Guiana. *Mem*

 Inst Oswaldo Cruz, 106(3):346-352.

Dyar HG, Knab F. 1907. The species of mosquitoes in the genus *Megarhinus*. *Smithson*

 Misc Collect, 48(17):241-258.

Engber B, Sone PF, Pillai JS. 1978. The occurrence of *Toxorhynchites amboinensis* in

 Western Samoa. *Mosquito News*, 38:295-296.

Epstein PR, Diaz HF, Elias S, Grabherr G, Graham NE, *et al*. 1998. Biological and

 physical signs of climate change: focus on mosquito-borne diseases. *B Am*

 Meteorol Soc, 79(3):409-417.

Fang J. 2010. A world without mosquitoes. *Nature*, 466(7305):432-434.

Faraji A, Unlu I. 2016. The eye of the tiger, the thrill of the fight: effective larval and

 adult control measures against the Asian tiger mosquito, *Aedes albopictus*

 (Diptera: Culicidae), in North America. *J Med Entomol*, 53(5):1029-1047.

Farajollahi A, Crans WJ, Bryant P, Wolf B, Burkhalter KL, *et al*. 2005. Detection of

 West Nile viral RNA from an overwintering pool of *Culex pipiens pipiens*

 (Diptera: Culicidae) in New Jersey, 2003. *J Med Entomol*, 42(3):490-494.

Farajollahi A, Fonseca DM, Kramer LD, Kilpatrick AM. 2011. "Bird biting" mosquitoes

 and human disease: a review of the role of *Culex pipiens* complex mosquitoes in

 epidemiology. *Infect Gen Evol*, 11:1577-1585.

Fauci AS, Morens DM. 2016. Zika virus in the Americas – yet another arbovirus threat. *N Engl J Med*, 374(7):601-604.

Fischer S, Pereyra D, Fernandez L. 2012. Predation ability and non-consumptive effects of *Notonecta sellata* (Heteroptera: Notonectidae) on immature stages of *Culex pipiens* (Diptera: Culicidae). *J Vector Ecol*, 37(1):245-251.

Focks DA. 2007. *Toxorhynchites* as biocontrol agents. *J Am Mosquito Contr*, 23(2):118-127.

Focks DA, Dame DA, Cameron AL, Boston MD. 1980. Predator-prey interaction between insular populations of *Toxorhynchites rutilus rutilus* and *Aedes aegypti*. *Environ Entomol*, 9(1):37-42.

Focks DA, Sackett SR, Bailey DL. 1982. Field experiments on the control of *Aedes aegypti* and *Culex quinquefasciatus* by *Toxorhynchites rutilus rutilus* (Diptera: Culicidae). *J Med Entomol*, 19(3):336-339.

Focks DA, Sackett SR, Dame DA, Bailey DL. 1983. *Toxorhynchites rutilus rutilus* (Diptera: Culicidae): field studies on dispersal and oviposition in the context of the biocontrol of urban container-breeding mosquitoes. *J Med Entomol*, 20(4):383-390.

Fonseca DM, Keyghobadi N, Malcolm CA, Mehmet C, Schaffner F, Mogi M, Fleischer RC, Wilkerson RC. 2004. Emerging vectors in the *Culex pipiens* complex. *Science*, 303(5663):1535-1538.

Frank JH, Curtis GA, O'Meara GF. 1984. On the bionomics of bromeliad-inhabiting mosquitoes. *J Med Entomol*, 21(2):149-158.

Franz AWE, Kantor AM, Passarelli AL, Clem RJ. 2015. Tissue barriers to arbovirus infection in mosquitoes. *Viruses*, 7:3741-3767.

Ginsberg HS, Bargar TA, Hladik ML, Lubelczyk C. 2017. Management of arthropod pathogen vectors in North America: minimizing adverse effects on pollinators. *J Med Entomol*, 54(6):1463-1475.

Go YY, Balasuriya UBR, Lee C. 2014. Zoonotic encephalitides caused by arboviruses: transmission and epidemiology of alphaviruses and flaviviruses. *Clin Exp Vaccine Res*, 3:58-77.

Grill CP, Juliano SA. 1996. Predicting species interactions based on behaviour: predation and competition in container-dwelling mosquitoes. *J Anim Ecol*, 65(1):63-76.

Gunathilaka N, Upulika H, Udayanga L, Amarasinghe D. 2019. Effect of larval nutritional regimes on morphometry and vectorial capacity of *Aedes aegypti* for dengue transmission. *BioMed Res Int*, 2019:3607342.

Guzman C, Aguilar-Fenollosa E, Sahun RM, Boyero JR, Vela JM, *et al*. 2016. Temperature-specific competition in predatory mites: implications for biological pest control in a changing climate. *Agr Ecosyst Environ*, 216:89-97.

Hahn MB, Eisen L, McAllister J, Savage HM, Mutebi J, Eisen RJ. 2017. Updated reported distribution of *Aedes* (*Stegomyia*) *aegypti* and *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) in the United States, 1995-2016. *J Med Entomol*, 54(5):1420-1424.

Hall-Mendelin S, McLean BJ, Bielefeldt-Ohmann H, Hobson-Peters J, Hall RA, Van den Hurk AF. 2016. The insect-specific Palm Creek virus modulates West Nile virus infection in and transmission by Australian mosquitoes. *Parasit Vectors*, 9:414.

Hamer GL, Kitron UD, Brawn JD, Loss SR, Ruiz MO, *et al*. 2008. *Culex pipiens* (Diptera: Culicidae): a bridge vector of West Nile virus to humans. *J Med Entomol*, 45(1):125-128.

Harbach RE. 2007. The Culicidae (Diptera): a review of taxonomy, classification and phylogeny. *Zootaxa* 1668:591-638.

Hardy JL, Houk EJ, Kramer LD, Reeves WC. 1983. Intrinsic factors affecting vector competence of mosquitoes for arboviruses. Annu *Rev Entomol*, 28:229-262.

Healy K, Hamilton G, Crepeau T, Healy S, Unlu I, *et al*. 2014. Integrating the public in mosquito management: active education by community peers can lead to significant reduction in peridomestic container mosquito habitats. *PLoS ONE*, 9(9):e108504.

Hintz HW. 1951. The role of certain arthropods in reducing mosquito populations of permanent ponds in Ohio. *Ohio J Sci*, 51(5):277-279.

Hobson-Peters J, Yam AWY, Lu JWF, Setoh YX, May FJ, *et al*. 2013. A new insect-specific flavivirus from northern Australis suppresses replication of West Nile virus and Murray Valley encephalitis virus in co-infected mosquito cells. *PLoS ONE*, 8(2):e56534.

Holderman CJ, Gezan SA, Stone AES, Connelly CR, Kaufman PE. 2018. Mosquitoes (Diptera: Culicidae) collected from residential yards and dog kennels in Florida using two aspirators, a sweep net, or a CDC Trap. *J Med Entomol*, 55(1):230-236.

Holzapfel CM, Bradshaw WE. 1976. Rearing of *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae) from Florida and Pennsylvania with notes on their pre-diapause and pupal development. *Ann Entomol Soc Am*, 69(6):1062-1064.

Hongoh V, Berrang-Ford L, Scott ME, Lindsay LR. 2012. Expanding geographical

distribution of the mosquito, *Culex pipiens,* in Canada under climate change. *Appl Geogr*, 33:53-62.

Hu SMK. 1955. Progress report on biological control of *Aedes albopictus* Skuse in

Hawaii. *Proceedings of the California Mosquito Control Association*, 1955:23.

Huang YS, Higgs S, Vanlandingham DL. 2017. Biological control strategies for

mosquito vectors of arboviruses. *Insects*, 8(21).

Johnson DM, Akre BG, Crowley PH. 1975. Modeling arthropod predation: wasteful

killing by damselfly naiads. *Ecology*, 56(5):1081-1093.

Jones CJ. 1993. Larval growth rates and adult reproduction of *Toxorhynchites splendens*

(Diptera: Culicidae) with restricted dietary intake. *Environ Entomol*, 22(1):174-182.

Jones C, Schreiber E. 1994. The carnivores, *Toxorhynchites*. *Wing Beats*, 5(4):4.

Juliano SA, Westby KM, Ower GD. 2019. Know your enemy: effects of a predator on

native and invasive container mosquitoes. *J Med Entomol*, 56(2):320-328.

Kar S, Aditya AK. 2003. Biological control of mosquitoes by aquatic planaria. *TISCIA*,

34:15-18.

Kean J, Rainey SM, McFarlane M, Donald CL, Schnettler E, *et al*. 2015. Fighting

arbovirus transmission: natural and engineered control of vector competence in *Aedes* mosquitoes. *Insects*, 6:236-278.

Kesavaraju B, Juliano SA. 2004. Differential behavioral responses to water-borne cues to

predation in two container-dwelling mosquitoes. *Ann Entomol Soc Am*, 97(1):194-201.

Kilpatrick AM, Kramer LD, Campbell SR, Alleyne EO, Dobson AP, Daszak P. 2005. West Nile virus risk assessment and the bridge vector paradigm. *Emerg Infect Dis*, 11(3):425-429.

Kilpatrick AM, Kramer LD, Jones MJ, Marra PP, Daszak P. 2006. West Nile virus epidemics in North America are driven by shifts in mosquito feeding behavior. *PLoS Biology*, 4(4):e82.

Kilpatrick AM, Fonseca DM, Ebel GD, Reddy MR, Kramer LD. 2010. Spatial and temporal variation in vector competence of *Culex pipiens* and *Cx. restuans* mosquitoes for West Nile virus. *Am J Trop Med Hyg*, 83(3):607-613.

Kilpatrick AM. 2011. Land use, and the invasion of West Nile virus. *Science*, 334(6054):323-327.

Knipling EF. 1955. Possibilities of insect control or eradication through the use of sexually sterile males. *J Econ Entomol*, 48(4):459-462.

Kothera L, Godsey MS, Doyle MS, Savage HM. 2012. Characterization of *Culex pipiens* complex (Diptera: Culicidae) populations in Colorado, USA using microsatellites. *PLoS ONE*, 7(10):e47602.

Kraemer MUG, Reiner RC, Brady OJ, Messina JP, Gilbert M, *et al*. 2019. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nature Microbiology*, 4:854-863.

Kroeger I, Liess M, Dziock F, Duquesne S. 2013. Sustainable control of mosquito larvae in the field by the combined actions of the biological insecticide Bti and natural competitors. *J Vector Ecol*, 38(1):82-89.

Kuno G, Chang GJ. 2005. Biological transmission of arboviruses: reexamination of and new insights into components, mechanisms, and unique traits as well as their evolutionary trends. *Clin Microbiol Rev*, 18(4):608-637.

Lacroix R, McKemey AR, Raduan N, Wee LK, Ming WH, e*t al.* 2012. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. *PLoS ONE*, 7(8):e42771.

Lambrechts L. 2011. Quantitative genetics of *Aedes aegypti* vector competence for dengue viruses: towards a new paradigm? *Trends Parasitol*, 27(3):111-114.

Legner EF, Yu HS, Medved RA, Badgley ME. 1975. Mosquito and chironomid midge control by planaria. *Calif Agr*, 29(11):3-6.

Lehane M. 2005. *The Biology of Blood-Sucking in Insects*. 2nd Edition. New York, USA: Cambridge University Press.

Linley JR. 1990. The predatory behavior of *Toxorhynchites amboinensis* and *Tx. brevipalpis* larvae (Diptera: Culicidae) in response to subsurface prey. *Fla Entomol*, 73(1):9-51.

Linley JR, Darling K. 1993. Search behavior associated with egg cannibalism in *Toxorhynchites amboinensis* and *Toxorhynchites rutilus rutilus* (Diptera: Culicidae). *J Med Entomol*, 30(3):561-570.

Linley JR, Duzak D. 1989. Egg cannibalism and carnivory among three species of *Toxorhynchites*. *J Am Mosquito Contr*, 5(3):359-362.

Lord CC. 2007. Modeling and biological control of mosquitoes. *J Am Mosquito Contr*, 23(2):252-264.

Lounibos LP. 1979. Temporal and spatial distribution, growth and predatory behaviour of

    Toxorhynchites brevipalpis (Diptera: Culicidae) on the Kenya coast. *J Anim Ecol*,

    48(1):213-236.

Lounibos LP, Frank JH, Machado-Allison CE, Ocanto P, Navarro JC. 1987. Survival,

    development and predatory effects of mosquito larvae in Venezuelan

    phytotelmata. *J Trop Ecol*, 3(3):221-242.

Lounibos LP, Escher RL, Duzak D, Martin EA. 1996. Body size, sexual receptivity and

    larval cannibalism in relation to protandry among *Toxorhynchites* mosquitoes.

    Oikos, 77(2):309-316.

Lounibos LP, Escher RL, Nishimura N, Juliano SA. 1997. Long-term dynamics of a

    predator used for biological control and decoupling from mosquito prey in a

    subtropical treehole ecosystem. *Oecologia*, 111(2):189-200.

Lounibos LP, Martin EA, Duzak D, Escher RL. 1998. Daylength and temperature control

    of predation, body size, and rate of increase in *Toxorhynchites rutilus* (Diptera:

    Culicidae). *Ann Entomol Soc Am*, 91(3):308-314.

Lounibos LP, Makhni S, Alto BW, Kesavaraju B. 2008. Surplus killing by predatory

    larvae of *Corethrella appendiculata:* prepupal timing and site-specific attack on

    mosquito prey. *J Insect Behav*, 21:47-54.

Manning JE, Morens DM, Kamhawi S, Valenzuela JG, Memoli M. 2018. Mosquito

    saliva: the hope for a universal arbovirus vaccine? *J Infect Dis*, 218:7-15.

Mantke OD. 2011. Arboviral encephalitis – epidemiology, diagnostics and surveillance in

    the face of changing environments. University of South Bohemia, habilitation

    thesis.

Marten GG. 1989. A survey of cyclopoid copepods for control of *Aedes albopictus* larvae. *Bull Soc Vector Ecol*, 14(2):232-236.

Meadows AJ, Owen JP, Snyder WE. 2017. Keystone nonconsumptive effects within a diverse predator community. *Ecol Evol*, 7:10315-10325.

Mellor PS. 2000. Replication of arboviruses in insect vectors. *J Comp Pathol*, 123:231-247.

Mills MK, Michel K, Pfannenstiel RS, Ruder MG, Veronesi E, Nayduch D. 2017. *Culicoides* – virus interactions: infection barriers and possible factors underlying vector competence. *Curr Opin Insect Sci*, 22:7-15.

Muturi EJ, Kim C, Alto BW, Berenbaum MR, Schuler MA. 2011. Larval environmental stress alters *Aedes aegypti* competence for Sindbis virus. *Trop Med Int Health*, 16(8):955-964.

Nakagawa PY. 1963. Status of *Toxorhynchites* in Hawaii. *Proc Hawaii Entomol Soc*, 18:291-294.

Nannini MA, Juliano SA. 1997. Effects of developmental asynchrony between *Aedes triseriatus* (Diptera: Culicidae) and its predator *Toxorhynchites rutilus* (Diptera: Culicidae). *J Med Entomol*, 34(4):457-460.

Nelms BM, Kothera L, Thiemann T, Macedo PA, Savage HM, Reisen WK. 2013. Phenotypic variation among *Culex pipiens* complex (Diptera: Culicidae) populations from the Sacramento Valley, California: horizontal and vertical transmission of West Nile virus, diapause potential, autogeny, and host selection. *Am J Trop Med Hyg*, 89(6):1168-1178.

Patterson GM. 2016. Looking backward, looking forward: the long, torturous struggle with mosquitoes. *Insects*, 7(56).

Peach DAH, Gries G. 2019. Mosquito phytophagy – sources exploited, ecological function, and evolutionary transition to haematophagy. *Entomol Exp Appl*, 2019:1-17.

Peacor SD, Werner EE. 2001. The contribution of trait-mediated indirect effects to the net effects of a predator. *PNAS*, 98(7):3904-3908.

Petersen JJ. 1981. Comparative susceptibility of larval mosquitoes exposed separately by instar or in mixed populations to the nematode *Romanomermis culicivorax*. *J Nematol*, 13(2):228-229.

Petersen JM, Mead PS, Schriefer ME. 2009. *Francisella tularensis*: an arthropod-borne pathogen. *Vet Res*, 40(2):7.

Peterson GD. 1956. The introduction of mosquitoes of the genus *Toxorhynchites* into American Samoa. *J Econ Entomol*, 49(6):786-789.

Pezzin A, Sy V, Puggioli A, Veronesi R, Carrieri M, *et* al. 2016. Comparative study on the effectiveness of different mosquito traps in arbovirus surveillance with a focus on WNV detection. *Acta Trop*, 153:93-100.

Platzer EG. 1980. Biological control of mosquitoes with mermithids. *J Nematol*, 13(3):257-262.

Ponlawat A, Harrington LC. 2005. Blood feeding patterns of *Aedes aegypti* and *Aedes albopictus* in Thailand. *J Med Entomol*, 42(5):844-849.

Powell JR, Tabachnick WJ. 2013. History of domestication and spread of *Aedes aegypti* – a review. *Mem Inst Oswaldo Cruz*, 108(1):11-17.

Preisser EL, Bolnick DI, Benard MF. 2005. Scared to death? The effects of intimidation and consumption in predator-prey interactions. *Ecology*, 86(2):501-509.

Pyke GH. 2005. A review of the biology of *Gambusia affinis* and *G. holbrooki*. *Rev Fish Biol Fisher*, 15:339-365.

Reisen WK, Milby MM, Presser SB, Hardy JL. 1992. Ecology of mosquitoes and St. Louis encephalitis virus in the Los Angeles Basin of California, 1987-1990. *J Med Entomol*, 29(4):582-598.

Relyea RA. 2000. Trait-mediated indirect effects in larval anurans: reversing competition with the threat of predation. *Ecology*, 81(8):2278-2289.

Richards SL, Balanay JAG, White AV, Hope J, Vandock K, *et al*. 2018. Insecticide susceptibility screening against *Culex* and *Aedes* (Diptera: Culicidae) mosquitoes from the United States. *J Med Entomol*, 55(2):398-407.

Riviere F, Kay BH, Klein JM, Sechan Y. 1987. *Mesocyclops aspericornis* (Copepoda) and *Bacillus thuringienses* var. *Israelensis* for the biological control of *Aedes* and *Culex* vectors (Diptera: Culicidae) breeding in crab holes, tree holes, and artificial containers. *J Med Entomol*, 24(4):425-430.

Rodhain F. 2015. Insects as vectors: systematics and biology. *Rev Sci Tech Off Int Epiz*, 34(1):83-96.

Rubio Y, Ayesta C. 1984. Laboratory observations on the biology of *Toxorhynchites theobaldi*. *Mosquito News*, 44(1):86-90.

Ruiz MO, Chaves LF, Hamer GL, Sun T, Brown WM, *et al*. 2010. Local impact of temperature and precipitation on West Nile virus infection in *Culex* species mosquitoes in northeast Illinois, USA. *Parasit Vectors*, 3:19.

Russo R. 1986. Comparison of predatory behavior in five species of *Toxorhynchites* (Diptera: Culicidae). *Ann Entomol Soc Am*, 79:715-722.

Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR. 2019. Global expansion and redistribution of *Aedes*-borne virus transmission risk with climate change. *PLoS Negl Trop Dis*, 13(3):e0007213.

Sardelis MR, Turell MJ, Dohm DJ, O'Guinn ML. 2001. Vector competence of selected North American *Culex* and *Coquillettidia* mosquitoes for West Nile virus. *Emerg Infect Dis*, 7(6):1018-1022.

Savage HM, Anderson M, Gordon E, McMillen L, Colton L, *et al*. 2006. Oviposition activity patterns and West Nile virus infection rates for members of the *Culex pipiens* complex at different habitat types within the hybrid zone, Shelby County, TN, 2002 (Diptera: Culicidae). *J Med Entomol*, 43(6):1227-1238.

Schiller A. 2015. Developing rearing cages for *Toxorhynchites rutilus septentrionalis*. *Wing Beats*, 26(2):27-30.

Schiller A, Allen M, Coffey J, Fike A, Carballo F. 2019. Updated methods for the production of *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae) for use as biocontrol agent against container breeding pest mosquitoes in Harris County, Texas. *J Insect Sci*, 19(2).

Shah PA, Pell JK. 2003. Entomopathogenic fungi as biological control agents. *Appl Microbiol Biotechnol*, 61:413-423.

Shope RE. 1980. Arbovirus-related encephalitis. *Yale J Biol Med*, 53:93-99.

Short SM, Mongodin EF, MacLeod HJ, Talyuli OAC, Dimopoulos G. 2017/ Amino acid

metabolic signaling influences *Aedes aegypti* midgut microbiome variability.

PLoS Negl Trop Dis, 11(7):e0005677.

Simon RB. 2017. West Nile virus. *Nursing2017*, 47(8):58-60.

Sivagnaname N. 2009. A novel method of controlling a dengue mosquito vector, *Aedes

aegypti* (Diptera: Culicidae) using an aquatic mosquito predator, *Diplonychus

indicus* (Hemiptera: Belostomatidae) in tyres. *Dengue Bull*, 33:148-160.

Steffan WA. 1975. Systematics and biological control potential of *Toxorhynchites*

(Diptera: Culicidae). *Mosquito Systematics*, 7(1):59-67.

Steffan WA, Evenhuis NL. 1985. Classification of the subgenus *Toxorhynchites* (Diptera:

Culicidae). *J Med Entomol*, 22(4):421-446.

Tabachnick WJ. 2013. Nature, nurture and evolution of intra-species variation in

mosquito arbovirus transmission competence. *Int J Environ Res Public Health*,

10:249-277.

Thomas MB. 2018. Biological control of human disease vectors: a perspective on

challenges and opportunities. *BioControl*, 63:61-69.

Thurman JH, Crowder DW, Northfield TD. 2017. Biological control agents in the

Anthropocene: current risks and future options. *Curr Opin Insect Sci*, 23:59-64.

Tranchida MC, Pelizza SA, Micieli MV, Macia A. 2014. Consequences of the

introduction of the planarian *Girardia anceps* (Tricladida: Dugesiidae) in artificial

containers with larvae of the mosquitoes *Aedes aegypti* and *Culex pipiens*

(Diptera: Culicidae) from Argentina. *Biol Control*, 71:49-55.

Trimble RM, Corbet PS. 1974. Laboratory colonization of *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae). *Ann Entomol Soc Am*, 68(2):217-219.

Trpis M. 1972. Development and predatory behavior of *Toxorhynchites brevipalpis* (Diptera: Culicidae) in relation to temperature. *Environ Entomol*, 1(5):537-546.

Trpis M. 1981. Survivorship and age-specific fertility of *Toxorhynchites brevipalpis* females (Diptera: Culicidae). *J Med Entomol*, 18(6):481-486.

Turell MJ, O'Guinn ML, Dohm DJ, Jones JW. 2001. Vector competence of North American mosquitoes (Diptera: Culicidae) for West Nile virus. *J Med Entomol*, 38(2):130-134.

Turell MJ, Dohm DJ, Sardelis MR, O'Guinn ML, Andreadis TG, Blow JA. 2005. An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. *J Med Entomol*, 42(1):57-62.

Turell MJ. 2012. Members of the *Culex pipiens* complex as vectors of viruses. *J Am Mosquito Contr*, 28(4):123-126.

Turner AM. 2004. Non-lethal effects of predators on prey growth rates depend on prey density and nutrient additions. *OIKOS*, 104(3):561-569.

Vezzani D, Albicocco AP. 2009. The effect of shade on the container index and pupal productivity of the mosquitoes *Aedes aegypti* and *Culex pipiens* breeding in artificial containers. *Med Vet Entomol*, 23:78-84.

Waage JK. 1979. The evolution of insect/vertebrate associations. *Biol J Linnean Soc*, 12:187-224.

Weaver SC, Reisen WK. 2010. Present and future arboviral threats. *Antiviral Res*, 85(2):328.

Weaver SC, Lecuit M. 2015. Chikungunya virus and the global spread of a mosquito-borne disease. *N Engl J Med*, 372:1231-1239.

WHO [World Health Organization]. 1959. Annual report of the director-general to the World Health Assembly and to the United Nations. Geneva, Switzerland: World Health Organization.

WHO [World Health Organization]. 2018. Global vector control response 2017-2030 [Internet]. Available from World Health Organization, Geneva [accessed March 6, 2020]. https://www.who.int/vector-control/publications/global-control-response/en/.

WHO [World Health Organization]. 2019. Guidelines for malaria vector control. [Internet]. Available from World Health Organization, Geneva [accessed March 6, 2020]. https://www.who.int/malaria/publications/atoz/9789241550499/en/

Wilson AL, Dhiman RC, Kitron U, Scott TW, van den Berg H, Lindsay SW. 2014. Benefit of insecticide-treated nets, curtains and screening on vector borne diseases, excluding malaria: a systematic review and meta-analysis. *PLoS Negl Trop Dis*, 8(10):e3228.

Yap HH, Foo AES. 1984. Laboratory studies on the oviposition site preference of *Toxorhynchites splendens* (Diptera: Culicidae). *J Med Entomol*, 21(2):183-187.

Yasuda H, Hashimoto T. 1995. Prey density effect on cannibalism by *Toxorhynchites towadensis* (Diptera: Culicidae). *J Med Entomol*, 32(5):650-653.

Yee DA, Kesavaraju B, Juliano SA. 2004. Larval feeding behavior of three co-occurring species of container mosquitoes. *J Vector Ecol*, 29(2):315-322.

Zavortink TJ, Chaverri LG. 2009. Resurrection of the names *Toxorhynchites Moctezuma*
(Dyar & Knab) and *Toxorhynchites hypoptes* (Knab) from synonymy with
Toxorhynchites theobaldi (Dyar & Knab) (Diptera: Culicidae). *Proc Entomol Soc
Wash*, 111(4):890-897.

Zuharah WF, Fadzly N, Yusof NA, Dieng H. 2015. Risky behaviors: effects of
Toxorhynchites splendens (Diptera: Culicidae) predator on the behavior of three
mosquito species. *J Insect Sci*, 15(1):128.

SURVIVAL OF *AEDES AEGYPTI* AND *CULEX QUINQUEFASCIATUS* IN THE PRESENCE OF *TOXORHYNCHITES RUTILUS SEPTENTRIONALIS*

DANIEL S. MARSHALL

DEPARTMENT OF BIOLOGY, UNIVERSITY OF CENTRAL OKLAHOMA,

EDMOND, OK 73034

ABSTRACT

Incidence of mosquito-borne disease has increased globally in recent years. The most effective way to reduce disease incidence is to control mosquitoes. A control technique used in the United States is the inundative release of the predatory mosquito, *Toxorhynchites rutilus*. The predators colonize the aquatic habitats of their vector counterparts where they feed on mosquito larvae. It is unclear if *Tx. rutilus* preferentially feeds on one of the commonly encountered vectors in the U.S. I measured predation rate and survival of *Aedes aegypti* and *Culex quinquefasciatus* in the presence of *Tx. rutilus*. My analyses revealed no significant difference in predation rate or survivorship. The predation rate for *Ae. aegypti* was 0.363 and 0.381 for *Cx. quinquefasciatus*. The cumulative probability of survival at 12 hours for *Ae. aegypti* was 55.6% and 54.3% for *Cx. quinquefasciatus*. These results indicate that *Tx. rutilus* does not have a preference between these two prey choices. Inundative mass releases for the purpose of general mosquito control will exert equal pressure on both *Ae. aegypti* and *Cx. quinquefasciatus* in habitats where they occur with *Tx. rutilus*. Targeted releases intended for control of

epidemic zoonoses will be ineffectual due to weakened predation pressure caused by the presence of heterospecific prey.

KEYWORDS *Toxorhynchites rutilus*, *Aedes aegypti*, *Culex quinquefasciatus*, predatory efficacy, natural enemies

INTRODUCTION

Incidence of mosquito-borne illnesses, particularly arboviruses, have increased in recent years despite many decades of work dedicated to halting their transmission (Fernandes et al. 2018). Factors contributing to the increasing incidence of mosquito-borne disease include climate change, increased globalization (Powell 2016, Kraemer et al. 2019), and the development of insecticide resistance (Hemingway et al. 2016). Mosquito-borne disease prevention takes many forms. Some diseases such as malaria and yellow fever are preventable by means of vaccines or chemoprophylaxis (CDC 2017); although, some *Plasmodium* spp. populations have become resistant to medications (Kain et al. 2001). One of the most effective ways to limit mosquito-borne illness is to control mosquito populations. This is accomplished through elimination of larval habitat (Frank 1981, Carlson et al. 1991), larvicide and adulticide application (Fonseca et al. 2013), release of genetically modified adults (Lacroix 2012, Kean 2015), and biological control applications including the use of natural enemies (Murdoch et al. 1985, Scholte 2004, Becker et al. 2010).

Among these natural enemies are mosquitoes of the genus *Toxorhynchites* Theobald. In their larval stadiums they are predators of aquatic invertebrates and reduce

numbers of emerging adult mosquitoes (Focks et al. 1979; 1982, Bailey et al. 1983).

Adults feed on nectar and do not take blood (Jenkins 1946). Larvae co-occur with vectors

in natural and artificial containers and thereby provide a natural means of disease control

(Focks et al. 1982). Many studies have observed *Toxorhynchites* spp. feeding behavior in

the presence of multiple prey species. Zuharah et al. (2015) observed that *Tx. splendens*

(Wied.) fed more frequently on *Aedes aegypti* (L.) than *Ae. albopictus* (Skuse) and

Anopheles sinensis Wied. The authors attributed this mortality to prey behavior. *Aedes*

albopictus and *An. sinensis* adopted less risky behaviors in response to the presence of

predators, but *Ae. aegypti* was more vulnerable to predation due to its aggressive foraging

behavior. Pramanik et al. (2017) found that *Tx. splendens* did not prey upon *Ae. aegypti*

more than *Cx. quinquefasciatus* Say. Digma et al. (2019) reported similar results for *Tx.*

amboinensis (Doleschall) offered the same prey choices. Some older reports of

Toxorhynchites spp. effect on wild mosquito populations claim seasonal eradication of

vectors (Trpis 1972, Focks et al. 1980). However, this extreme effect is likely isolated to

a narrow range of habitats and prey species as most investigations do not report complete

control (Focks 1982, Bailey et al. 1983a, Focks et al. 1983, Annis et al. 1990a). Campos

and Lounibos (2000b) analyzed gut contents from wild-caught *Tx. rutilus* (Coq.) larvae.

They found that mosquitoes only made up 6% of the larval diet in tree holes and 8% in

tires with the rest comprised of non-culicid arthropods. This indicates that wild

populations of *Tx. rutilus* do not exert predation pressures on mosquitoes to the same

extent seen in the laboratory due to the availability of other prey. Other field studies

reported large reductions in vector survivorship in the presence of *Tx. rutilus*. The release

of the predator into a New Orleans neighborhood, resulted in a 74% reduction in *Cx.*

44

quinquefasciatus and *Ae. aegypti* (Focks et al. 1982). A later study by Bailey et al. (1983) observed a 50% reduction in *Ae. aegypti* in tire dumps due to *Tx. rutilus* predation. Not all *Tx. rutilus* releases have been successful (Focks et al. 1979; 1983). Despite the failures, some mosquito abatement organizations currently utilize this predator for vector control (Schiller et al. 2019).

In the United States vector control professionals mass rear *Tx. rutilus* and release gravid females or immature life stages to control wild mosquito populations (Focks et al. 1979, Bailey et al. 1983, Schiller et al. 2019). The benefit of inundative releases is that females oviposit into mosquito breeding sites otherwise difficult to reach by traditional control means (Focks et al. 1979, Collins and Blackwell 2000). However, this method of control is short-lived. Because *Tx. rutilus* does not develop and reproduce as quickly as other mosquitoes and asynchrony with prey habitats and phenology, predators do not keep vectors in check indefinitely (Collins and Blackwell 2000, Focks 2007). Mosquito control workers circumvent this problem by repeat treatments of inundative releases throughout a season so that *Tx. rutilus* provides more complete control over vector mosquitoes (Bailey et al. 1983b, Focks 2007). Whether *Tx. rutilus* provides even control over multiple mosquito species is unclear.

Here I inferred the predatory preference of *Tx. rutilus* subspecies *septentrionalis* Dyar and Knab on two common vectors of human and animal concern by analyzing predation rate and survivorship curves. The extent of predation on a species approximates prey preference (Zuharah et al. 2015). My intention was to inform mosquito control professionals of the level of control inundative releases of *Tx. r. septentrionalis* exert on *Ae. aegypti* and *Cx. quinquefasciatus*. I found no difference in survivorship of these two

45

species in the presence of *Tx. r. septentrionalis*. The predation rate also does not differ. My results imply no preference in prey between those offered. When releasing *Tx. r. septentrionalis,* mosquito control professionals should consider that larvae will act indiscriminately on these prey species and targeted releases will likely be ineffectual.

MATERIALS AND METHODS

Mosquito Rearing

I obtained *Tx. rutilus septentrionalis* individuals from the laboratory strain colony at the Harris County Precinct 4 Biocontrol Initiative (Texas, USA). To prevent cannibalism, I raised larvae individually. I reared early instars in seasoned tap water and later instars in seasoned tap water treated with Melafix (Mars Fishcare North America Inc., Chalfont, PA) according to manufacturer instructions. To create seasoned tap water, I filtered tap water through activated charcoal, ceramic bio-rings (SunSun Group Ltd., Zhejiang, China), and ammonia scrubber media (Hagen Inc., Montreal, Canada) for a minimum of 24 hours. First instar larvae fed on live nematodes (*Panagrellus redivivus*; www.insectsales.com). Second instar larvae fed on live lab-reared oligochaetes (*Dero* sp.) and third and fourth instars fed on frozen bloodworms (*Chironomus plumosus*; San Francisco Bay Brand Inc., Newark, CA). I housed adult mosquitoes in 72"x36" (1.8 m x 0.9 m) cylindrical rearing cages (Bioquip Products Inc., Rancho Dominguez, CA; Schiller 2015) inside of a pop-up walk-in insectary (Mylar hydroponic grow tent; Vivosun, Los Angeles, CA). I maintained insectary conditions at 24°C ± 2°C and 75% ± 5% relative humidity with 14:10 light cycle. Adults fed *ad libitum* on 1:1 honey-water solution and 1:10 sugar-water solution. Gravid females oviposited in black stadium cups

hung approximately 1 meter above the floor of the rearing cage. To avoid cannibalism, I removed eggs from ovicups daily.

I purchased *Ae. aegypti* and *Cx. quinquefasciatus* from Benzon Research (Carlisle, PA) as eggs and first instar larvae, respectively. Larvae fed on tropical fish food (Spectrum Brands Pet LLC, Blacksburg, VA). I batch-reared larvae in seasoned tap water in one-quart (946 mL) storage containers at insectary conditions.

Predatory observations

I held a single fourth instar *Tx. r. septentrionalis* and ten fourth instar prey larvae of a single species in separate two fluid ounce (59 mL) cups (Solo Cup Company, Lake Forest, IL) containing approximately 25 mL seasoned tap water. I starved *Tx. r. septentrionalis* larvae for 24 hours prior to observation. Predator and prey acclimated to room temperature and lighting conditions for one hour prior to observation. After acclimation, I introduced *Tx. r. septentrionalis* into the prey cup and recorded predatory behavior for 12 hours using a high definition camcorder (JVC Everio GZ-E505BU; JVC, Yokohama, Japan). I repeated observations 42 times with *Ae. aegypti* and 36 times with *Cx. quinquefasciatus.* I did not record a *Tx. r. septentrionalis* larvae more than once.

Visual analysis of recordings

I viewed recordings for prey mortality, recording time of capture and attack outcome (e.g. prey death, prey survival). Predatory larvae occasionally attacked but did not consume prey. I observed these larvae after their release to verify survival and scored them accordingly. I discarded samples in which *Tx. r. septentrionalis* pupated during observation. In total, I retained 39 *Ae. aegypti* and 35 *Cx. quinquefasciatus* recordings for analysis.

Statistical analyses

Predation rate (PR) was calculated for each recording. Predation rate was calculated as the number of prey killed divided by the observation time of 12 hours (Digma et al. 2019). A Mann-Whitney U test was used to compare the non-normally distributed PR between prey species (Zar 2009). Kaplan-Meier survivorship analysis was used to compare survival times of the two species. I used a log-rank test to analyze survivorship with censored data. Analyses were performed in R v3.6.2 (R Core Team 2019) using the packages, survival (Thurneau and Grambsch 2000, Thurneau 2015) and survminer (Kassambara et al. 2019). A total of 390 *Ae. aegypti*, 350 *Cx. quinquefasciatus* larvae, and 333 predatory events were used in my analyses.

RESULTS

The average PR by *Tx. r. septentrionalis* on *Ae. aegypti* was 0.363 deaths per hour (0 – 0.833). The average PR for *Cx. quinquefasciatus* was 0.381 deaths per hour (0 – 0.833; Figure 1). Predatory larvae killed 44.4% of *Ae. aegypti* and 45.7% of *Cx. quinquefasciatus* larvae (Table 1). In total, *Tx. r. septentrionalis* larvae killed 333 prey. Of these, 52.0% were *Ae. aegypti* and 48.0% were *Cx. quinquefasciatus*. The Mann-Whitney U test indicated no significant difference between *Ae. aegypti* and *Cx. quinquefasciatus* PR (W = 651.5, p = 0.739).

Log-rank analysis indicated no significant difference in survivorship between *Ae. aegypti* and *Cx. quinquefasciatus* ($\chi^2 = 0.1$, p = 0.772). Kaplan-Meier survivorship curves showed sharp drops in survivorship probability for both prey species during the first hour of observation. (Figure 2). For most of the observation time, *Ae. aegypti* maintained

greater survival probability than *Cx. quinquefasciatus* except for hours 1 – 3. The minimum cumulative probability of survival was 0.556 (SE = 0.025) for *Ae. aegypti* and 0.543 (SE = 0.027) for *Cx. quinquefasciatus*. I did not observe the median survival time (LT$_{50}$) for either prey as the length of observation did not allow for 50% mortality. A total of 217 (55.6%) *Ae. aegypti* larvae and 190 (54.3%) *Cx. quinquefasciatus* larvae survived beyond 12 hours (Table 1).

DISCUSSION

The PR analysis revealed no significant difference between *Tx. r. septentrionalis* feeding on *Ae. aegypti* and *Cx. quinquefasciatus*. My results lend support to previous work that demonstrated *Toxorhynchites* larvae as generalist predators (Campos and Lounibos 2000b; Digma et al. 2019). Campos & Lounibos (2000b) found that wild *Tx. rutilus* preyed on many aquatic invertebrates with mosquitoes being a small portion of their diet. This suggests that *Tx. rutilus* polyphagous habits may be inhibitory to its ability to control mosquitoes. Others report that a *Toxorhynchites* larva will attack upwards of 5000 prey over the course of its development and that differences in prey numbers are a results container dimensions, temperature, prey species and behavior (Focks 2007). Zuharah et al. (2015) hypothesized that anti-predator behaviors made *Ae. aegypti* more susceptible to predation by *Tx. splendens* compared to *Ae. albopictus* and *Anopheles sinensis*. While I did not measure prey behavior, my data do not reflect these findings in *Tx. r. septentrionalis*. Others have reported similar results. Pramanik et al. (2017) reported that in the presence of *Tx. splendens*, *Ae. aegypti* and *Cx. quinquefasciatus* predation did not vary significantly. On multiple occasions I observed both *Ae. aegypti*

and *Cx. quinquefasciatus* feeding directly from *Tx. r. septentrionalis*. *Aedes aegypti* larvae often attempted to feed on captured larvae as *Tx. r. septentrionalis* fed or swarmed the anus during excretion. I observed *Cx. quinquefasciatus* larvae grazing on *Tx. r. septentrionalis* setae. These behaviors certainly exposed larvae to predation as many were preyed upon while they attempted to feed from *Tx. r. septentrionalis.* My results indicate that fourth instar *Tx. r. septentrionalis* larvae focus on gaining mass for pupation and are less concerned with prey of a specific type. Indeed, Digma et al. (2019) found that fourth instar of *Tx. amboinensis* is the most predacious stadium. Further investigation is needed to determine if predation by I – III instar *Tx. r. septentrionalis* larvae is greater on specific prey.

I observed no significant difference in prey survival in the presence of *Tx. r. septentrionalis* using Kaplan-Meier survivorship analysis. During the first hour of observation, I detected heightened mortality for both prey species. This was likely due to predatory larvae starvation prior to observation. During this period of heightened predatory activity, I saw a lower cumulative probability of survival (S) for *Ae. aegypti* (S = 76.2%) than *Cx. quinquefasciatus* (S = 84.6%; Figure 2). Survival probability reversed beginning in the fourth hour of observation when *Ae. aegypti* had a greater cumulative survival probability than *Cx. quinquefasciatus*. The final cumulative survival probability of *Ae. aegypti* (S = 55.6%) was higher than that of *Cx. quinquefasciatus* (S = 54.3%).

Aedes aegypti may have taken longer to digest resulting in cessation of predatory activity by *Tx. r. septentrionalis* larvae until the end of the observation window. Larvae may have digested *Cx. quinquefasciatus* more quickly or incompletely. Such a situation would prevent satiation and result in more frequent consumption. Timmermann and

50

Briegel (1999) found that late instar *Cx. pipiens* L. larvae reached a larger body size than *Ae. aegypti* when fed the same diet. Additionally, the authors found that fourth instar *Ae. aegypti* had a higher lipid content and lower protein content than *Cx. pipiens* larvae of the same stadium. *Culex quinquefasciatus* likely contains nearly the same nutritional content as *Cx. pipiens* due to their close relatedness (Dumas et al. 2016). Perhaps *Tx. r. septentrionalis* larvae digest lipids more completely than proteins resulting in a lengthened digestion time of *Ae. aegypti.* Digestion times are not the same across prey species and densities. Campos and Lounibos (2000b) found that fourth instar *Tx. rutilus* ingested prey more frequently in higher density communities and that excretion occurred 8 – 18 hours post ingestion. My observations differ in digestion times although I did not vary prey density. Predatory larvae often excreted previous prey prior to attacking new ones. At times I observed feces excreted within several hours of ingestion. Often these feces were so poorly digested that they were easily recognized as mosquito larvae. I also noted several instances of *Tx. r. septentrionalis* larvae engaging in coprophagy. I only observed attacks on poorly digested excreta.

These results show that fourth instar *Tx. r. septentrionalis* will feed indiscriminately on *Ae. aegypti* and *Cx. quinquefasciatus* larvae in habitats where the predator and one or both prey species occur. Through predation and trait-mediated effects, these predators reduce vector emergence and the occurrence of mosquito-borne illness. Inundative releases of *Tx. r. septentrionalis* adults will be equally effective against both species in theory, but likely not in practice as these species infrequently occur in the same larval habitats. The preferred oviposition sites for *Cx. quinquefasciatus* are storm drains, cisterns, and occasionally smaller artificial containers such as tires and

flowerpots (Leisnham et al. 2014). Sylvan populations occupy cavities in vegetation (Lapointe 2008). *Aedes aegypti* oviposits in peridomestic containers including flowerpots, tires, and storm drains (Winch et al. 1992). Tree holes are the preferred oviposition site for *Tx. r. septentrionalis*, although it occurs in artificial containers as well (Focks 2007). The three species share larval habitats to a limited extent with variations in occurrence due to spatial partitioning and phenology (Bonnet 1946, Albeny-Simoes et al. 2014, Leisnham et al. 2014, Baak-Baak et al. 2016). Surveillance is key to targeted mass releases as knowledge of local mosquito populations' habits will allow for informed decisions about mass release efficacy against a specific vector. *Toxorhynchites rutilus septentrionalis* released into urban habitats will prey on both *Ae. aegypti* and *Cx. quinquefasciatus* with equal intensity. The extent to which it will control these vectors depends on the degree of larval cohabitation and the density of heterospecific prey (Campos and Lounibos 2000b).

There is increased concern for the emergence of diseases such as dengue fever, yellow fever, and Zika in the United States. Using this predator, mosquito control professionals can limit the encroachment of these diseases in the U.S. Additionally, inundative releases of *Tx. r. septentrionalis* can reduce incidence of established diseases such as West Nile, Eastern equine encephalitis, St. Louis encephalitis, and dog heartworm. Broadly increased mosquito-borne disease incidence is likely occurring due in part to insecticide resistance (Rivero et al. 2010). However, models indicate incidence of these diseases will broadly increase globally due to climate change (Savic et al. 2014). To meet the challenge of increasing mosquito-borne disease incidence, novel and integrated control methods are necessary. Some researchers suggest utilizing

combinations of natural enemies to suppress mosquito populations and reserving

chemical pesticides for epidemic-level threat response (Huang et al. 2017). Others

propose alternating pesticide and natural enemy releases or using them concurrently

(Focks et al. 1986, Collins and Blackwell 2000a, Focks 2007). However, these results

clearly show that *Tx. r. septentrionalis* releases are effective at general mosquito

population suppression. Mass releases alone will not provide control over an on-going

zoonotic epidemic in which one or a few mosquitoes are responsible for transmission.

This is due to the predator's polyphagous habits and the presence of heterospecific prey.

More information is needed to accurately predict the outcome of integrated control

strategies and the effect *Tx. r. septentrionalis* has on prey within that framework.

In this study, I observed predation on *Ae. aegypti* and *Cx. quinquefasciatus*

presented as a single prey choice. Predator behavior changes in response to the

availability of multiple prey types and different prey densities (Amalraj and Das 1996,

Digma et al. 2019). Additionally, I recorded predation in a single-predator system. The

presence of con- and heterospecific predators changes predatory behavior and, therefore,

prey survivorship outcomes (McPeek 1998, Meadows et al. 2017). I observed mortality

within the aquatic system and did not measure the effect of non-consumptive or trait-

mediated interactions on longevity, fecundity, morphometrics, or vector competence of

adult mosquitoes. Prey alter their behavior in the presence of a predator (Preisser et al.

2005). For mosquito larvae this means increasing or decreasing foraging, increasing

predator-avoidance behaviors such as diving or avoiding high-risk areas of their habitat

(Meadows et al. 2017). These behaviors effect development time, ability to acquire

resources, body size, and survivorship (Costanzo et al. 2011, Roux et al. 2015). Less time

spent foraging leads to poor nutrient acquisition and prolonged development time. Mosquito larvae that are less successful foragers emerge as smaller adults with shortened longevity and reduced fecundity (Costanzo et al. 2011, Roux et al. 2015). Reduced longevity prevents transmission of many diseases of human importance as the incubation period within the vector is longer than the lifespan of the mosquito (LaDeau et al. 2015, Bellamy and Alto 2018). Additionally, smaller adult mosquitoes are less competent vectors compared to larger mosquitoes of the same species (Alto et al. 2008a). Future research should focus on accurately measuring predatory behavior in complex systems that approximate the field conditions in which *Tx. r. septentrionalis* are mass released. These systems should consist of varying prey and conspecific densities and species which match those observed in release habitats. Accurate behavioral analysis in complex systems are much needed by mosquito control professionals to estimate control level of released *Tx. r. septentrionalis*.

REFERENCES CITED

Albeny-Simoes D, Murrell EG, Elliot SL, Andrade MR, Lima E, *et al*. 2014. Attracted to the enemy: *Aedes aegypti* prefers oviposition sites with predator-killed conspecifics. *Oecologia,* 175(2):481-492.

Alto BW, Lounibos LP, Mores CN, Reiskind MH. 2008. Larval competition alters susceptibility of adult *Aedes* mosquitoes to dengue infection. *Proc R Soc B*, 275:463-471.

Amalraj DD, Das PK. 1996. Frequency-dependent prey selection by larvae of *Toxorhynchites splendens* (Diptera: Culicidae). *B Entomol Res*, 86:633-639.

Annis B, Nalim S, Hadisuwasono, Widiarti, Boewono DT. 1990. *Toxorhynchites amboinensis* larvae released in domestic containers fail to control dengue vectors in a rural village in central Java. *J Am Mosquito Contr*, 6(1):75-78.

Baak-Baak CM, Cigarroa-Toledo N, Arana-Guardia R, Chi Chim WA, Chan Orilla JA, *et al*. 2016. Mosquito fauna associated with *Aedes aegypti* (Diptera: Culicidae) in Yucatan State of southeastern Mexico, and checklist with new records. *Fla Entomol*, 99(4):703-709.

Bailey DL, Jones RG, Simmonds PR. 1983a. Effects of indigenous *Toxorhynchites rutilus rutilus* on *Aedes aegypti* breeding in tire dumps. *Mosq News*, 43(1):33-37.

Bailey DL, Adabbe D, Focks DA, Dame DA. 1983b. Field releases with adults and pupae of *Toxorhynchites rutilus rutilus* (Diptera: Culicidae). *J Med Entomol*, 20(3):308-312.

Becker N, Petric D, Zgomba M, Boase C, Madon M, *et al*. 2010. *Mosquitoes and Their Control*. 2nd Edition. Heidelberg: Springer.

Bellamy SK, Alto BW. 2018. Mosquito responses to trait- and density-mediated interactions of predation. *Oecologia*, 187:233-243.

Bonnet DD. 1946. The distribution of mosquito breeding by type of container in Honolulu, T.H. *Proc Hawn Ent Soc*, 13(1):43-49.

Campos RE, Lounibos LP. 2000. Natural prey and digestion times of *Toxorhynchites rutilus* (Diptera: Culicidae) in southern Florida. *Ann Entomol Soc Am*, 93(6):1280-1287.

Carlson DB, O'Bryan PD, Rey JR. 1991. A review of current salt marsh management issues in Florida. *J Am Mosquito Contr*, 7(1):83-88.

CDC [Centers for Disease Control and Prevention]. 2017. *CDC Yellow Book 2020: Health Information for International Travel*. New York: Oxford University Press.

Collins LE, Blackwell A. 2000. The biology of *Toxorhynchites* mosquitoes and their potential as biocontrol agents. *BioControl*, 21(4):105N-116N.

Costanzo KS, Muturi EJ, Alto BW. 2011. Trait-mediated effects of predation across life-history stages in container mosquitoes. *Ecol Entomol*, 36:605-615.

Digma JR, Sumalde AC, Salibay CC. 2019. Laboratory evaluation of predation of *Toxorhynchites amboinensis* (Diptera: Culicidae) on three mosquito vectors of arboviruses in the Philippines. *Biol Control*, 137:104009.

Dumas E, Atyame CM, Malcolm CA, Le Goff G, Unal S, *et al*. 2016. Molecular data reveal a cryptic species within the *Culex pipiens* mosquito complex. *Insect Mol Biol*, 25(6):800-809.

Fernandes JN, Moise IK, Maranto GL, Beier JC. 2018. Revamping mosquito-borne disease control to tackle future threats. *Trends Parasitol*, 34(5):359-368.

Focks DA. 2007. *Toxorhynchites* as biocontrol agents. *J Am Mosquito Contr*, 23(2):118-127.

Focks DA, Seawright JA, Hall DW. 1979. Field survival, migrations and oviposition characteristics of laboratory-reared *Toxorhynchites rutilus rutilus* (Diptera: Culicidae). *J Med Entomol,* 16(2):121-127.

Focks DA, Dame DA, Cameron AL, Boston MD. 1980. Predator-prey interaction between insular populations of *Toxorhynchites rutilus rutilus* and *Aedes aegypti*. *Environ Entomol*, 9(1):37-42.

Focks DA, Sackett SR, Bailey DL. 1982. Field experiments on the control of *Aedes aegypti* and *Culex quinquefasciatus* by *Toxorhynchites rutilus rutilus* (Diptera: Culicidae). *J Med Entomol*, 19(3):336-339.

Focks DA, Sackett SR, Dame DA, Bailey DL. 1983. *Toxorhynchites rutilus rutilus* (Diptera: Culicidae): field studies on dispersal and oviposition in the context of the biocontrol of urban container-breeding mosquitoes. *J Med Entomol*, 20(4):383-390.

Focks DA, Sackett SR, Kloter KO, Dame DA, Carmichael GT. 1986. The integrated use of *Toxorhynchites amboinensis* and ground-level ULV insecticide application to suppress *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol*, 23(4):513-519.

Fonseca DM, Unlu I, Crepeau T, Farajollahi A, Healy SP, *et al*. 2013. Area-wide management of *Aedes albopictus*. Part 2: Gauging the efficacy of traditional integrated pest control measures against urban container mosquitoes. *Pest Manag Sci*, 69:1351-1361.

Frank JH. 1981. Recycling of discarded tires for control of *Aedes aegypti*. *J Fla Anti-Mosq*, 52:44-48.

Hemingway J, Ranson H, Magill A, Kolaczinski J, Fornadel C, *et al*. 2016. Averting a malaria disaster: will insecticide resistance derail malaria control? *Lancet*, 23(387):1785-1788.

Huang YS, Higgs S, Vanlandingham DL. 2017. Biological control strategies for mosquito vectors of arboviruses. *Insects*, 8(21).

Jenkins DW, Carpenter SJ. 1946. Ecology of the tree hole breeding mosquitoes of Nearctic North America. *Ecol Monogr*, 16(1):31-47.

Kain KC, Shanks GD, Keystone JS. 2001. Malaria chemoprophylaxis in the age of drug resistance. I. Currently recommended drug regimens. *Clin Infect Dis*, 33:226-234.

Kassambara A, Kosinski M, Biecek P. 2019. survminer: Drawing Survival Curves using 'ggplot2'. R package version 0.4.6. Available from: https://CRAN.R-project.org/package=survminer.

Kean J, Rainey SM, McFarlane M, Donald CL, Schnettler E, *et al*. 2015. Fighting arbovirus transmission: natural and engineered control of vector competence in *Aedes* mosquitoes. *Insects*, 6:236-278.

Kraemer MUG, Reiner RC, Brady OJ, Messina JP, Gilbert M, *et al*. 2019. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nat Microbiol*, 4:854-863.

Lacroix R, McKemey AR, Raduan N, Wee LK, Ming WH, e*t al.* 2012. Open field release of genetically engineered sterile male *Aedes aegypti* in Malaysia. *PLoS ONE*, 7(8):e42771.

LaDeau SL, Allan BF, Leisnham PT, Levy MZ. 2015. The ecological foundations of

transmission potential and vector-borne disease in urban landscapes. *Funct Ecol*,

29:889-901.

Lapointe DA. 2008. Dispersal of *Culex quinquefasciatus* (Diptera: Culicidae) in a

Hawaiian rain forest. *J Med Entomol*, 45(4):600-609.

Leisnham PT, LaDeau SL, Juliano SA. 2014. Spatial and temporal habitat segregation of

mosquitoes in urban Florida. *PLoS ONE*, 9(3):e91655.

McPeek MA. 1998. The consequences of changing the top predator in a food web: a

comparative experimental approach. *Ecol Monogr*, 68(1):1-23.

Meadows AJ, Owen JP, Snyder WE. 2017. Keystone nonconsumptive effects within a

diverse predator community. *Ecol Evol*, 7:10315-10325.

Murdoch WW, Chesson J, Chesson PL. 1985. Biological control in theory and practice.

Am Nat, 125(3):344-366.

Powell JR. 2016. Mosquitoes on the move. *Science*, 354(6315):971-972.

Pramanik S, Banerjee S, Saha GK, Aditya G. 2017. Observations on the predation of

mosquito in presence of chironomid prey by *Toxorhynchites splendens*

Wiedemann, 1898 (Diptera: Culicidae): implications in biological control of

mosquito. *Eco Env & Cons*, 23(4):2163-2168.

Preisser EL, Bolnick DI, Benard MF. 2005. Scared to death? The effects of intimidation

and consumption in predator-prey interactions. *Ecology*, 86(2):501-509.

R Core Team. 2013. R: A language and environment for statistical computing. R

Foundation for Statistical Computing. Vienna, Austria. Available from:

http://www.R-project.org/.

Rivero A, Vezilier J, Weill M, Read AF, Gandon S. 2010. Insecticide control of vector-borne diseases: when is insecticide resistance a problem? *PLoS Pathog*, 6(8):e1001000.

Roux O, Vantaux A, Roche B, Yameogo KB, Dabire KR, *et al*. 2015. Evidence for carry-over effects of predator exposure on pathogen transmission potential. *Proc R Soc B*, 282:20152430.

Savic S, Vidic B, Grgic Z, Potkonjak A, Spasojevic L. 2014. Emerging vector-borne diseases – incidence through vectors. *Front Public Health*, 2:267.

Schiller A. 2015. Developing rearing cages for *Toxorhynchites rutilus septentrionalis*. *Wing Beats*, 26(2):27-30.

Schiller A, Allen M, Coffey J, Fike A, Carballo F. 2019. Updated methods for the production of *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae) for use as biocontrol agent against container breeding pest mosquitoes in Harris County, Texas. *J Insect Sci*, 19(2):8.

Scholte EJ, Knols BGJ, Samson RA, Takken W. 2004. Entomopathogenic fungi for mosquito control: a review. *J Insect Sci*, 4(19).

Therneau TM. 2015. A Package for Survival Analysis in R. R package version 2.38. Available from: https://CRAN.R-project.org/package=survival.

Therneau TM, Grambsch PM. 2000. *Modeling Survival Data: Extending the Cox Model*. New York: Springer.

Timmermann SE, Briegel H. 1999. Larval growth and biosynthesis of reserves in mosquitoes. *J Insect Physiol*, 45:461-470.

Trpis M. 1972. Development and predatory behavior of *Toxorhynchites brevipalpis* (Diptera: Culicidae) in relation to temperature. *Environ Entomol*, 1(5):537-546.

Winch PJ, Barrientos-Sanchez G, Puigserver-Castro E, Manzano-Cabrera L, Lloyd LS, Mendez-Galvan JF. 1992. Variation in *Aedes aegypti* larval indices over a one year period in a neighborhood of Merida, Yucatan, Mexico. *J Am Mosquito Contr*, 8(2):193-195.

Zar JH. 2009. *Biostatistical Analysis*. 5th Edition. London: Pearson Education International.

Zuharah WF, Fadzly N, Yusof NA, Dieng H. 2015. Risky behaviors: effects of *Toxorhynchites splendens* (Diptera: Culicidae) predator on the behavior of three mosquito species. *J Insect Sci*, 15(1):128.

FIGURES AND TABLES

Figure 1 – Boxplot of *Toxorhynchites rutilus septentrionalis* predation rate (PR) on *Aedes aegypti* and *Culex quinquefasciatus*. Average PR for *Ae. aegypti* was 0.363 deaths per hour (0 – 0.833) and *Cx. quinquefasciatus* was 0.381 deaths per hour (0 – 0.833).
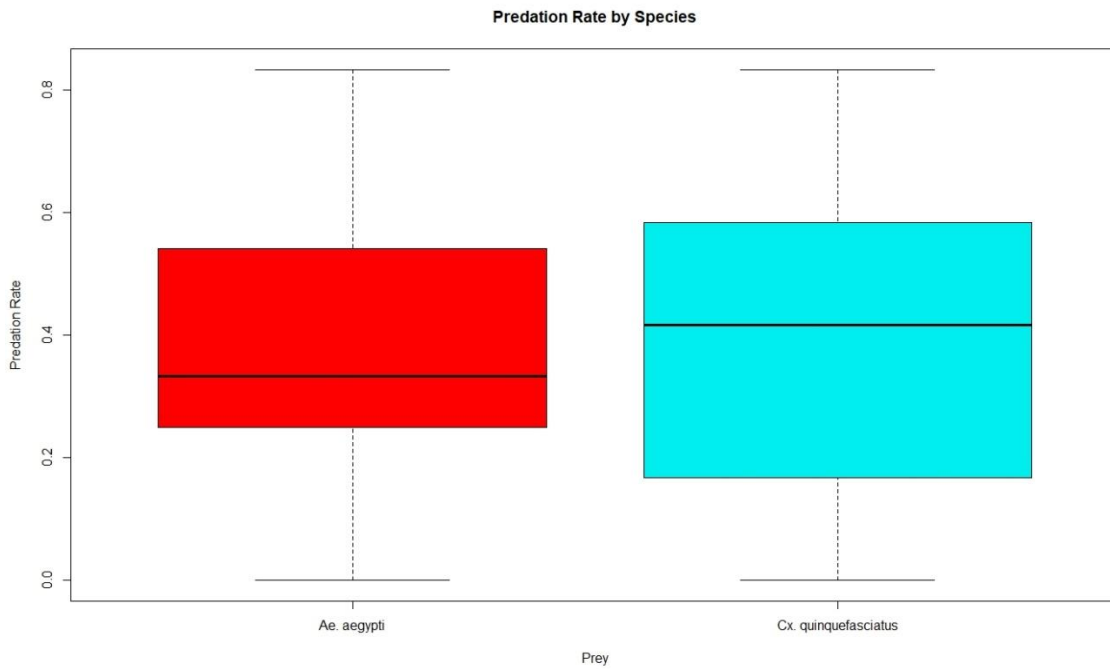
Figure 2 – Kaplan-Meier survivorship curves for *Aedes aegypti* and *Culex quinquefasciatus* in the presence of a single *Toxorhynchites rutilus septentrionalis* larvae. Shaded areas represent 95% confidence intervals. + indicates the presence of right censored individuals.
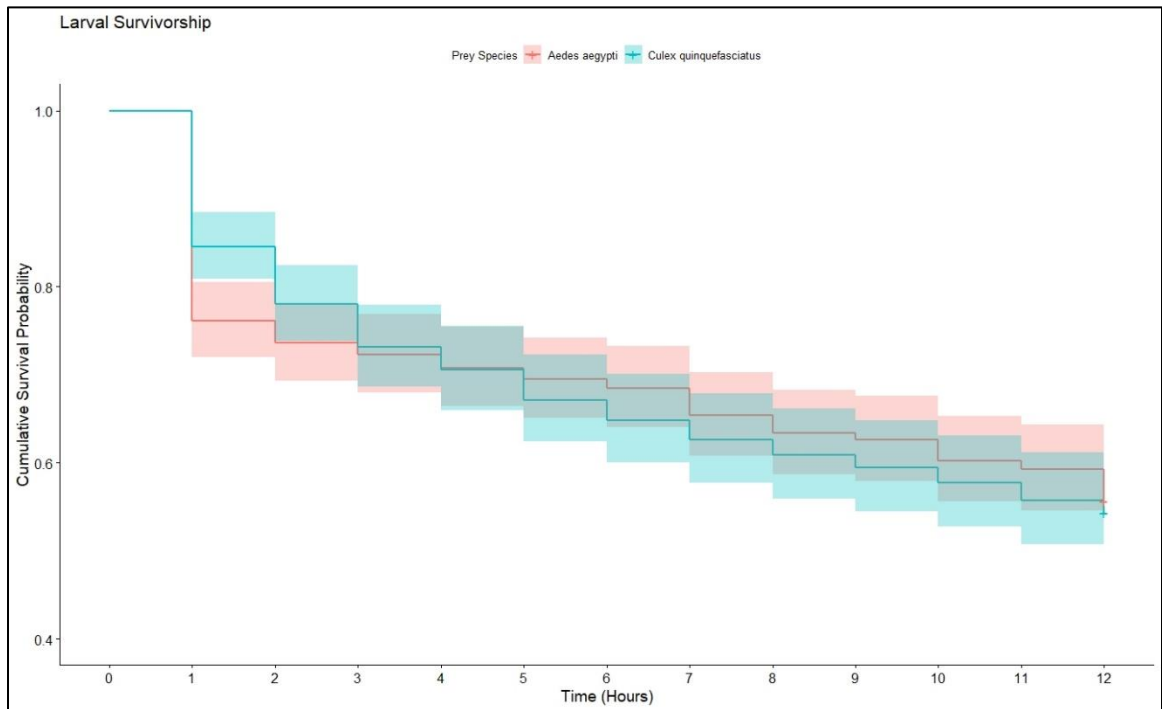
Table 1 – Results of the predation rate (PR) and Kaplan-Meier analyses and the total

number of individuals observed, killed, and censored of each prey type.

Prey	N Observed	N Killed	N Censored	% of Total Prey Killed	Mean PR	S_{12}
Aedes aegypti	390	173 (44.4%)	217 (55.6%)	0.52	0.363 (0 - 0.833)	0.556 (SE = 0.0252)
Culex quinquefasciatus	350	160 (45.7%)	190 (54.3%)	0.48	0.381 (0 - 0.833)	0.543 (SE = 0.0266)

POTENTIAL DISTRIBUTION OF THE BIOCONTROL AGENT

TOXORHYNCHITES RUTILUS BY 2070

DANIEL S. MARSHALL

DEPARTMENT OF BIOLOGY, UNIVERSITY OF CENTRAL OKLAHOMA,

EDMOND, OK 73034

ABSTRACT

Climate change projections indicate that mosquito distributions will expand to include new areas of North America increasing human exposure to mosquito-borne disease. Controlling these vectors is imperative, as mosquito-borne disease incidence will rise in response to expansion of mosquito range and increased seasonality. One means of mosquito control used in the United States is the biocontrol agent, *Toxorhynchites rutilus*. Climate change will open new habitats for its use by vector control organizations, but the extent of this change in habitat is currently unknown. I used a maximum entropy approach to create species distribution models for *Tx. rutilus* under four climate change scenarios by 2070. Mean temperature of warmest quarter (22.6°C to 29.1°C), annual precipitation (1025.15 mm to 1529.40 mm), and precipitation seasonality (≤ 17.86) were the most important bioclimatic variables for suitable habitat. The centroid of current possible habitat distribution of *Tx. rutilus* was found in central Tennessee. Depending upon the scenario, centroids shifted north-northeast 97.68 km to 280.16 km by 2070. The extreme change in area of greater than 50% suitable habitat probability was 141.14% with 99.44% area retained. My models indicate limited change in current habitat as well

as creation of new habitat. These results are promising for North American mosquito control programs for the continued and potential combat of vector mosquitoes using *Tx. rutilus*.

KEYWORDS *Toxorhynchites rutilus,* biological control, climate change, ecological niche model, Maxent

INTRODUCTION

The incidence of mosquito-borne illness is expected to increase in the future as vector populations expand due to climate change (Bouzid et al. 2014, Savic et al. 2014, Carvalho et al. 2017, IPCC 2018, Kraemer et al. 2019), increasing globalization (Kilpatrick 2011, Powell 2016), changes in land use, and urbanization (Bowden et al. 2011, Rogalski et al. 2017). As of 2017, the estimated number of annual cases of the major mosquito-vectored diseases (malaria, dengue fever, lymphatic filariasis, chikungunya, Zika virus, yellow fever, Japanese encephalitis, and West Nile virus) was over 347 million with nearly 448,000 estimated deaths (WHO 2018). Controlling mosquito populations is critical in the prevention and elimination of the diseases they vector. Chemical pesticides (Faraji and Unlu 2016), entomopathogenic bacteria, viruses, and fungi (Atyame et al. 2011, Kean et al. 2015), sterilized male mosquitoes (Alphey et al. 2010), reproduction-inhibiting bacteria, genetically modified adults, and natural enemies (Benelli et al. 2016) are employed to kill or otherwise prevent the reproduction of these insects and limit mosquito-borne illness.

One such natural enemy, the mosquito *Toxorhynchites rutilus* (Coq.), is a biocontrol

agent used in the United States (Focks et al. 1982). The use of *Tx. rutilus* as a biocontrol

involves mass breeding of the predator and inundative releases of adults. Also known as

the elephant mosquito and the mosquito assassin, the larvae prey on aquatic invertebrates

in tree holes and artificial containers (Dodge 1964, Focks 2007, Schiller et al. 2019).

Adults feed on nectar and never blood and are thus incapable of transmitting human or

animal pathogens (Collins and Blackwell 2000, Burkett-Cadena 2013). *Toxorhynchites*

rutilus is widely distributed across eastern North America, ranging from northern Mexico

to New England and the Atlantic coast to the Great Plains (Darsie and Ward 2005).

Many studies have modeled the potential distributions of vector mosquitoes due

to climate change (Epstein et al. 1998, Hongoh et al. 2012, Brown et al. 2015). However,

recent work suggests that some native and established natural enemy populations may be

at risk of extinction due to climate change (Thurman et al. 2017). The current study

represents the only of its kind for *Tx. rutilus* and addresses a critical gap in knowledge, as

biocontrol deployments of *Tx. rutilus* will be less effective in habitats where climatic

variables limit oviposition and embryonic and larval development (Campos and Lounibos

2000, Focks 2007; Anita Schiller, personal communication). Similar models found that

warmer and wetter habitats are projected to move northward in North America

(McKenney et al. 2007, Butler et al. 2016). Over the next 50 years higher latitudes and

higher elevations will likely experience greater numbers of mosquitoes for longer periods

each year (Ryan et al. 2019). Affected regions will benefit from increased *Tx. rutilus*

habitat and vector control organizations in these areas may begin using the predator as a

biocontrol agent. Areas already utilizing *Tx. rutilus* as a biocontrol will likely have to

deploy the predator for longer periods throughout the year due to increased vector

seasonality. Breeding adequate numbers of these mosquitoes to achieve vector control

requires considerable effort in both labor and resources (Schiller et al 2019; Schiller,

personal communication). To maximize production and release efficiency, vector control

organizations require new and more accurate information to make better-informed

decisions on the release of *Tx. rutilus*. Additionally, the possibility that natural

populations of the predator may experience extinction events due to climate change needs

to be addressed as releases of *Tx. rutilus* will be ineffective in those conditions. I used

Maxent to model the niches of *Tx. rutilus* and then modeled future niches under four

climate change scenarios in order to identify areas that will be suitable for this biocontrol

agent by 2070.

MATERIALS AND METHODS

Occurrence data were downloaded from Global Biodiversity Information Facility

(GBIF.org). I mapped these data and visually identified and removed aberrant

occurrences. Data were resampled using ENMTools (Warren et al. 2019) such that one

record occurred per 100 km^2 (Warren et al. 2010). Elevation and bioclimatic variables

were obtained from WorldClim (http://www.worldclim.org/; Table 1) at a 5 arc-minutes

resolution (Hijmans et al. 2005). I trimmed the spatial extent of elevation and climatic

variables from central Mexico to northern Canada using ArcGIS v10.4 (ESRI Inc.,

Redlands, CA; ESRI 2016). A total of 258 *Tx. rutilus* occurrence records were

downloaded from GBIF. After resampling, I used a total of 123 records for constructing

my species distribution model.

I modeled the current and projected distributions of *Tx. rutilus* using the maximum entropy (Maxent) approach (Phillips et al. 2006). All bioclimatic variables were modeled initially. I retained the variables that had the greatest effect on the gain in order to observe the strongest predictive model (Butler et al. 2016). This included variables that greatly increased the gain as well as those that caused the greatest decrease in gain. I avoided overfitting by penalizing increased model complexity using a regularization approach (Phillips et al. 2006, Merckx et al. 2011). I evaluated regularized models with Akaike's information criterion (AIC; Warren and Seifert 2011) using variables that did not exhibit high multicollinearity (Jones et al. 2010). Sensitivity versus specificity were plotted to create receiver operating characteristic (ROC) curves. I used cross-validation area under the curve (AUC) scores to evaluate accuracy of the model along with AIC scores and model weights (So and Sham 2010).

Predicted climatic models from the Intergovernmental Panel on Climate Change (IPCC) were used to project the potential distribution of *Tx. rutilus* by 2070. I obtained these climatic models from WorldClim (Hijmans et al. 2005). Intergovernmental Panel on Climate Change scenarios include representative concentration pathway (RCP) 2.6, RCP 4.5, RCP 6.0, and RCP 8.5. In the RCP 2.6 scenario carbon dioxide emissions peak by 2020 and then decline, these emissions peak by 2040 in the RCP 4.5 scenario, by 2080 in RCP 6.0. Emissions continue to rise throughout the 21st century in RCP 8.5. I used model averages under each RCP scenario to create projected suitable habitat models.

RESULTS

The best model for *Tx. rutilus* (i.e. with the lowest AICc score) included the variables mean temperature of warmest quarter, annual precipitation, and precipitation seasonality (Table 2). The AUC for this model was 0.960 ± 0.006. Areas that were predicted to have suitability >50% had a mean temperature of warmest quarter of 22.6°C to 29.1°C, annual precipitation of 1025.15 mm to 1529.40 mm, and precipitation seasonality of less than 17.86. The current modeled suitable distribution ranged from northern Mexico to southern Canada through central and eastern United States (Figure 1). Highly suitable areas (i.e. those of >50% suitability) were restricted to south Texas through central Illinois and southern Georgia to central Pennsylvania excluding Appalachia.

The median projected increase in highly suitable conditions for all *Tx. rutilus* was 84.03% (range 9.61% – 141.14%); although there was considerable variation among RCP scenarios (Table 3). Under the RCP 2.6 scenario, the amount of suitable habitat increased by only 9.61%. In contrast, under RCP scenarios 4.5, 6.0, and 8.5, the amount of suitable habitat increased by 70.76%, 114.62%, and 141.14%, respectively. The amount of currently highly suitable habitat retained in future projections for this species was 89.78% to 99.65%, with a median of 96.92%. The RCP 2.6 scenario had the least suitable habitat retention with range losses mostly in Mississippi, Alabama, and Kentucky (Figure 2). Under RCP scenarios 4.5 and 6.0, 99.65% and 98.80% suitable habitat was retained, respectively. These scenarios indicate highly suitable conditions will expand into southern Canada and peninsular Florida. The RCP 8.5 scenario retained 99.44% of current habitat. The probable range under this scenario extended further south than

71

previous models and also expanded in geographic distribution around the Great Lakes and New England.

The centroid of the current suitable *Tx. rutilus* distribution according to the best model was in central Tennessee (Figure 3). Centroids shifted generally north-northeast in successive scenarios. The median projected centroid shift for each scenario was 29.0 km per decade (range 16 km/decade – 47 km/decade), but variability existed in the response rate. The centroids for the RCP 2.6 and 4.5 scenarios were relatively close together, only 97.68 km north and 126.91 km north-northeast from the current probable centroid, respectively (Table 4). Centroids for RCP 6.0 and 8.5 scenarios were farther apart, in southern and northern Kentucky, respectively. Scenarios RCP 6.0 and RCP 8.5 were 194.52 km north-northeast and 280.16 km north-northeast from the current probably centroid, respectively.

DISCUSSION

My models showed that the current range of *Tx. rutilus* will expand northward by 2070. The RCP 2.6 scenario showed a slight north and northwestward increase into the southern Great Lakes region and central New England. Scenarios RCP 4.5 and RCP 6.0 demonstrated a moderate increase in northward and westward ranges. The RCP 8.5 scenario indicated a strong northward and westward expansion from the current range into southern Quebec and Ontario and northern New England. Plotted centroids showed a north northeastward trend moving from central Tennessee to the Kentucky-Indiana border in the extreme scenario. These centroids showed a small change in potential *Tx. rutilus* habitat.

Toxorhynchites rutilus response to mean temperature of warmest quarter, annual precipitation, and precipitation seasonality was likely due to an increase in suitable vegetation and prey, a greater number of individuals reaching maturity, and a reduced length of overwintering seasons. Because *Tx. rutilus* is sylvatic it may be that increased potential habitat was due partially to an increase in suitable woody vegetation in which its oviposition sites are located. Generally, temperate forests will benefit from increased temperatures and precipitation (Saxe et al. 2001). Eastern North American forests are less sensitive to climate change than their western counterparts (Phipps 1982) which, ignoring land use changes, explains the extent of habitat retention in my models. Additionally, increased precipitation will keep tree holes and other oviposition sites filled with water thereby increasing larval habitat. An increased length of warmer portions of the year will result in increased food abundance (i.e. vector mosquitoes and flowering plants). This greater seasonality also contributes to an increased number of generations per year of both *Tx. rutilus* and vector mosquitoes as well as reduced diapause periods. Insectary temperatures recommended for optimal growth of *Tx. rutilus* are between 21°C and 29°C (Schiller et al. 2019). The increase in mean temperature of warmest quarter to 22.6°C to 29.1°C will allow more mosquitoes to reach maturity.

Vector control organizations currently using *Tx. rutilus* in the southern United States will be unaffected by climate change as the four RCP scenarios had little effect on the species' southern habitat. This is good news for mosquito control organizations as deployments of *Tx. rutilus* will remain effective as vector populations increase and temperatures rise (Collins and Blackwell 2000). However, the need for bolstered control methods is apparent when considering the potential for increased vector populations and

seasonality. Increased length of mosquito season will require greater numbers of captive

Tx. rutilus bred annually. This puts an increasing financial burden on control

organizations in both resources and labor to effectively combat mosquitoes. Novel

mosquito controls, particularly integrated vector management will undoubtedly be

necessary in the near future (WHO 2012, Fernandes et al. 2018).

Some research indicates that native biocontrols are at risk of extinction (Thurman

et al. 2017). This does not appear to be that case for *Tx. rutilus*. I do not anticipate

extinction events of *Tx. rutilus* as my models indicated high retention of current habitat

under the four climate change scenarios. This may be due in large part to the generalist

feeding habits of this predator (Collins and Blackwell 2000). Additionally, Deutsch et al.

(2008) found that non-tropical insect species will exhibit limited negative responses to

increased global temperatures through the 21st century. Collier and Steenwyk identified

environmental conditions that are unfavorable for augmentative biocontrol releases

(Collier and Van Steenwyk 2004). They found that, generally, too hot and dry conditions

lowered efficacy in agricultural settings. These findings should be of concern to mosquito

control professionals, but my models show that this will not be of concern in eastern

North America. Most research on biocontrol responses to climate change have focused on

agricultural pests. More work is needed to evaluate the efficacy of biocontrols of

mosquitoes and other vectors of emerging and re-emerging infectious disease.

Much research has focused on vector-borne disease risk under climate change.

Vector-borne disease occurrence will broadly increase due to increasing globalism

(Berrang-Ford et al. 2009) and vector dependence on the warmer and wetter conditions

predicted by climate change models (Lafferty 2009). In areas where these conditions will

occur, naïve populations are at risk of exposure to emerging and re-emerging vector-borne diseases such as dengue fever and malaria (Berrang-Ford et al. 2009, Bouzid et al. 2014). Increased seasonality is another concern for establishment of endemic vector-borne disease. In areas where autochthonous infections are rare, increased seasonality will heighten the risk of establishment by lengthening the time of the year in which vectors are active. Areas on the fringe of endemic transmission areas and those with occasional outbreaks are of particular concern for establishment of vector-borne disease (Butterworth et al. 2017).

These models consider bioclimatic variables under climate change scenarios. They do not account for habitat loss due to anthropogenic factors such as deforestation and urbanization. Further study is needed to adequately identify potential future habitats based on bioclimatic variables, urbanization, and vegetation modelling (Bowden et al. 2011, Khazan et al. 2015, Rogalski et al. 2017, Kraemer et al. 2019). Occurrence records tend to be clustered around areas easily accessed near roadways, towns and cities, and bodies of water near homes or areas of interest to collectors and observers (Newbold 2010). Low sample size coupled with potentially biased occurrence records may have resulted in skewed habitat predictions (Feeley and Silman 2011). Additionally, I did not account for interspecific and conspecific competition when creating my models. These factors are of great importance when projecting potential distributions of organisms and further study will be needed to maximize these models in accordance with inter- and conspecific competition (Yoshioka et al. 2012, Wasserberg et al. 2014, Lounibos and Juliano 2018). In the case of *Tx. rutilus*, I expect introduction into new habitats to be by anthropogenic means in the form of inundative control releases. Mass releases of

predatory mosquitoes will suffer losses by competition but will likely not be limiting due to the sheer numbers of individuals released.

All four scenarios showed the potential for the use of *Tx. rutilus* as a biocontrol in an expanded range over the next 50 years. Notably, communities in New England and the American Midwest will be able to take advantage of this increase in habitat suitability. Under the RCP 8.5 scenario, cities as far north as Montreal, Quebec could support populations of *Tx. rutilus.* In these northern climes where the incidence of vector-borne disease is projected to rise, sustainable *Tx. rutilus* habitat and the benefits of natural vector reduction will certainly be welcome (Berrang-Ford et al. 2009, Hongoh et al. 2012). Additionally, retention of *Tx. rutilus* habitat in the southern United States is a reassuring sign in a time when the future of mosquito-borne illness in North America is of great concern.

REFERENCES CITED

Alphey L, Benedict M, Bellini R, Clark GG, Dame DA, Service MW, Dobson SL. 2010.
Sterile-insect methods for control of mosquito-borne diseases: an analysis.
Vector-borne and Zoonotic Dis 10(3):295-311.

Atyame CM, Pasteur N, Dumas E, Tortosa P, Tantely ML, Pocquet N, Licciardi S,
Bheecarry A, Zumbo B, Weill M, Duron O. 2011. Cytoplasmic incompatibility as
a means of controlling *Culex pipiens quinquefasciatus* mosquito in the islands of
the south-western Indian Ocean. *PLoS Negl Trop Dis* 5(12): e1440.

Benelli G, Jeffries CL, Walker T. 2016. Biological control of mosquito vectors: past,
present, and future. *Insects*, 7(52).

Berrang-Ford L, MacLean JD, Gyorkos TW, Ford JD, Ogden NH. 2009. Climate change
and malaria in Canada: a systems approach. *Interdiscip Persp Inf Dis*
2009:385487.

Bouzid M, Colon-Gonzalez FJ, Lung T, Lake IR, Hunter PR. 2014. Climate change and
the emergence of vector-borne diseases in Europe: case study of dengue fever.
BMC Public Health 14:781.

Bowden SE, Magori K, Drake JM. 2011. Regional differences in the association between
land cover and West Nile virus disease incidence in humans in the United States.
Am J Trop Med Hyg 84(2):234-238.

Brown HE, Young A, Lega J, Andreadis TG, Schurich J, Comrie A. 2015. Projection of
climate change influences on U.S. West Nile virus vectors. *Earth Interact*
19(18):1-18.

Burkett-Cadena ND. 2013. *Mosquitoes of the Southeastern United States.* Tuscaloosa,
AL: The University of Alabama Press.

Butler C J, Stanila BD, Iverson JB, Stone PA, Bryson M. 2016. Projected changes in
climatic suitability for *Kinosternon* turtles by 2050 and 2070. *Ecol Evol* 6:7690-
7705.

Butterworth MK, Morin CW, Comrie AC. 2017. An analysis of the potential impact of
climate change on dengue transmission in the southeastern United States. *Environ
Health Perspect* 125(4):579-585.

Campos RE, Lounibos LP. 2000. Life tables of *Toxorhynchites rutilus* (Diptera:
Culicidae) in nature in southern Florida. *J Med Entomol* 37(3):385-392.

Carvalho BM, Rangel EF, Vale MM. 2017. Evaluation of the impacts of climate change
on disease vectors through ecological niche modelling. *Bull Entomol Res*
107:419-430.

Collier T, Van Steenwyk R. 2004. A critical evaluation of augmentative biological
control. *Biol Control* 31:245-256.

Collins LE, Blackwell A. 2000. The biology of *Toxorhynchites* mosquitoes and their
potential as biocontrol agents. *Biocontrol* 21(4):105N-116N.

Darsie RF, Ward RA. 2005. *Identification and Geographical Distribution of the
Mosquitoes of North America, North of Mexico.* Gainesville, FL: University Press
of Florida.

Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin
PR. 2008. Impacts of climate warming on terrestrial ectotherms across latitude. *P
Natl Acad Sci USA* 105(18):6668-6672.

Dodge HR. 1964. Larval chaetotaxy and notes on the biology of *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae). *Ann Entomol Soc Am* 57:46-53.

Epstein PR, Diaz HF, Elias S, Grabherr G, Graham NE, Martens WJM, Mosley-Thompson E, Susskind J. 1998. Biological and physical signs of climate change: focus on mosquito-borne diseases. *B Am Meteorol Soc* 79(3):409-417.

ESRI. 2016. *ArcGIS 10.4*. Environmental Systems Research Institute. Redlands, CA, USA.

Faraji A, Unlu I. 2016. The eye of the tiger, the thrill of the fight: effective larval and adult control measures against the Asian tiger mosquito, *Aedes albopictus* (Diptera: Culicidae), in North America. *J Med Entomol* 53(5):1029-1047.

Feeley KJ, Silman MR. 2011. Keep collecting: accurate species distribution modelling requires more collections than previously thought. *Diversity Distrib* 17:1132-1140.

Fernandes JN, Moise IK, Maranto GL, Beier JC. 2018. Revamping mosquito-borne disease control to tackle future threats. *Trends Parasitol* DOI: 10.1016/j.pt.2018.01.005.

Focks DA, Sackett SR, Bailey DL. 1982. Field experiments on the control of *Aedes aegypti* and *Culex quinquefasciatus* by *Toxorhynchites rutilus rutilus* (Diptera: Culicidae). *J Med Entomol* 19(3):336-339.

Focks DA. 2007. *Toxorhynchites* as biocontrol agents. *J Am Mosquito Contr*, 23(2):118-127.

GBIF.org. GBIF Occurrence Download https://doi.org/10.15468/dl.6hji3j. [Accessed February 4, 2019].

Hijmans R J, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol* 25:1965-1978.

Hongoh V, Berrang-Ford L, Scott ME, Lindsay LR. 2012. Expanding geographical distribution of the mosquito, *Culex pipiens*, in Canada under climate change. *Appl Geogr* 33:53-62.

IPCC [Intergovernmental Panel on Climate Change]. 2018. *Global warming of 1.5°C: Summary for Policymakers* [Internet]. Geneva, Switzerland: Intergovernmental Panel on Climate Change [Accessed November 25, 2019]. Available from: https://www.ipcc.ch/sr15/.

Jones CC, Acker SA, Halperin CB. 2010. Combining local- and large-scale models to predict the distributions of invasive plant species. *Ecol Appl* 20:311-326.

Kean J, Rainey SM, McFarlane M, Donald CL, Schnettler E, Kohl A, Pondeville E. 2015. Fighting arbovirus transmission: natural and engineered control of vector competence in *Aedes* mosquitoes. *Insects* 6:236-278.

Khazan ES, Bright EG, Beyer JE. 2015. Land management impacts on tree hole invertebrate communities in a Neotropical rainforest. *J Insect Conserv* 19:681-690.

Kilpatrick AM. 2011. Globalization, land use, and the invasion of West Nile virus. *Science* 334(6054):323-327.

Kraemer MUG, Reiner RC Jr., Brady OJ, Messina JP, Gilbert M, Pigott DM, Yi D, Johnson K, Earl L, Marczak LB, Shirude S, Weaver ND, Bisanzio D, Perkins TA, Lai S, Lu X, Jones P, Coelho GE, Carvalho RG, Bortel WV, Marsboom C, Hendrickx G, Schaffner F, Moore CG, Nax HH, Bengtsson L, Wetter E, Tatem

AJ, Brownstein JS, Smith DL, Lambrechts L, Cauchemez S, Linard C, Faria NR, Pybus OG, Scott TW, Liu Q, Yu H, Wint GRW, Hay SI, Golding N. 2019. Past and future spread of the arbovirus vectors *Aedes aegypti* and *Aedes albopictus*. *Nat Microbiol* 4:854-863.

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

Lounibos LP, Juliano SA. 2018. Where vectors collide: the importance of mechanisms shaping the realized niche for modeling ranges of invasive *Aedes* mosquitoes. *Biol Invasions* 20:1913-1929.

McKenney DW, Pedlar JH, Lawrence K, Campbell K, Hutchinson MF. 2007. Potential impacts of climate change on the distribution of North American trees. *BioScience* 57(11):939-948.

Merckx B, Steyaert M, Vanreusel A, Vincx M, Vanaverbeke J. 2011. Null models reveal preferential sampling, spatial autocorrelation and overfitting in habitat suitability modelling. *Ecol Model* 222:588-597.

Newbold T. 2010. Applications and limitations of museum data for conservation and ecology, with particular attention to species distribution models. *Prog Phys Geog* 34(1):3-22.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modelling of species distributions. *Ecol Model* 190:231-259.

Phipps RL. 1982. Comments of interpretation of climatic information from tree rings, eastern North America. *Tree-Ring Bulletin* 42:11-22.

Powell JR. 2016. Mosquitoes on the move. *Science* 354(6315):971-972.

Rogalski MA, Gowler CD, Shaw CL, Hufbauer RA, Duffy MA. 2017. Human drivers of

ecological and evolutionary dynamics in emerging and disappearing infectious

disease systems. *Phil Trans R Soc B* 372:20160043.

Ryan SJ, Carlson CJ, Mordecai EA, Johnson LR. 2019. Global expansion and

redistribution of *Aedes*-borne virus transmission risk with climate change. *PLoS

Negl Trop Dis* 13(3):e0007213.

Savic S, Vidic B, Grgic Z, Potkonjak A, Spasojevic L. 2014. Emerging vector-borne

diseases – incidence through vectors. *Front Public Health* 2:267.

Saxe H, Cannell MGR, Johnsen O, Ryan MG, Vourlitis G. 2001. Tree and forest

functioning in response to global warming. *New Phytol* 149:369-400.

Schiller A, Allen M, Coffey J, Fike A, Carballo F. 2019. Updated methods for the

production of *Toxorhynchites rutilus septentrionalis* (Diptera: Culicidae) for use

as biocontrol agent against container breeding pest mosquitoes in Harris County,

Texas. *J Insect Sci* 19(2):8.

So HC, Sham PC. 2010. A unifying framework for evaluating the predictive power of

genetic variants based on the level of heritability explained. *PLoS Genetics*

6(12):e1001230.

Thurman JH, Crowder DW, Northfield TD. 2017. Biological control agents in the

Anthropocene: current risks and future options. *Curr Opin Insect Sci* 23:59-64.

Warren DL, Glor RE, Turelli M. 2010. ENMTools: a toolbox for comparative studies of

environmental niche models. *Ecography* 33(3):607-611.

Warren DL, Seifert SN. 2011. Ecological niche modeling in Maxent: the importance of model complexity and the performance of model selection criteria. *Ecol Appl* 21(2):335-342.

Warren, DL, Matzke N, Cardillo M, Baumgartner J, Beaumont L, Huron N, Simoes M, Dinnage R. 2019. ENMTools (Software Package). [accessed December 4, 2019]. https://github.com/danlwarren/ENMTools.

Wasserberg G, Bailes N, Davis C, Yeoman K. 2014. Hump-shaped density-dependent regulation of mosquito oviposition site-selection by conspecific immature stages: theory, field test with *Aedes albopictus*, and a meta-analysis. *PLoS ONE* 9(3):e92658.

WHO [World Health Organization]. 2012. *Handbook for Integrated Vector Management.* Geneva: WHO Press.

WHO [World Health Organization]. 2018. *Annex 1. Global Burden of Major Vector-Borne Diseases* [Internet]. Geneva, Switzerland: World Health Organization [accessed October 22, 2019]. Available from: http://www.who.int/ vector-control/burden_vector-borne_diseases.pdf.

Yoshioka M, Couret J, Kim F, McMillan J, Burkot TR, Dotson EM, Kitron U, Vazquez-Prokopec GM. 2012. Diet and density dependent competition affect larval performance and oviposition site selection in the mosquito species *Aedes albopictus* (Diptera: Culicidae). *Parasites & Vectors* 5:225.

Table 1 Summary of bioclimatic variables used in this study.

Variable	Definition
BIO 1	Annual mean temperature
BIO 2	Mean diurnal range (Mean of monthly [max temp - min temp])
BIO 3	Isothermality (BIO 2 / BIO 7) * 100
BIO 4	Temperature seasonality (standard deviation * 100)
BIO 5	Max temperature of warmest month
BIO 6	Min temperature of coldest month
BIO 7	Temperature annual range (BIO 5 - BIO 6)
BIO 8	Mean temperature of wettest quarter
BIO 9	Mean temperature of driest quarter
BIO 10	Mean temperature of warmest quarter
BIO 11	Mean temperature of coldest quarter
BIO 12	Annual precipitation
BIO 13	Precipitation of wettest month
BIO 14	Precipitation of driest month
BIO 15	Precipitation seasonality (coefficient of variation)
BIO 16	Precipitation of wettest quarter
BIO 17	Precipitation of driest quarter
BIO 18	Precipitation of warmest quarter
BIO 19	Precipitation of coldest quarter
Elevation	Elevation above sea level

Table 2. A comparison of the top five model runs for *Toxorhynchites rutilus*. Log-likelihood is the natural log of the probability of the data given in the model. AIC_c is a corrected AIC score, used for a small sample size by increasing the cost for each parameter. The difference between the model with the lowest score (the "best" model) and the AIC_c score for each model is given by ΔAIC_c. The model weight ($wAIC_c$) is the relative likelihood for each model, divided by the total relative likelihood for all models that were considered. AUC (area under the curve) is a measure of the accuracy of the model.

Variables	Log likelihood	AIC_c score	ΔAIC_c	$wAIC_c$	Mean AUC
BIO 10, BIO 12, BIO 15	-1286.863	2608.211	0.000	0.955	0.960
Elevation, BIO 1, BIO 12, BIO 15,	-1285.202	2615.783	7.572	0.022	0.958
BIO 9, BIO 10, BIO 12, BIO 15	-1275.230	2617.085	8.874	0.011	0.964
Elevation, BIO 10, BIO 12, BIO 15	-1286.878	2619.135	10.924	0.004	0.959
BIO 10, BIO 14, BIO 15	-1290.101	2620.031	11.82	0.003	0.957

Table 3. The total area predicted to have >50% probability of suitable conditions for *Toxorhynchites rutilus* under each climate change scenario by 2070. Percent area of retention and change of highly suitable habitat relative to the current predicted distribution.

Scenario	Area (km^2)	% change in area from current	Area common to current (km^2)	% current distribution retained
Current	1,359,647.02			
RCP 2.6	1,490,357.88	9.61%	1,220,641.98	89.78%
RCP 4.5	2,321,781.88	70.76%	1,354,870.71	99.65%
RCP 6.0	2,918,007.51	114.62%	1,343,359.31	98.80%
RCP 8.5	3,278,635.34	141.14%	1,352,042.22	99.44%

Table 4 A summary of the distance from each centroid for each scenario to the current

centroid and the rate per decade.

Scenario	Distance (km) and direction from current	Rate per decade
RCP 2.6	97.68 (N)	16 km / decade
RCP 4.5	126.91 (NNE)	21 km / decade
RCP 6.0	194.52 (NNE)	32 km / decade
RCP 8.5	280.16 (NNE)	47 km / decade

Figure 1 The current possible distribution of *Toxorhynchites rutilus* according to the best
model. Blue circles indicate occurrence points. Habitat suitability probability is indicated
by black intensity, areas of white are unsuitable.
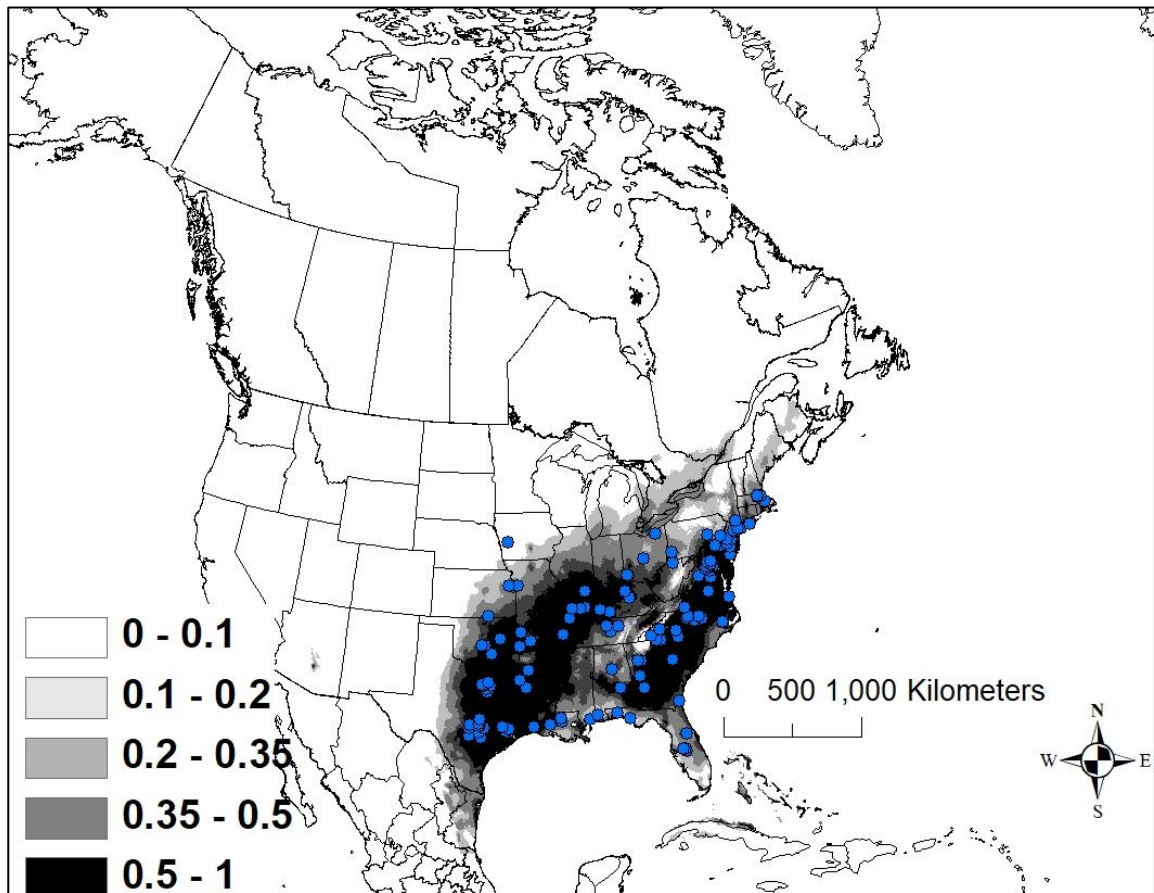
Figure 2 Predicted suitable habitat of *Toxorhynchites rutilus* for (A) RCP2.6, (B) RCP4.5, (B) RCP6.0, and (D) RCP8.5 scenarios. Habitat suitability probability is indicated by black intensity, areas of white are unsuitable.
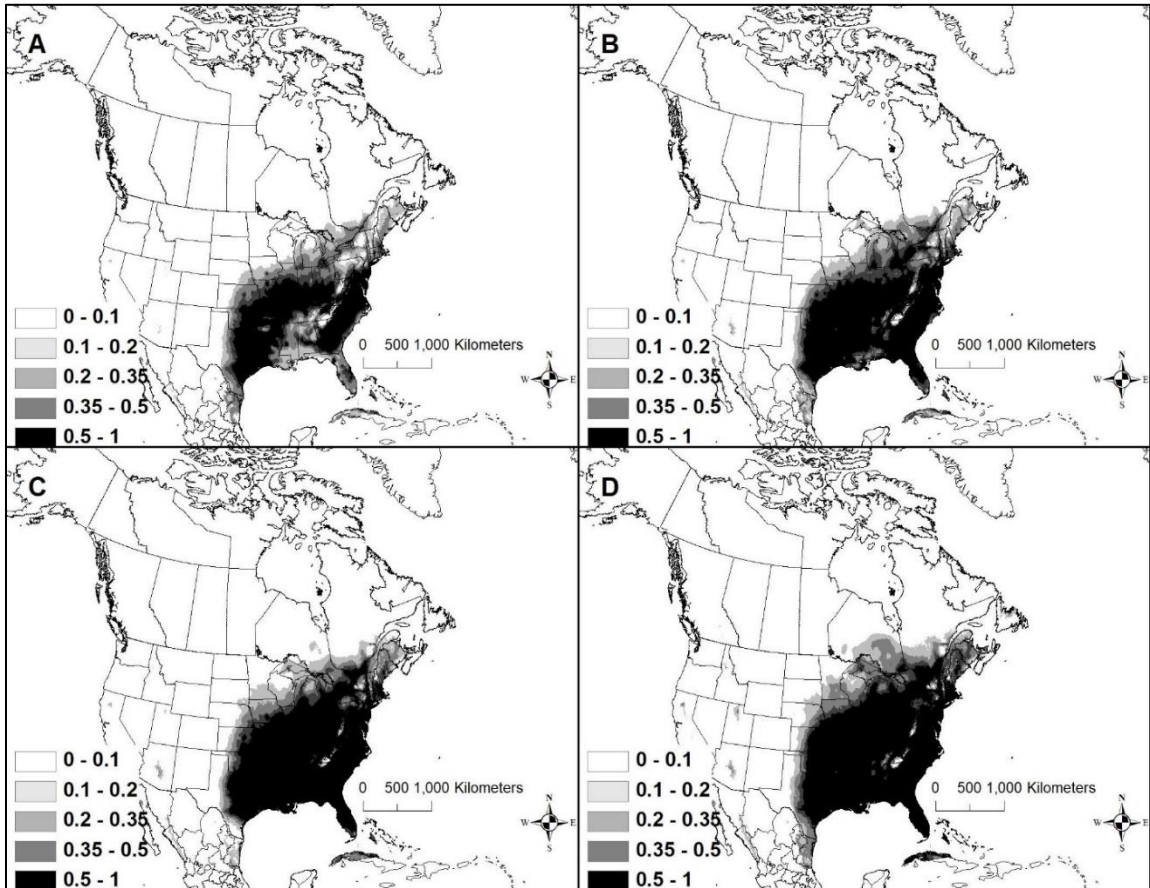
Figure 3 Centroids for the current and 2070 *Toxorhynchites rutilus* distributions under

each of the four climate change scenarios.
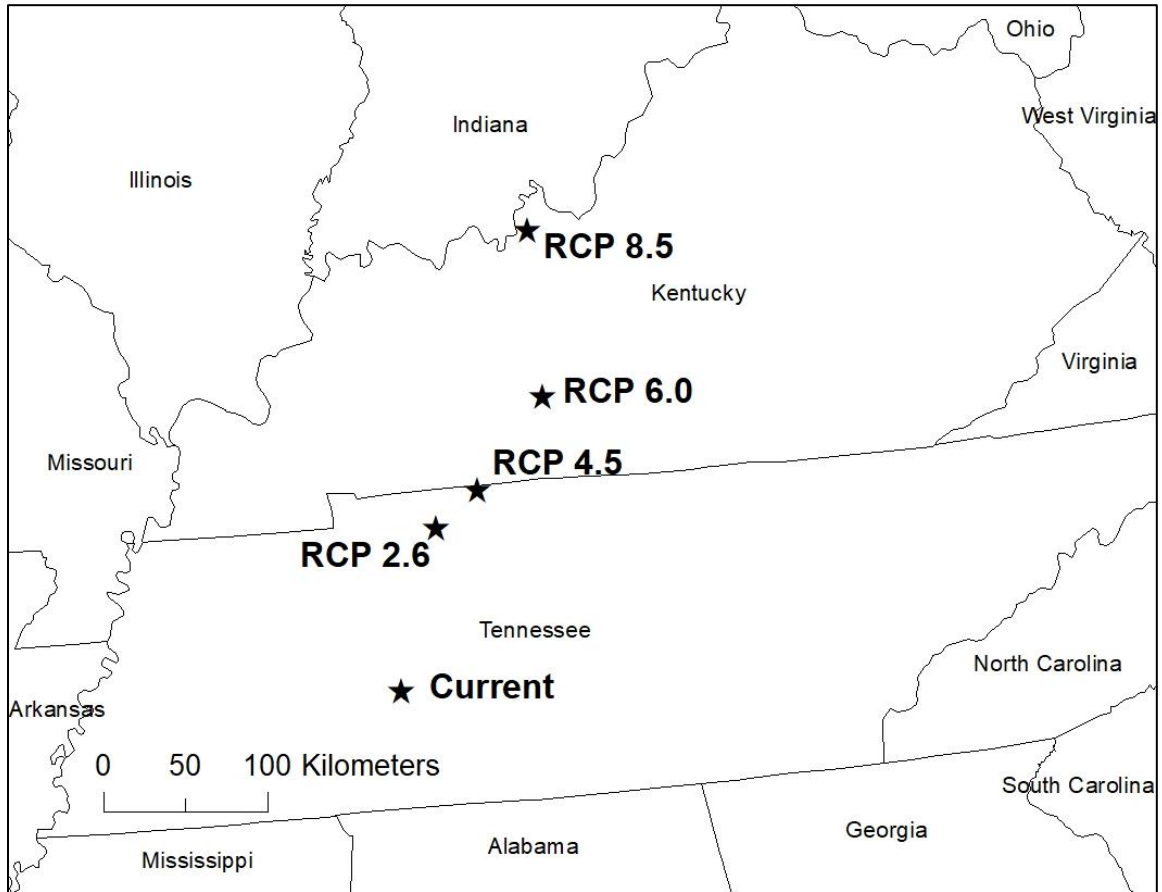
GENERAL SUMMARY

My thesis adds to the scant body of knowledge on which *Toxorhynchites* biological controls are based. The species on which this document focuses, *Toxorhynchites rutilus,* is of economic and public health importance in the United States. In this chapter I conclude my thesis by summarizing the work presented in the preceding chapters, discussing how my results contribute to the science of mosquito control, and suggest areas of future research.

In Chapter Two I observed fourth instar *Toxorhynchites rutilus septentrionalis* predation on *Aedes aegypti* and *Culex quinquefasciatus* as well as survivorship of the two prey. My analyses indicated no significant difference in predation rate or survivorship between prey. These results lend support to previous work demonstrating *Toxorhynchites* larvae as generalist predators (Campos and Lounibos 2000; Digma et al. 2019). *Toxorhynchites rutilus* polyphagy may contribute to broad mosquito control rather than species-specific control. However, a generalist diet may be inhibitory to its ability to control mosquitoes in the presence of other potential prey. Further research is needed to determine how predation changes between predator instars, habitats, prey diversity, and prey density.

Through predation and trait-mediated effects, these predators reduce survival of larvae, longevity, fecundity, vector capacity of adults, and ultimately the occurrence of mosquito-borne illness. Further study in field or near-field systems including complex aquatic communities is needed to determine the extent to which trait-mediated effects impact wild mosquitoes. Some historic releases of *Toxorhynchites* spp. have failed due to asynchrony in oviposition sites with target mosquitoes (Collins and Blackwell 2000).

Aedes aegypti, Cx. quinquefasciatus, and *Tx. r. septentrionalis* share breeding sites to a limited extent with variations in occurrence due to spatial partitioning and phenology (Bonnet 1946, Albeny-Simoes *et al*. 2014, Leisnham *et al*. 2014, Baak-Baak *et al*. 2016). Combined control strategies utilizing *Toxorhynchites* spp. offers a promising solution to compensate for asynchronous oviposition sites. Integrated mosquito management will become increasingly important in the coming decades as vector populations shift and disease incidence increases in response to climate change and globalization. Additional research is needed to evaluate the efficacy of *Toxorhynchites* mass releases in integrated mosquito control systems.

In Chapter 3 I showed that the current distribution of *Tx. rutilus* will expand northward by 2070 while the southern range will remain relatively intact. The intent of this chapter was to inform mosquito control professionals of the potential future areas in which *Tx. rutilus* may be successfully used as a biological control agent. My models will aid mosquito control professionals and policy makers in deciding mosquito abatement options and funding allotments over the next 50 years. Distribution models of the mosquito responded to three abiotic factors: mean temperature of warmest quarter, annual precipitation, and precipitation seasonality. Due to its sylvatic habit, *Tx. rutilus* response to these factors may have been a function of changes in preferred vegetation (*i.e.* oviposition sites and food plants). Additionally, lengthened suitable seasons will increase the number of vector mosquitoes which will increase the predator population as well. It is currently unclear why *Tx. rutilus* responds to these variables. This chapter represents the only ecological niche model of *Tx. rutilus*. Most research on biocontrol responses to climate change have focused on agricultural pests. More work is needed to evaluate the

efficacy of biocontrols of mosquitoes and other vectors of emerging and re-emerging infectious disease. Researchers should focus on how natural enemies disperse once released under different climate scenarios, how climate change will affect their ability to locate and capture prey, and how fecundity and longevity of these biocontrols will change.

Vector control organizations currently using *Tx. rutilus* in the southern United States will be able to continue using the predator as my models indicated little effect on the species' southern habitat. However, increased length of mosquito season, increased number of vectors, invasive mosquitoes, and insecticide resistance indicate a need for bolstered and novel control methods. Novel mosquito controls, particularly integrated mosquito management will become increasingly necessary in the future (WHO 2012, Fernandes et al. 2018).

REFERENCES CITED

Albeny-Simoes D, Murrell EG, Elliot SL, Andrade MR, Lima E, *et al*. 2014. Attracted to
the enemy: *Aedes aegypti* prefers oviposition sites with predator-killed conspecifics.
Oecologia, 175(2):481-492.

Baak-Baak CM, Cigarroa-Toledo N, Arana-Guardia R, Chi Chim WA, Chan Orilla JA, *et
al*. 2016. Mosquito fauna associated with *Aedes aegypti* (Diptera: Culicidae) in
Yucatan State of southeastern Mexico, and checklist with new records. *Fla Entomol*,
99(4):703-709.

Bonnet DD. 1946. The distribution of mosquito breeding by type of container in
Honolulu, T.H. Proc Hawn Ent Soc, 13(1):43-49.

Campos RE, Lounibos LP. 2000. Natural prey and digestion times of *Toxorhynchites
rutilus* (Diptera: Culicidae) in Southern Florida. *Ann Entomol Soc Am*, 93(6):1280-
1287.

Collins LE, Blackwell A. 2000. The biology of *Toxorhynchites* mosquitoes and their
potential as biocontrol agents. *Biocontrol*, 21(4):105N-116N.

Digma JR, Sumalde AC, Salibay CC. 2019. Laboratory evaluation of predation of
Toxorhynchites amboinensis (Diptera: Culicidae) on three mosquito vectors of
arboviruses in the Philippines. *Biol Control*, 137:104009.

Fernandes JN, Moise IK, Maranto GL, Beier JC. 2018. Revamping mosquito-borne
disease control to tackle future threats. *Trends Parasitol*, DOI:
10.1016/j.pt.2018.01.005.

Leisnham PT, LaDeau SL, Juliano SA. 2014. Spatial and temporal habitat segregation of
mosquitoes in urban Florida. *PLoS ONE*, 9(3):e91655.

WHO [World Health Organization]. 2012. *Handbook for Integrated Vector Management*.

Geneva: WHO Press.

APPENDICES

Appendix A1. Raw survivorship data from predation video analysis (Chapter 2). Status is either 1 (death) or 0 (censored). All Status = 0 occur in Hour = 12 as mosquitoes were not permitted to prematurely withdraw from the study. The number of live larvae at the start of each observation Hour is represented by n.

Dataset	Status	n	Hour	Prey
20190123	1	10	1	AE
20190123	1	10	1	AE
20190123	1	10	1	AE
20190123	1	10	1	AE
20190123	1	6	2	AE
20190123	1	5	3	AE
20190123	1	4	9	AE
20190123	0	3	12	AE
20190123	0	3	12	AE
20190123	0	3	12	AE
20190224	1	10	1	AE
20190224	1	10	1	AE
20190224	1	8	2	AE
20190224	1	7	4	AE
20190224	1	6	5	AE
20190224	1	5	6	AE
20190224	1	4	9	AE
20190224	0	3	12	AE
20190224	0	3	12	AE
20190224	0	3	12	AE
20190126	1	10	1	AE
20190126	1	10	1	AE
20190126	1	8	7	AE
20190126	0	7	12	AE
20190126	0	7	12	AE
20190126	0	7	12	AE
20190126	0	7	12	AE
20190126	0	7	12	AE
20190126	0	7	12	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190126	0	7	12	AE
20190127	1	10	1	AE
20190127	1	10	1	AE
20190127	1	10	1	AE
20190127	1	7	5	AE
20190127	1	6	7	AE
20190127	1	5	10	AE
20190127	0	4	12	AE
20190127	0	4	12	AE
20190127	0	4	12	AE
20190127	1	4	12	AE
20190128	1	10	1	AE
20190128	1	10	1	AE
20190128	1	8	3	AE
20190128	1	7	4	AE
20190128	1	6	8	AE
20190128	1	5	11	AE
20190128	0	4	12	AE
20190128	0	4	12	AE
20190128	0	4	12	AE
20190128	0	4	12	AE
20190129	1	10	1	AE
20190129	1	10	1	AE
20190129	1	8	8	AE
20190129	0	7	12	AE
20190129	0	7	12	AE
20190129	0	7	12	AE
20190129	0	7	12	AE
20190129	0	7	12	AE
20190129	0	7	12	AE
20190129	0	7	12	AE
20190130	1	10	1	AE
20190130	1	10	1	AE
20190130	1	8	2	AE
20190130	1	8	2	AE
20190130	1	6	6	AE
20190130	1	5	7	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190130	1	4	10	AE
20190130	1	3	11	AE
20190130	1	3	11	AE
20190130	1	1	12	AE
20190201	1	10	1	AE
20190201	0	9	12	AE
20190201	0	9	12	AE
20190201	0	9	12	AE
20190201	0	9	12	AE
20190201	0	9	12	AE
20190201	0	9	12	AE
20190201	0	9	12	AE
20190201	0	9	12	AE
20190201	0	9	12	AE
20190202	1	10	4	AE
20190202	1	9	8	AE
20190202	1	8	9	AE
20190202	0	7	12	AE
20190202	0	7	12	AE
20190202	0	7	12	AE
20190202	0	7	12	AE
20190202	0	7	12	AE
20190202	0	7	12	AE
20190202	0	7	12	AE
20190203	1	10	10	AE
20190203	0	9	12	AE
20190203	0	9	12	AE
20190203	0	9	12	AE
20190203	0	9	12	AE
20190203	0	9	12	AE
20190203	0	9	12	AE
20190203	0	9	12	AE
20190203	0	9	12	AE
20190203	0	9	12	AE
20190205	1	10	1	AE
20190205	1	10	1	AE
20190205	1	10	1	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190205	1	10	1	AE
20190205	1	6	4	AE
20190205	1	5	8	AE
20190205	1	4	10	AE
20190205	0	3	12	AE
20190205	0	3	12	AE
20190205	0	3	12	AE
20190207	1	10	1	AE
20190207	1	10	1	AE
20190207	1	8	10	AE
20190207	0	7	12	AE
20190207	0	7	12	AE
20190207	0	7	12	AE
20190207	0	7	12	AE
20190207	0	7	12	AE
20190207	0	7	12	AE
20190207	0	7	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190208	0	10	12	AE
20190210	1	10	1	AE
20190210	1	9	8	AE
20190210	0	8	12	AE
20190210	0	8	12	AE
20190210	0	8	12	AE
20190210	0	8	12	AE
20190210	0	8	12	AE
20190210	0	8	12	AE
20190210	0	8	12	AE
20190210	0	8	12	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190211	1	10	1	AE
20190211	1	9	7	AE
20190211	0	8	12	AE
20190211	0	8	12	AE
20190211	0	8	12	AE
20190211	0	8	12	AE
20190211	0	8	12	AE
20190211	0	8	12	AE
20190211	0	8	12	AE
20190211	0	8	12	AE
20190212	1	10	1	AE
20190212	1	10	1	AE
20190212	1	8	5	AE
20190212	0	7	12	AE
20190212	0	7	12	AE
20190212	0	7	12	AE
20190212	0	7	12	AE
20190212	0	7	12	AE
20190212	0	7	12	AE
20190212	0	7	12	AE
20190213	1	10	1	AE
20190213	1	9	7	AE
20190213	0	8	12	AE
20190213	0	8	12	AE
20190213	0	8	12	AE
20190213	0	8	12	AE
20190213	0	8	12	AE
20190213	0	8	12	AE
20190213	0	8	12	AE
20190213	0	8	12	AE
20190214	1	10	1	AE
20190214	1	10	1	AE
20190214	1	8	10	AE
20190214	0	7	12	AE
20190214	0	7	12	AE
20190214	0	7	12	AE
20190214	0	7	12	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190214	0	7	12	AE
20190214	0	7	12	AE
20190214	0	7	12	AE
20190215	1	10	1	AE
20190215	1	10	1	AE
20190215	1	8	10	AE
20190215	0	7	12	AE
20190215	0	7	12	AE
20190215	0	7	12	AE
20190215	0	7	12	AE
20190215	0	7	12	AE
20190215	0	7	12	AE
20190215	1	7	12	AE
20190216	1	10	1	AE
20190216	1	10	1	AE
20190216	1	10	1	AE
20190216	1	7	2	AE
20190216	1	6	5	AE
20190216	1	5	6	AE
20190216	0	4	12	AE
20190216	0	4	12	AE
20190216	0	4	12	AE
20190216	1	4	12	AE
20190309	1	10	1	AE
20190309	1	10	1	AE
20190309	1	10	1	AE
20190309	0	7	12	AE
20190309	0	7	12	AE
20190309	0	7	12	AE
20190309	0	7	12	AE
20190309	0	7	12	AE
20190309	0	7	12	AE
20190309	0	7	12	AE
20190308	1	10	1	AE
20190308	1	10	1	AE
20190308	1	10	1	AE
20190308	1	10	1	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190308	0	6	12	AE
20190308	0	6	12	AE
20190308	0	6	12	AE
20190308	0	6	12	AE
20190308	0	6	12	AE
20190308	0	6	12	AE
20190318	1	10	1	AE
20190318	1	10	1	AE
20190318	1	8	2	AE
20190318	1	8	2	AE
20190318	1	8	2	AE
20190318	1	5	7	AE
20190318	0	4	12	AE
20190318	0	4	12	AE
20190318	0	4	12	AE
20190318	0	4	12	AE
20190310	1	10	1	AE
20190310	1	10	1	AE
20190310	1	10	1	AE
20190310	0	7	12	AE
20190310	0	7	12	AE
20190310	0	7	12	AE
20190310	0	7	12	AE
20190310	0	7	12	AE
20190310	0	7	12	AE
20190310	1	7	12	AE
20190313	1	10	1	AE
20190313	1	9	11	AE
20190313	0	8	12	AE
20190313	0	8	12	AE
20190313	0	8	12	AE
20190313	0	8	12	AE
20190313	0	8	12	AE
20190313	0	8	12	AE
20190313	0	8	12	AE
20190313	0	8	12	AE
20190314	1	10	1	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190314	1	10	1	AE
20190314	1	10	1	AE
20190314	1	10	1	AE
20190314	1	10	1	AE
20190314	1	5	2	AE
20190314	1	4	3	AE
20190314	1	3	5	AE
20190314	1	2	10	AE
20190314	0	1	12	AE
20190315	1	10	1	AE
20190315	1	10	1	AE
20190315	1	10	1	AE
20190315	1	7	4	AE
20190315	1	6	7	AE
20190315	0	5	12	AE
20190315	0	5	12	AE
20190315	0	5	12	AE
20190315	0	5	12	AE
20190315	0	5	12	AE
20190320	1	10	1	AE
20190320	1	10	1	AE
20190320	1	10	1	AE
20190320	1	7	7	AE
20190320	0	6	12	AE
20190320	0	6	12	AE
20190320	0	6	12	AE
20190320	0	6	12	AE
20190320	0	6	12	AE
20190320	0	6	12	AE
20190424	1	10	1	AE
20190424	1	10	1	AE
20190424	1	10	1	AE
20190424	1	10	1	AE
20190424	1	6	3	AE
20190424	0	5	12	AE
20190424	0	5	12	AE
20190424	0	5	12	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190424	0	5	12	AE
20190424	0	5	12	AE
20190415	1	10	1	AE
20190415	1	10	1	AE
20190415	1	10	1	AE
20190415	0	7	12	AE
20190415	0	7	12	AE
20190415	0	7	12	AE
20190415	0	7	12	AE
20190415	0	7	12	AE
20190415	0	7	12	AE
20190415	0	7	12	AE
20190416	1	10	1	AE
20190416	1	10	1	AE
20190416	1	10	1	AE
20190416	0	7	12	AE
20190416	0	7	12	AE
20190416	0	7	12	AE
20190416	0	7	12	AE
20190416	0	7	12	AE
20190416	0	7	12	AE
20190416	1	7	12	AE
20190419	1	10	1	AE
20190419	1	10	1	AE
20190419	1	10	1	AE
20190419	1	7	2	AE
20190419	1	6	10	AE
20190419	0	5	12	AE
20190419	0	5	12	AE
20190419	0	5	12	AE
20190419	0	5	12	AE
20190419	0	5	12	AE
20190423	1	10	1	AE
20190423	1	10	1	AE
20190423	1	8	8	AE
20190423	0	7	12	AE
20190423	0	7	12	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190423	0	7	12	AE
20190423	0	7	12	AE
20190423	0	7	12	AE
20190423	0	7	12	AE
20190423	0	7	12	AE
20190425	1	10	1	AE
20190425	1	10	1	AE
20190425	1	10	1	AE
20190425	1	10	1	AE
20190425	1	10	1	AE
20190425	1	10	1	AE
20190425	1	4	7	AE
20190425	1	4	7	AE
20190425	0	2	12	AE
20190425	0	2	12	AE
20190708	1	10	1	AE
20190708	1	9	8	AE
20190708	1	9	8	AE
20190708	0	7	12	AE
20190708	0	7	12	AE
20190708	0	7	12	AE
20190708	0	7	12	AE
20190708	0	7	12	AE
20190708	0	7	12	AE
20190708	1	7	12	AE
20190710	1	10	1	AE
20190710	0	9	12	AE
20190710	0	9	12	AE
20190710	0	9	12	AE
20190710	0	9	12	AE
20190710	0	9	12	AE
20190710	0	9	12	AE
20190710	0	9	12	AE
20190710	0	9	12	AE
20190710	0	9	12	AE
20190713	1	10	1	AE
20190713	1	10	1	AE

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190713	1	10	1	AE
20190713	1	10	1	AE
20190713	1	10	1	AE
20190713	1	5	3	AE
20190713	1	4	4	AE
20190713	0	3	12	AE
20190713	0	3	12	AE
20190713	1	3	12	AE
20190714	1	10	1	AE
20190714	1	9	7	AE
20190714	1	9	7	AE
20190714	0	7	12	AE
20190714	0	7	12	AE
20190714	0	7	12	AE
20190714	0	7	12	AE
20190714	0	7	12	AE
20190714	0	7	12	AE
20190714	1	7	12	AE
20190715	1	10	1	AE
20190715	1	10	1	AE
20190715	1	10	1	AE
20190715	1	10	1	AE
20190715	1	6	6	AE
20190715	1	5	12	AE
20190715	1	5	12	AE
20190715	1	5	12	AE
20190715	1	5	12	AE
20190715	1	5	12	AE
20190218	1	10	1	CX
20190218	0	9	12	CX
20190218	0	9	12	CX
20190218	0	9	12	CX
20190218	0	9	12	CX
20190218	0	9	12	CX
20190218	0	9	12	CX
20190218	0	9	12	CX
20190218	0	9	12	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190218	0	9	12	CX
20190220	1	10	1	CX
20190220	1	10	1	CX
20190220	1	10	1	CX
20190220	1	7	3	CX
20190220	1	6	6	CX
20190220	0	5	12	CX
20190220	0	5	12	CX
20190220	0	5	12	CX
20190220	0	5	12	CX
20190220	0	5	12	CX
20190221	1	10	1	CX
20190221	1	9	2	CX
20190221	1	9	2	CX
20190221	1	7	3	CX
20190221	1	7	3	CX
20190221	1	5	9	CX
20190221	0	4	12	CX
20190221	0	4	12	CX
20190221	0	4	12	CX
20190221	1	4	12	CX
20190223	1	10	1	CX
20190223	1	10	1	CX
20190223	0	8	12	CX
20190223	0	8	12	CX
20190223	0	8	12	CX
20190223	0	8	12	CX
20190223	0	8	12	CX
20190223	0	8	12	CX
20190223	0	8	12	CX
20190223	0	8	12	CX
20190617	1	10	1	CX
20190617	1	9	2	CX
20190617	0	8	12	CX
20190617	0	8	12	CX
20190617	0	8	12	CX
20190617	0	8	12	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190617	0	8	12	CX
20190617	0	8	12	CX
20190617	0	8	12	CX
20190617	0	8	12	CX
20190618	1	10	1	CX
20190618	1	9	2	CX
20190618	1	8	3	CX
20190618	1	7	4	CX
20190618	1	6	5	CX
20190618	1	5	6	CX
20190618	0	4	12	CX
20190618	0	4	12	CX
20190618	0	4	12	CX
20190618	0	4	12	CX
20190619	1	10	2	CX
20190619	0	9	12	CX
20190619	0	9	12	CX
20190619	0	9	12	CX
20190619	0	9	12	CX
20190619	0	9	12	CX
20190619	0	9	12	CX
20190619	0	9	12	CX
20190619	0	9	12	CX
20190619	0	9	12	CX
20190620	1	10	2	CX
20190620	0	9	12	CX
20190620	0	9	12	CX
20190620	0	9	12	CX
20190620	0	9	12	CX
20190620	0	9	12	CX
20190620	0	9	12	CX
20190620	0	9	12	CX
20190620	0	9	12	CX
20190620	0	9	12	CX
20190621	1	10	1	CX
20190621	1	9	2	CX
20190621	1	8	3	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190621	1	7	5	CX
20190621	0	6	12	CX
20190621	0	6	12	CX
20190621	0	6	12	CX
20190621	0	6	12	CX
20190621	0	6	12	CX
20190621	1	6	12	CX
20190622	1	10	1	CX
20190622	1	10	1	CX
20190622	1	10	1	CX
20190622	1	7	5	CX
20190622	0	6	12	CX
20190622	0	6	12	CX
20190622	0	6	12	CX
20190622	0	6	12	CX
20190622	0	6	12	CX
20190622	0	6	12	CX
20190623	0	10	12	CX
20190623	0	10	12	CX
20190623	0	10	12	CX
20190623	0	10	12	CX
20190623	0	10	12	CX
20190623	0	10	12	CX
20190623	0	10	12	CX
20190623	0	10	12	CX
20190623	0	10	12	CX
20190623	1	10	12	CX
20190624	1	10	1	CX
20190624	1	10	1	CX
20190624	1	10	1	CX
20190624	1	10	1	CX
20190624	1	6	3	CX
20190624	1	5	4	CX
20190624	1	4	5	CX
20190624	1	3	6	CX
20190624	1	2	8	CX
20190624	1	1	11	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190625	1	10	1	CX
20190625	1	9	3	CX
20190625	1	8	7	CX
20190625	1	7	10	CX
20190625	1	6	11	CX
20190625	0	5	12	CX
20190625	0	5	12	CX
20190625	0	5	12	CX
20190625	0	5	12	CX
20190625	0	5	12	CX
20190626	1	10	4	CX
20190626	1	9	6	CX
20190626	1	9	6	CX
20190626	0	7	12	CX
20190626	0	7	12	CX
20190626	0	7	12	CX
20190626	0	7	12	CX
20190626	0	7	12	CX
20190626	0	7	12	CX
20190626	1	7	12	CX
20190627	1	10	1	CX
20190627	1	10	1	CX
20190627	1	10	1	CX
20190627	1	7	2	CX
20190627	1	7	2	CX
20190627	1	5	3	CX
20190627	1	4	4	CX
20190627	1	3	5	CX
20190627	1	2	9	CX
20190627	1	1	10	CX
20190628	1	10	3	CX
20190628	1	9	8	CX
20190628	0	8	12	CX
20190628	0	8	12	CX
20190628	0	8	12	CX
20190628	0	8	12	CX
20190628	0	8	12	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190628	0	8	12	CX
20190628	0	8	12	CX
20190628	0	8	12	CX
20190629	1	10	1	CX
20190629	1	10	1	CX
20190629	1	10	1	CX
20190629	1	7	2	CX
20190629	1	6	3	CX
20190629	1	5	4	CX
20190629	1	4	5	CX
20190629	1	3	9	CX
20190629	1	2	11	CX
20190629	0	1	12	CX
20190630	1	10	1	CX
20190630	0	9	12	CX
20190630	0	9	12	CX
20190630	0	9	12	CX
20190630	0	9	12	CX
20190630	0	9	12	CX
20190630	0	9	12	CX
20190630	0	9	12	CX
20190630	0	9	12	CX
20190630	0	9	12	CX
20190701	1	10	1	CX
20190701	1	10	1	CX
20190701	1	10	1	CX
20190701	1	7	2	CX
20190701	1	6	5	CX
20190701	1	5	8	CX
20190701	0	4	12	CX
20190701	0	4	12	CX
20190701	0	4	12	CX
20190701	1	4	12	CX
20190702	1	10	1	CX
20190702	1	10	1	CX
20190702	1	8	2	CX
20190702	1	8	2	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190702	1	8	2	CX
20190702	1	5	3	CX
20190702	1	4	4	CX
20190702	1	3	7	CX
20190702	0	2	12	CX
20190702	0	2	12	CX
20190703	1	10	1	CX
20190703	1	10	1	CX
20190703	1	8	4	CX
20190703	1	7	8	CX
20190703	1	6	9	CX
20190703	0	5	12	CX
20190703	0	5	12	CX
20190703	0	5	12	CX
20190703	0	5	12	CX
20190703	0	5	12	CX
20190716	1	10	2	CX
20190716	1	10	2	CX
20190716	1	8	3	CX
20190716	1	7	7	CX
20190716	0	6	12	CX
20190716	0	6	12	CX
20190716	0	6	12	CX
20190716	0	6	12	CX
20190716	0	6	12	CX
20190716	0	6	12	CX
20190717	1	10	1	CX
20190717	1	10	1	CX
20190717	1	10	1	CX
20190717	1	7	3	CX
20190717	1	6	5	CX
20190717	1	5	8	CX
20190717	0	4	12	CX
20190717	0	4	12	CX
20190717	0	4	12	CX
20190717	0	4	12	CX
20190718	1	10	1	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190718	1	9	4	CX
20190718	1	8	6	CX
20190718	1	7	7	CX
20190718	1	6	11	CX
20190718	0	5	12	CX
20190718	0	5	12	CX
20190718	0	5	12	CX
20190718	0	5	12	CX
20190718	0	5	12	CX
20190719	1	10	6	CX
20190719	1	9	10	CX
20190719	0	8	12	CX
20190719	0	8	12	CX
20190719	0	8	12	CX
20190719	0	8	12	CX
20190719	0	8	12	CX
20190719	0	8	12	CX
20190719	0	8	12	CX
20190719	0	8	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190720	0	10	12	CX
20190721	1	10	1	CX
20190721	1	10	1	CX
20190721	1	8	2	CX
20190721	1	7	3	CX
20190721	1	6	5	CX
20190721	1	5	6	CX
20190721	1	4	11	CX
20190721	0	3	12	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190721	0	3	12	CX
20190721	0	3	12	CX
20190722	1	10	1	CX
20190722	1	10	1	CX
20190722	1	10	1	CX
20190722	1	7	2	CX
20190722	1	6	3	CX
20190722	1	5	7	CX
20190722	1	4	9	CX
20190722	0	3	12	CX
20190722	0	3	12	CX
20190722	0	3	12	CX
20190724	1	10	4	CX
20190724	1	9	5	CX
20190724	1	8	11	CX
20190724	0	7	12	CX
20190724	0	7	12	CX
20190724	0	7	12	CX
20190724	0	7	12	CX
20190724	0	7	12	CX
20190724	0	7	12	CX
20190724	0	7	12	CX
20190725	1	10	8	CX
20190725	0	9	12	CX
20190725	0	9	12	CX
20190725	0	9	12	CX
20190725	0	9	12	CX
20190725	0	9	12	CX
20190725	0	9	12	CX
20190725	0	9	12	CX
20190725	0	9	12	CX
20190725	0	9	12	CX
20190727	1	10	1	CX
20190727	1	10	1	CX
20190727	1	10	1	CX
20190727	1	10	1	CX
20190727	1	6	2	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190727	1	5	3	CX
20190727	1	4	5	CX
20190727	0	3	12	CX
20190727	0	3	12	CX
20190727	0	3	12	CX
20190729	1	10	1	CX
20190729	1	10	1	CX
20190729	1	8	2	CX
20190729	1	7	3	CX
20190729	1	6	7	CX
20190729	1	5	11	CX
20190729	0	4	12	CX
20190729	0	4	12	CX
20190729	0	4	12	CX
20190729	0	4	12	CX
20190730	1	10	1	CX
20190730	1	10	1	CX
20190730	1	10	1	CX
20190730	1	10	1	CX
20190730	1	6	2	CX
20190730	1	5	5	CX
20190730	1	4	7	CX
20190730	0	3	12	CX
20190730	0	3	12	CX
20190730	0	3	12	CX
20190801	1	10	1	CX
20190801	1	10	1	CX
20190801	1	8	2	CX
20190801	1	7	10	CX
20190801	0	6	12	CX
20190801	0	6	12	CX
20190801	0	6	12	CX
20190801	0	6	12	CX
20190801	0	6	12	CX
20190801	0	6	12	CX
20190802	1	10	1	CX
20190802	1	9	2	CX

Appendix A1 (continued). Raw survivorship data from predation video analysis (Chapter 2).

Dataset	Status	n	Hour	Prey
20190802	1	8	7	CX
20190802	1	7	10	CX
20190802	1	7	10	CX
20190802	0	5	12	CX
20190802	0	5	12	CX
20190802	0	5	12	CX
20190802	0	5	12	CX
20190802	0	5	12	CX

Appendix A2. Predation rate (PR) for each 12-hour observation (Chapter 2).

Dataset	Prey	PR	Dataset	Prey	PR
20190218	CX	0.083	20190126	AE	0.250
20190220	CX	0.417	20190127	AE	0.583
20190221	CX	0.583	20190128	AE	0.500
20190223	CX	0.167	20190129	AE	0.250
20190617	CX	0.167	20190130	AE	0.833
20190618	CX	0.500	20190201	AE	0.083
20190619	CX	0.083	20190202	AE	0.250
20190620	CX	0.083	20190203	AE	0.083
20190621	CX	0.417	20190205	AE	0.583
20190622	CX	0.333	20190207	AE	0.250
20190623	CX	0.083	20190208	AE	0.000
20190624	CX	0.833	20190210	AE	0.167
20190625	CX	0.417	20190211	AE	0.167
20190626	CX	0.333	20190212	AE	0.250
20190627	CX	0.833	20190213	AE	0.167
20190628	CX	0.167	20190214	AE	0.250
20190629	CX	0.750	20190215	AE	0.333
20190630	CX	0.083	20190216	AE	0.583
20190701	CX	0.583	20190309	AE	0.250
20190702	CX	0.667	20190308	AE	0.333
20190703	CX	0.417	20190318	AE	0.500
20190716	CX	0.333	20190310	AE	0.333
20190717	CX	0.500	20190313	AE	0.167
20190718	CX	0.417	20190314	AE	0.750
20190719	CX	0.167	20190315	AE	0.417
20190720	CX	0.000	20190320	AE	0.333
20190721	CX	0.583	20190424	AE	0.417
20190722	CX	0.583	20190415	AE	0.250
20190724	CX	0.250	20190416	AE	0.333
20190725	CX	0.083	20190419	AE	0.417
20190727	CX	0.583	20190423	AE	0.250
20190729	CX	0.500	20190425	AE	0.667
20190730	CX	0.583	20190708	AE	0.333
20190801	CX	0.333	20190710	AE	0.083
20190802	CX	0.417	20190713	AE	0.667
20190123	AE	0.583	20190714	AE	0.333
20190224	AE	0.583	20190715	AE	0.583

Appendix A3. Occurrence data of *Toxorhynchites rutilus* (Chapter 3). The total number of records used to create ecological niche models was 165.

Species	Latitude	Longitude
Toxorhynchites rutilus	27.7361	-81.6159
Toxorhynchites rutilus	27.8836	-81.8299
Toxorhynchites rutilus	27.9531	-81.6928
Toxorhynchites rutilus	28.8106	-81.4258
Toxorhynchites rutilus	28.8106	-81.4258
Toxorhynchites rutilus	28.8106	-81.4258
Toxorhynchites rutilus	29.4851	-97.4503
Toxorhynchites rutilus	29.6009	-98.4876
Toxorhynchites rutilus	29.8516	-97.6951
Toxorhynchites rutilus	29.8573	-95.3930
Toxorhynchites rutilus	29.8573	-95.3930
Toxorhynchites rutilus	29.8573	-95.3930
Toxorhynchites rutilus	29.8573	-95.3930
Toxorhynchites rutilus	29.9236	-95.5238
Toxorhynchites rutilus	30.0290	-95.2999
Toxorhynchites rutilus	30.0911	-97.1717
Toxorhynchites rutilus	30.1038	-98.4255
Toxorhynchites rutilus	30.1038	-98.4259
Toxorhynchites rutilus	30.1665	-95.7631
Toxorhynchites rutilus	30.1868	-93.2457
Toxorhynchites rutilus	30.2065	-92.0642
Toxorhynchites rutilus	30.2348	-97.6411
Toxorhynchites rutilus	30.2376	-85.6314
Toxorhynchites rutilus	30.2395	-97.6913
Toxorhynchites rutilus	30.2395	-97.6913
Toxorhynchites rutilus	30.2418	-97.6987
Toxorhynchites rutilus	30.2473	-97.6941
Toxorhynchites rutilus	30.2656	-98.4000
Toxorhynchites rutilus	30.2779	-97.7040
Toxorhynchites rutilus	30.2780	-97.7039
Toxorhynchites rutilus	30.2780	-97.7041
Toxorhynchites rutilus	30.2780	-97.7041
Toxorhynchites rutilus	30.3101	-97.7043
Toxorhynchites rutilus	30.3128	-97.6985
Toxorhynchites rutilus	30.4061	-88.8019
Toxorhynchites rutilus	30.4263	-91.0390

Appendix A3 (continued). Occurrence data of *Toxorhynchites rutilus* (Chapter 3).

Species	Latitude	Longitude
Toxorhynchites rutilus	30.5440	-91.0932
Toxorhynchites rutilus	30.6659	-86.5942
Toxorhynchites rutilus	30.6811	-97.6361
Toxorhynchites rutilus	30.6846	-88.1966
Toxorhynchites rutilus	30.6846	-88.1966
Toxorhynchites rutilus	30.9133	-81.6420
Toxorhynchites rutilus	32.0422	-84.2043
Toxorhynchites rutilus	32.2502	-86.0888
Toxorhynchites rutilus	32.3114	-97.0149
Toxorhynchites rutilus	32.3114	-97.0149
Toxorhynchites rutilus	32.3116	-97.0160
Toxorhynchites rutilus	32.3116	-97.0161
Toxorhynchites rutilus	32.3118	-97.0159
Toxorhynchites rutilus	32.3118	-97.0160
Toxorhynchites rutilus	32.3118	-97.0159
Toxorhynchites rutilus	32.3119	-97.0161
Toxorhynchites rutilus	32.3122	-97.0149
Toxorhynchites rutilus	32.5659	-97.1481
Toxorhynchites rutilus	32.5772	-93.8824
Toxorhynchites rutilus	32.7670	-96.7784
Toxorhynchites rutilus	32.7720	-97.2913
Toxorhynchites rutilus	32.7720	-97.2913
Toxorhynchites rutilus	32.7745	-97.3746
Toxorhynchites rutilus	32.7746	-97.3747
Toxorhynchites rutilus	32.8586	-97.4737
Toxorhynchites rutilus	32.8591	-84.4863
Toxorhynchites rutilus	32.9551	-96.9325
Toxorhynchites rutilus	33.0837	-94.3576
Toxorhynchites rutilus	33.4327	-86.7060
Toxorhynchites rutilus	33.5500	-81.6330
Toxorhynchites rutilus	33.6577	-93.6087
Toxorhynchites rutilus	33.7707	-84.2263
Toxorhynchites rutilus	33.7900	-84.4682
Toxorhynchites rutilus	34.7211	-96.6927
Toxorhynchites rutilus	34.8854	-82.7234
Toxorhynchites rutilus	34.8926	-82.3721
Toxorhynchites rutilus	34.9527	-80.8311
Toxorhynchites rutilus	35.1970	-94.2750

Appendix A3 (continued). Occurrence data of *Toxorhynchites rutilus* (Chapter 3).

Species	Latitude	Longitude
Toxorhynchites rutilus	35.2064	-97.3231
Toxorhynchites rutilus	35.2118	-97.5019
Toxorhynchites rutilus	35.2865	-83.1306
Toxorhynchites rutilus	35.3546	-76.9696
Toxorhynchites rutilus	35.3623	-80.9184
Toxorhynchites rutilus	35.5133	-93.3266
Toxorhynchites rutilus	35.5444	-82.3710
Toxorhynchites rutilus	35.6258	-79.3096
Toxorhynchites rutilus	35.6469	-95.9646
Toxorhynchites rutilus	35.6469	-95.9646
Toxorhynchites rutilus	35.7050	-79.2515
Toxorhynchites rutilus	35.7787	-78.8201
Toxorhynchites rutilus	35.7898	-78.6506
Toxorhynchites rutilus	35.8283	-90.6304
Toxorhynchites rutilus	35.8434	-86.4172
Toxorhynchites rutilus	36.0362	-79.8399
Toxorhynchites rutilus	36.0366	-78.8779
Toxorhynchites rutilus	36.0366	-78.8779
Toxorhynchites rutilus	36.0366	-78.8779
Toxorhynchites rutilus	36.0366	-78.8779
Toxorhynchites rutilus	36.0695	-94.1731
Toxorhynchites rutilus	36.0791	-79.7887
Toxorhynchites rutilus	36.0791	-79.7887
Toxorhynchites rutilus	36.0926	-85.8107
Toxorhynchites rutilus	36.1691	-86.7848
Toxorhynchites rutilus	36.6206	-79.9807
Toxorhynchites rutilus	36.8529	-75.9780
Toxorhynchites rutilus	36.8554	-89.9417
Toxorhynchites rutilus	36.9956	-86.4236
Toxorhynchites rutilus	37.0689	-97.0406
Toxorhynchites rutilus	37.2779	-87.2114
Toxorhynchites rutilus	37.3762	-88.9304
Toxorhynchites rutilus	37.3839	-89.6849
Toxorhynchites rutilus	37.4172	-88.5424
Toxorhynchites rutilus	37.4524	-77.5847
Toxorhynchites rutilus	37.7664	-84.6219
Toxorhynchites rutilus	38.2009	-84.8733
Toxorhynchites rutilus	38.2665	-77.1808

Appendix A3 (continued). Occurrence data of *Toxorhynchites rutilus* (Chapter 3).

Species	Latitude	Longitude
Toxorhynchites rutilus	38.4121	-78.2770
Toxorhynchites rutilus	38.4320	-88.4321
Toxorhynchites rutilus	38.4320	-88.4321
Toxorhynchites rutilus	38.6654	-77.2332
Toxorhynchites rutilus	38.8010	-77.5552
Toxorhynchites rutilus	38.8259	-76.8473
Toxorhynchites rutilus	38.8331	-77.3369
Toxorhynchites rutilus	38.8337	-77.2761
Toxorhynchites rutilus	38.8405	-77.1280
Toxorhynchites rutilus	38.8839	-94.8223
Toxorhynchites rutilus	38.9412	-77.3661
Toxorhynchites rutilus	38.9665	-77.0526
Toxorhynchites rutilus	38.9717	-95.2350
Toxorhynchites rutilus	39.0072	-94.3425
Toxorhynchites rutilus	39.0072	-94.3425
Toxorhynchites rutilus	39.0072	-94.3425
Toxorhynchites rutilus	39.0072	-94.3425
Toxorhynchites rutilus	39.1189	-84.4158
Toxorhynchites rutilus	39.1374	-77.2031
Toxorhynchites rutilus	39.1485	-84.5146
Toxorhynchites rutilus	39.1969	-84.5442
Toxorhynchites rutilus	39.2523	-76.9244
Toxorhynchites rutilus	39.3284	-75.1216
Toxorhynchites rutilus	39.5058	-80.2434
Toxorhynchites rutilus	39.8024	-74.9613
Toxorhynchites rutilus	39.8024	-74.9613
Toxorhynchites rutilus	39.8024	-74.9613
Toxorhynchites rutilus	39.9740	-75.7497
Toxorhynchites rutilus	40.0420	-76.2502
Toxorhynchites rutilus	40.0571	-75.0948
Toxorhynchites rutilus	40.0722	-82.8775
Toxorhynchites rutilus	40.1309	-80.2416
Toxorhynchites rutilus	40.2825	-74.7037
Toxorhynchites rutilus	40.2825	-74.7037
Toxorhynchites rutilus	40.2825	-74.7037
Toxorhynchites rutilus	40.2825	-74.7037
Toxorhynchites rutilus	40.3369	-75.1071
Toxorhynchites rutilus	40.6142	-75.5906

Appendix A3 (continued). Occurrence data of *Toxorhynchites rutilus* (Chapter 3).

Species	Latitude	Longitude
Toxorhynchites rutilus	40.6142	-75.5906
Toxorhynchites rutilus	40.6599	-74.3087
Toxorhynchites rutilus	40.7297	-73.5894
Toxorhynchites rutilus	40.7484	-74.3232
Toxorhynchites rutilus	40.8515	-76.7099
Toxorhynchites rutilus	40.8686	-73.8781
Toxorhynchites rutilus	40.8687	-73.8781
Toxorhynchites rutilus	40.9436	-72.6922
Toxorhynchites rutilus	41.3195	-73.9887
Toxorhynchites rutilus	41.5018	-81.4853
Toxorhynchites rutilus	41.6790	-95.3089
Toxorhynchites rutilus	41.9872	-70.7419
Toxorhynchites rutilus	42.3871	-71.1471
Toxorhynchites rutilus	42.4461	-71.4755
Toxorhynchites rutilus	42.4795	-71.3965

Appendix A4. Scores of variable combinations with the greatest effect on model gain (Chapter 3). AIC (Akaike information criterion), AICc (corrected Akaike information criterion), BIC (Bayesian information criterion), AUC (are under the curve) scores were all used in model selection.

Model variables	AIC score	AICc score	BIC score	AUC score
Bio10, Bio12, Bio15	2603.725	2608.211	2645.908	0.961
Elev, Bio1, Bio12, Bio15	2608.405	2615.783	2661.836	0.962
Bio9, Bio10, Bio12, Bio15	2602.460	2617.085	2675.577	0.965
Elev, Bio10, Bio12, Bio15	2611.756	2619.135	2665.188	0.961
Bio10, Bio14, Bio15	2614.203	2620.031	2662.010	0.960
Elev, Bio9, Bio10, Bio15	2612.978	2620.356	2666.409	0.961
Elev, Bio10, Bio14, Bio15	2616.076	2622.653	2666.696	0.960
Bio1, Bio12, Bio15	2613.646	2622.795	2672.702	0.961
Bio9, Bio10, Bio14, Bio15	2609.890	2623.292	2680.195	0.963
Elev, Bio5, Bio14, Bio15	2619.519	2623.408	2658.889	0.958
Bio5, Bio12, Bio15	2620.762	2623.599	2654.508	0.957
Elev, Bio9, Bio10, Bio14, Bio15	2610.459	2626.375	2686.388	0.964
Bio9, Bio10, Bio15	2623.154	2626.494	2659.713	0.958
Bio1, Bio14, Bio15	2620.047	2626.624	2670.666	0.959
Elev, Bio9, Bio10, Bio12, Bio15	2608.432	2628.649	2692.798	0.966
Elev, Bio1, Bio14, Bio15	2621.021	2629.257	2677.265	0.960
Bio5, Bio14, Bio15	2625.527	2630.659	2670.522	0.958
Bio10, Bio15, Bio17	2623.822	2632.971	2682.878	0.959
Bio9, Bio10, Bio15, Bio17	2616.809	2634.086	2695.550	0.963
Elev, Bio10, Bio15, Bio17	2625.624	2635.744	2687.492	0.959
Bio5, Bio15, Bio17	2630.044	2636.621	2680.664	0.958
Elev, Bio5, Bio12, Bio15	2629.398	2636.776	2682.829	0.958
Bio9, Bio10, Bio14	2629.768	2640.920	2694.448	0.959
Bio1, Bio15, Bio17	2629.970	2641.122	2694.650	0.959
Bio9, Bio10, Bio12	2632.155	2642.275	2694.023	0.958
Bio10, Bio14	2638.327	2642.813	2680.510	0.955
Elev, Bio5, Bio15, Bio17	2634.080	2643.229	2693.136	0.958
Bio5, Bio14	2642.695	2645.532	2676.441	0.953
Bio9, Bio10, Bio17	2634.508	2645.660	2699.188	0.958
Bio10, Bio15	2644.982	2647.818	2678.728	0.956
Bio1, Bio14	2643.142	2648.274	2688.137	0.953
Bio10, Bio17	2643.991	2648.477	2686.174	0.954

Appendix A4 (continued). Model scores of variable combinations (Chapter 3).

Model variables	AIC score	AICc score	BIC score	AUC score
Elev, Bio9, Bio10, Bio14	2631.305	2648.581	2710.046	0.961
Elev, Bio1, Bio15, Bio17	2634.751	2650.667	2710.680	0.960
Bio5, Bio17	2649.017	2652.906	2688.388	0.952
Elev, Bio10, Bio14	2646.017	2654.252	2702.260	0.956
Elev, Bio10, Bio15	2647.117	2655.353	2703.361	0.956
Elev, Bio10, Bio17	2649.984	2657.363	2703.416	0.956
Bio1, Bio17	2651.260	2657.837	2701.879	0.952
Elev, Bio1, Bio17	2650.150	2658.385	2706.394	0.953
Elev, Bio9, Bio10	2653.903	2663.052	2712.959	0.956
Bio1, Bio15	2658.585	2663.071	2700.768	0.953
Elev, Bio1, Bio14	2652.374	2663.525	2717.054	0.953
Elev, Bio9, Bio10, Bio17	2642.908	2664.710	2730.086	0.960
Elev, Bio5, Bio15	2658.338	2664.915	2708.958	0.953
Elev, Bio9, Bio10, Bio15, Bio17	2632.366	2665.448	2736.417	0.964
Bio1, Bio12	2659.328	2665.905	2709.947	0.949
Elev, Bio1, Bio15	2655.464	2667.708	2722.956	0.955
Elev, Bio5, Bio14	2657.649	2667.769	2719.517	0.954
Bio10, Bio12	2660.665	2668.043	2714.096	0.950
Elev, Bio5, Bio17	2659.257	2668.406	2718.313	0.954
Elev, Bio1, Bio12	2662.501	2674.746	2729.993	0.952
Bio5, Bio12	2677.630	2683.458	2725.437	0.944
Elev, Bio9, Bio12	2682.096	2687.925	2729.904	0.948
Elev, Bio9, Bio14	2683.632	2689.461	2731.439	0.946
Bio9, Bio12, Bio15	2687.757	2692.243	2729.940	0.946
Elev, Bio9, Bio17	2686.630	2692.458	2734.437	0.946
Elev, Bio10, Bio12	2677.278	2695.988	2758.831	0.952
Elev, Bio1	2693.363	2696.702	2729.921	0.941
Elev, Bio9, Bio12, Bio15	2684.870	2699.495	2757.986	0.952
Elev, Bio9, Bio15	2691.313	2699.549	2747.557	0.947
Elev, Bio9, Bio14, Bio15	2687.990	2700.235	2755.483	0.951
Bio5, Bio15	2699.216	2702.556	2735.775	0.947
Elev, Bio5, Bio12	2687.042	2704.319	2765.783	0.950
Bio9, Bio12	2707.683	2711.023	2744.242	0.936
Elev, Bio9, Bio15, Bio17	2696.264	2712.180	2772.193	0.950
Elev, Bio9, Bio10, Bio12	2665.486	2716.255	2789.222	0.961
Elev, Bio10	2713.462	2719.290	2761.269	0.942
Elev, Bio9	2716.129	2720.018	2755.500	0.939

Appendix A4 (continued). Model scores of variable combinations (Chapter 3).

Model variables	AIC score	AICc score	BIC score	AUC score
Bio9, Bio14	2727.519	2732.651	2772.514	0.934
Bio9, Bio15, Bio17	2725.059	2735.179	2786.927	0.943
Bio9, Bio14, Bio15	2727.508	2739.753	2795.000	0.944
Bio9, Bio17	2733.570	2740.147	2784.189	0.932
Bio9, Bio10	2772.446	2776.335	2811.816	0.924
Elev, Bio5	2757.081	2780.548	2847.071	0.937
Bio9, Bio15	2783.994	2787.334	2820.552	0.922
Bio1	2790.320	2791.294	2810.006	0.916
Bio10	2793.943	2794.456	2808.004	0.904
Elev, Bio17	2798.230	2804.059	2846.038	0.915
Bio12, Bio15	2809.043	2813.529	2851.226	0.908
Bio15, Bio17	2810.067	2814.553	2852.250	0.916
Bio12	2815.155	2816.418	2837.652	0.900
Bio17	2816.551	2817.276	2833.425	0.904
Elev, Bio14, Bio15	2635.120	2821.796	2884.640	0.925
Bio14, Bio15	2803.086	2822.041	2857.523	0.912
Bio14	2818.152	2823.523	2839.672	0.900
Elev, Bio15, Bio17	2822.799	2826.788	2893.312	0.929
Elev, Bio14	2803.322	2829.072	2885.974	0.915
Elev, Bio12, Bio15	2815.670	2851.056	2923.141	0.926
Elev, Bio12	2813.466	2851.688	2920.257	0.918
Elev, Bio15	2824.643	2864.536	2910.589	0.900
Bio5	2857.157	2865.301	2881.450	0.864
Bio9	2864.577	2880.357	2906.515	0.871
Bio15	2878.393	2920.466	2934.014	0.854
Elev	2919.954	3044.075	3097.604	0.794