ABSTRACT

Gregarine Parasitism in Dragonfly Populations of Central Texas with an Assessment of Fitness Costs in *Erythemis simplicicollis*

Jason L. Locklin, Ph.D.

Mentor: Darrell S. Vodopich, Ph.D.

Dragonfly parasites are widespread and frequently include gregarines (Phylum Apicomplexa) in the gut of the host. Gregarines are ubiquitous protozoan parasites that infect arthropods worldwide. More than 1,600 gregarine species have been described, but only a small percentage of invertebrates have been surveyed for these apicomplexan parasites. Some consider gregarines rather harmless, but recent studies suggest otherwise. Odonate-gregarine studies have more commonly involved damselflies, and some have considered gregarines to rarely infect dragonflies. In this study, dragonfly populations were surveyed for gregarines and an assessment of fitness costs was made in a common and widespread host species, *Erythemis simplicicollis*. Adult dragonfly populations were surveyed weekly at two reservoirs in close proximity to one another and at a flow-through wetland system. Gregarine prevalences and intensities were compared within host populations between genders, among locations, among wing loads, and through time. Host fitness parameters measured included wing load, egg size, clutch size, and total egg count. Of the 37 dragonfly species surveyed, 14 species (38%) hosted

gregarines. Thirteen of those species were previously unreported as hosts. Gregarine prevalences ranged from 2% – 52%. Intensities ranged from 1 – 201. Parasites were aggregated among their hosts. Gregarines were found only in individuals exceeding a minimum wing load, indicating that gregarines are likely not transferred from the naiad to adult during emergence. Prevalence and intensity exhibited strong seasonality during both years at one of the reservoirs, but no seasonal trend was detected at the wetland. The seasonal trend at the reservoir suggests that gregarine oocyst viability parallels increasing host population densities and may be short-lived. Prevalence and intensity also differed between dragonfly populations at the locations. Regression analyses revealed that host species, host gender, month, and year were significant explanatory variables related to gregarine prevalence and intensity. The fitness parameters measured were not correlated with presence or intensity of gregarines, suggesting that either gregarines do not affect wing loading and egg production in *E. simplicicollis*, or that virulence depends on parasite intensity and/or the specific gregarine species infecting the hosts. Our results emphasize the importance of considering season, hosts, and habitat when studying gregarine-dragonfly ecology.

Gregarine Parasitism in Dragonfly Populations of Central Texas with an Assessment of Fitness Costs in *Erythemis simplicicollis*

by

Jason L. Locklin, A.A., B.S., M.S.

A Dissertation

Approved by the Department of Biology

_____
Robert D. Doyle, Ph.D., Chairperson

Submitted to the Graduate Faculty of
Baylor University in Partial Fulfillment of the
Requirements for the Degree
of
Doctor of Philosophy

Approved by the Dissertation Committee

_____
Darrell S. Vodopich, Ph.D., Chairperson

_____
Tamarah L. Adair, Ph.D.

_____
Robert S. Baldridge, Ph.D.

_____
Rena M. Bonem, Ph.D.

_____
Patrick D. Danley, Ph.D.

_____
Robert D. Doyle, Ph.D.

Accepted by the Graduate School
August 2010

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J. Larry Lyon, Ph.D., Dean

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGMENTS

I am indebted to many individuals who have worked on this particular project, supported me during this study, and/or guided me into a career as awarding as this.

Many thanks to David Cummings, Joshua Huckabee, Sherry Stewart, Justin Clanton, Victoria Prescott, Isis Dominguez, Edgar Alvarez, Tamara Strohm, Sal LaMastra, and Austin Cunningham for your help in the field and lab. This project would not have happened without all of you and I am grateful for your tireless efforts.

My advisor, Dr. Darrell Vodopich, far exceeded my expectations as a Ph.D. mentor. I am grateful for your unending enthusiasm, support, and encouragement. The time and effort that you invested in my program will never be forgotten. I also wish to extend my sincere appreciation to Dr. Tammy Adair, Dr. R.S. Baldridge, Dr. Rena Bonem, Dr. Pat Danley, and Dr. Robert Doyle for serving on my committee. I especially appreciate Dr. Baldridge's critical review of the manuscripts and his advice on a number of issues which had to be addressed during the project.

I appreciate Terry Austin, biology department chairman at Temple College, for your support and flexibility during this work. Temple College's Faculty Development Leave Grant was also much appreciated.

Finally, I thank those individuals that sparked my interest in biology and guided me into this field. Thank you Mom and Dad for your relentless efforts in getting us outdoors and involved in nature. I will never forget those wet and cold camping trips. Thank you Dr. Ben Liles and Dr. Ed Morgan for teaching those influential biology

courses. Your enthusiasm for the field and love of teaching was contagious. You have both inspired me personally and professionally.

Most importantly, I thank my family for the many years of support. Anna, your encouragement and sacrifices made this dissertation work possible. I only hope that I am as supportive of your endeavors as you have been of mine. Ava and Morgan, thank you for those hugs and kisses. Without you, accomplishments are meaningless.

DEDICATION

For Ava and Morgan

CHAPTER ONE

Introduction and Background

Odonata

Odonates are one of the most primitive extant insect groups. The oldest odonate fossils date back more than 250 million years, and members of the Protodonata (the likely ancestors of Odonata) lived more than 325 million years ago (Corbet 1999). Some fossilized odonates had wingspans greater than 71cm (2 feet) (Abbott 2005). Not only have odonates stimulated human interest and intrigue for centuries, but they have also spawned fear – their rapid buzzing flight patterns inspire such names as snake doctors, devil's darning needles, mosquito hawks, and horse stingers (Abbott 2005, Beaton 2007).

Odonates have been prime subjects for ecological and evolutionary research for decades. Despite the relatively low diversity of Odonata (compared to Coleoptera, Diptera, and Lepidoptera, for example), their use in the ecological and evolutionary disciplines is immense (Abbott 2005).

Distribution

Dragonflies and damselflies (Odonata) occur worldwide except Antarctica. Approximately 5,500 species have been described, yet only 433 species occur in North America north of Mexico (Abbott 2005). Three suborders within Odonata include Zygoptera, Anisoptera, and Anisozygoptera. These suborders are based on the relative shapes of the forewings and hindwings. Damselflies (Zygoptera) have forewings and hindwings of the same shape. Dragonflies (Anisoptera) have hindwings which are

broader than their forewings. And members of Anisozygoptera (only two extant species which are restricted to East Asia) have a mix of characters found in the two former suborders (Abbott 2005).

Life History

Odonates are hemimetabolous insects with aquatic naiads and terrestrial adults. Females typically lay their eggs in the water although some species will lay eggs in a dry habitat in advance of rains that will submerge the eggs (Beaton 2007). Eggs of most species hatch in three to four weeks (Corbet 1999, Beaton 2007).

Naiads commonly occupy marshes, lakes, and streams. They are voracious predators that feed on worms, small crustaceans, insect larvae, and may even consume small fish and amphibians (Corbet 1999, Abbott 2005). Naiadal development ranges from one month to several years in others depending on species and environment (Beaton 2007). Because odonates are among the largest aquatic insect orders, they play an ecologically significant role in aquatic ecosystems (Abbott 2005, Corbet 1999, Quiroz-Martinez and Rodriguez-Castro 2007, Remsburg and Turner 2009). Naiads typically emerge from the aquatic habitat by crawling onto emergent or shoreline vegetation. Some species walk up to 30 m from the water body where they developed before finding a suitable place for ecdysis (Corbet 1999). As the adult pulls itself free from the exuviae, it remains suspended from its perch until the wings inflate and the body begins to harden. An emerged teneral typically lacks the vibrant coloration of many mature adults, yet it is highly vulnerable to predation during this time of limited mobility (Abbott 2005).

Emerged adults may require as little as one day or up to several weeks (depending on the species, temperature, food availability, etc.) to become sexually mature (Abbott

2005, McVey 1985). Much of this time is spent away from the breeding site where they feed and mature. Once mature, males of most species establish a territory near the water for mating. The strategy for males is to select a suitable section of breeding habitat that they patrol or defend against other males and wait for a female to arrive (Beaton 2007). Receptive females are seized by the male commonly in mid-air for copulation. In dragonflies, the male grasps the head of the female and curls his abdomen to transfer sperm from his genital pore (ventral side of segment nine) to the accessory genitalia (on the ventral side of segment two). After transfer, he straightens his abdomen and the receptive female curls her abdomen so that her genital opening on segment eight contacts his accessory genitalia. This "wheel position" last from several minutes to hours depending on the species (Corbet 1999, Abbott 2005). Males then 1) transport the female in tandem to the water to ensure that his sperm does the fertilizing and that she is not captured by another male, 2) release her to oviposit in his territory as he guards her from intruder males, or 3) release her to oviposit without guarding her. These strategies by males vary by species and mate quality (Corbet 1999, Cordoba-Aguilar et al 2003). Females will typically mate more than once per clutch and in some species males are known to remove sperm of other males from previous matings (Waage 1979).

Odonate Parasites

Odonates are diverse, abundant, easily collected, and exhibit a wide range of behaviors. Therefore, odonates are commonly used in studies investigating biological processes prominent in ecology, evolution, and behavior (see Córdoba-Aguilar and Cordero-Rivera 2005). They also host a variety of ecto- and endoparasites, most of which influence their hosts' fitness (Bonn et al. 1996, Canales-Lazcano et al. 2005,

Córdoba-Aguilar 2002, Córdoba-Aguilar et al. 2003, Marden and Cobb 2004, Reinhardt 1996, Rolff et al. 2000). Dragonflies host several major parasitic groups, including water mites, tapeworms, flukes, roundworms, horsehair worms, and gregarines (Corbet 1999, Forbes and Robb 2008). Because dragonflies are commonly used in ecological, evolutionary, and behavioral studies, information about their parasite fauna and related fitness costs are important to consider as they likely influence dragonfly biology.

Gregarine Parasites

Gregarine parasites are among the most ubiquitous and diverse groups of protozoan parasites (phylum Apicomplexa) (Smith and Clopton 2003). They infect a variety of invertebrates, particularly annelids and insects (Manwell 1961, Clopton 2002, Smith and Clopton 2003, Roberts and Janovy 2005). Among insect hosts, orthopterans and odonates are the most heavily infected (Åbro 1974 and Corbet 1999), and additional insect hosts include roaches (Blattodea), mosquitoes (Diptera), and beetles (Coleoptera) (Roberts and Janovy 2005). However, only a small percentage of invertebrates have been surveyed for these apicomplexan parasites (Roberts and Janovy 2005). Clopton (2006) estimates over 1 million gregarine species worldwide making them among the most diverse group of organisms infecting a broad range of hosts.

Gregarine systematics and taxonomy is complex, young, and rapidly developing. Clopton (2002) recognizes 1,656 species within 244 genera of gregarines in the order Eugregarinorida infecting more than 3,124 host species. However, many species of the North American gregarine fauna are undescribed (Clopton et al 1993).

Phylum Apicomplexa contains parasitic organisms without cilia/flagella (except for some gamete stages) (Roberts and Janovy 2005). Three classes are recognized by

Roberts and Janovy (2005): 1) Perkinsasidea, 2) Acnoidasida, and 3) Conoidasida. The gregarines of interest for this research are in the class Conoidasida and the order Eugregarinorida. Most parasitize their host's hemocoel, reproductive system, or intestinal tract (as in Odonata) (Manwell 1961, Roberts and Janovy 2005).

Morphology

Morphology of gregarines varies greatly. They range in size from a few mm to 16 mm (Manwell 1961) and can be so large that 19th century zoologists placed them with the worms (Roberts and Janovy 2005). A gregarine body is composed of ectoplasm and endoplasm. Endoplasm contains the organelles and is crowded with reserve food in the form of paraglycogen and fat globules (Manwell 1961). Two major body plans for gregarines are 1) acephaline and 2) cephaline. Acephaline gregarines have a simple body plan without well defined body regions (Manwell 1961), but may have an anterior anchoring device called a mucron (Roberts and Janovy 2005). Cephaline gregarines are more complex and the most frequently encountered. They have three body regions: 1) rostrum (anterior), 2) protomerite, and 3) deutomerite (posterior) (Manwell 1961, Roberts and Janovy 2005). An epimerite, the anchoring device for cephalines, is on the rostrum. This region may be reduced or lost when the gregarine detaches to become a gamont (free-roaming within the intestinal lumen) (Manwell 1961). The protomerite and deutomerite typically are divided by a septum (Manwell 1961, Roberts and Janovy 2005). Some deutomerites appear to be segmented ("polycystid") while others appear unsegmented ("monocystid") (Manwell 1961).

Life Cycle

The life cycle of eugregarinorid gregarines studied involves a single invertebrate host (Omoto et al 2004). Eugregarinorids have no merogony (asexual form of reproduction in which multiple mitoses and subsequent cytokineses produce many daughter cells) but undergo multiple fissions within a cyst (gametocyst) during gametogenesis (Roberts and Janovy 2005). During gametogenesis, two gamonts of opposite mating types coalesce to form a stable mating pair called a syzygys (Zuk 1987). Some gregarine species fuse in an anterior to posterior fashion whereas others fuse side to side (Roberts and Janovy 2005). In anterior-posterior fusion, the anterior cell is the "primate" and the posterior cell is the "satellite." Once fused, a cyst forms around the two gamonts to form the gametocyst. This cyst passes out with the host's feces (Zuk 1987) and the nucleus in each gamont divides repeatedly by binary fission to produce many nuclei that line the periphery of the membrane of each gamont. The nuclei give rise to many gametes inside each gamont. Once the gametes are mature, the membrane separating the two gamonts disintegrates and fertilization produces zygotes. The zygotes secrete a protective membrane around themselves to become oocysts (spores) (Roberts and Janovy 2005). Hundred of oocysts form within each gametocyst (Zuk 1987), and each nucleus within each oocyst undergoes multiple divisions to produce eight sickle-shaped sporozoites within each oocyst. The oocysts are liberated from the mature gametocyst either through spore-releasing ducts or the rupturing of the gametocyst.

When the oocyst is ingested by an odonate host, it ruptures and releases the sporozoites. The sporozoites then attach to the host gut epithelium and become trophozoites that feed on gut contents (Zuk 1987), grow, detach from the epithelium,

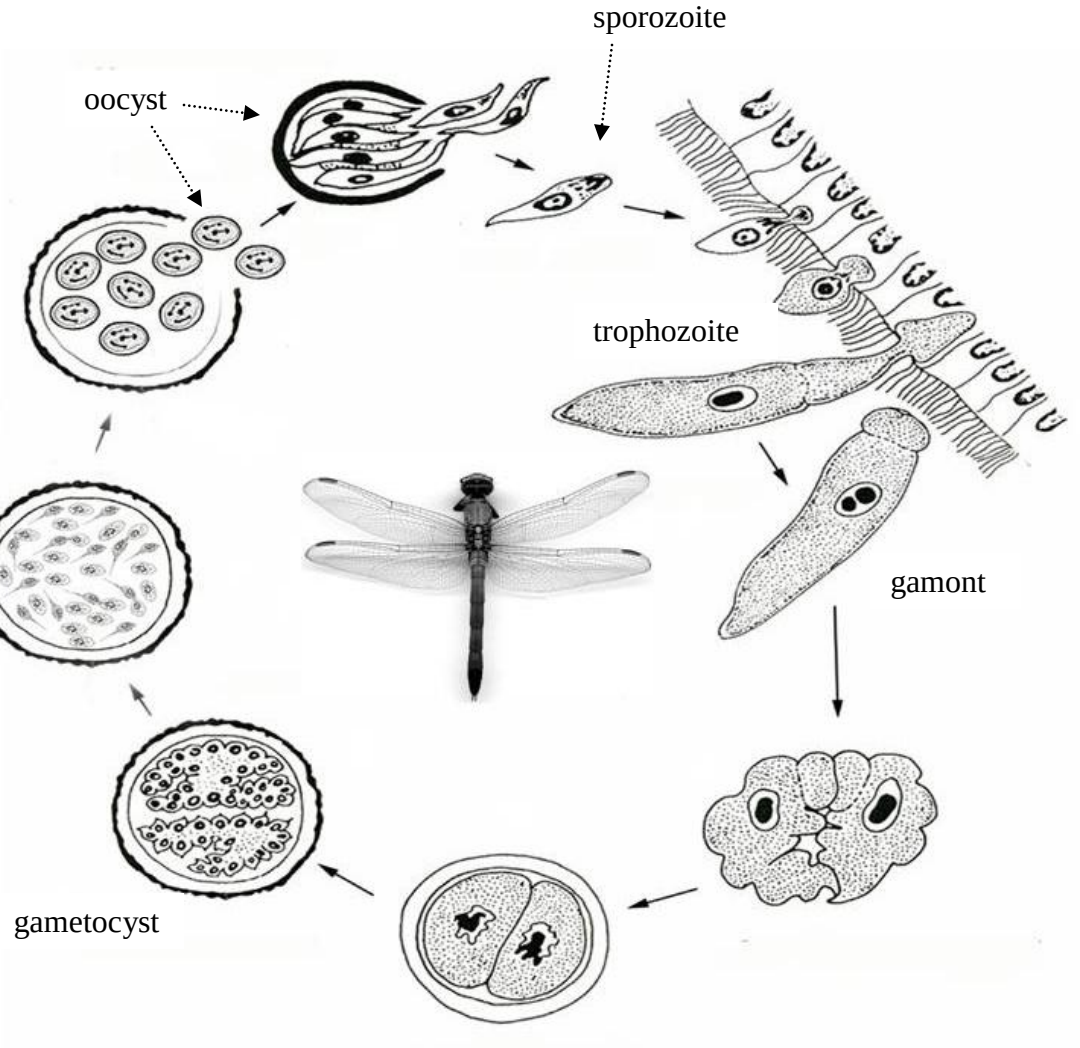

Figure 1.1: Life cycle of odonate-infecting gregarine parasites. When an oocyst is ingested by an odonate, excystation of the oocyst occurs and releases sporozoites. The sporozoites attach to the host's intestinal epithelium as trophozoites and absorb nutrients from the lumen. Trophozoites detach as gamonts and form mating pairs in a gametocyst. Nuclear divisions occur within each gamont to produce gametes that fuse to produce an oocyst. Within each oocyst, sporozoites develop that will be released when the oocyst is ingested. Image modified from Rueckert and Leander (2008).

and join others of the opposite mating type to form a gametocyst for reproduction (Cordoba-Aguilar et al 2003) (Figure 1.1).

Role of the Environment

Despite the many reports of gregarine infections in invertebrate populations, little is known about the environmental conditions/parameters that influence gregarine parasitism. Marden and Cobb (2004) reported prevalence variation for *Libellula pulchella* (Anisoptera: Libellulidae) in two habitats where gregarine prevalence and intensity were higher in one habitat compared to the other. Unfortunately, research on variation in gregarine prevalence and intensity among different habitats is lacking. Overall, while interactions between host species and their parasites have been well studied, the role of the environment in mediating the outcome of the interaction is still generally unknown (Belden 2006).

Fitness Costs

Some authors (Canning 1956, Manwell 1961) have regarded gregarines as harmless commensals. However, recent studies indicate detrimental effects of some gregarine infections (see Åbro 1971 and Zuk 1987).

Åbro (1971, 1974) first reported that gregarines negatively impact their odonate hosts. Gregarine trophozoites may form a barrier between the epithelium and the food in the lumen and interfere with digestion and absorption (Åbro 1971). Harmful alterations to the epithelium may be due to gregarine metabolites/toxins, or the intense parasite infections may cause lesions in the midgut wall during parasite movement. Åbro (1971) observed masses of discarded epithelial cells in damselfly guts with heavy infection.

Specimens of *Coenagrion hastulatum*, *Enallagma* spp., and *Pyrrhosoma* spp. (Zygoptera: Coenagrionidae) had eroded areas in the midgut with only the muscle layers remaining (Åbro 1974). Such specimens were reportedly easy to recognize in the field because they were poor fliers and their abdominal pigmentation was reduced. Reports of massive infections are not exclusive to damselfly adults, however. Damselfly naiads of *Ishnura heterosticta* (Zygoptera: Coenagrionidae) may also harbor many gregarine trophozoites (see Åbro 1971).

Åbro (1974) reported that the frequency of infected adult damselflies increased as the flight season progressed in western Norway. Such infections and tissue damage may strongly impact survivorship and fitness. Åbro (1971, 1974) speculated that infection may impair survival during rapid environmental changes or long-lasting weather conditions (dry/wet/cool/hot) that limit available food. Åbro (1974) also found gregarine intensity to be less in dragonflies compared to the damselflies, but the parasites' impact on behavior, fitness, and survivorship may be as severe. J.H. Marden (personal communication), for example, suggests that a single trophozoite likely impacts a host the same as does 100 trophozoites if a toxin is being released by the parasite.

As mentioned by Åbro (1971, 1974), food availability may be critical to the overall impact of infection. Since gregarine parasites appear to inhibit a host's absorption of nutrients (or may feed on the food in the lumen) (Åbro 1974), low food availability probably amplifies the parasites' detrimental effects. Tsubaki and Hooper (2004) found a negative coorelation between host survivorship and gregarine abundance under a low feeding regime but no relationship under high feeding regimes. Parasite burden, therefore, may have little effect on host survival and fitness when food availability is

abundant. If this is true, gregarine parasitism may hasten declines in host populations during the fall when prey items become more scarce.

Odonate competition for resources, territories, and mates involves ornaments, coloration patterns/intensity, chemical signals, etc. (Marden and Cobb 2004). Such external traits signal the overall condition of the individual to potential mates or competitors (Siva-Jothy and Plaistow 1999, Hooper et al 2006). In addition, performance-based activities (vocalizations, mating dances, fighting, etc.) also clearly communicate an individual's condition. All of these traits are more or less energy-dependent. Competition for mates and resources demands high energy expenditure (Marden and Cobb 2004). Such traits depend on the physiological health of the organism and can reveal a compromised physiological condition (Koskimäki et al 2004).

In many species where males compete for resources (e.g., quality oviposition sites for odonates) to attract mates, dominant males monopolize quality resources and consequently have a mating advantage (Qvarnström and Forsgren 1998). Among the several signals odonates are thought to use to communicate their condition/health to a potential competitor or mate are wing/body coloration and flight ability. Gregarine-infected hosts may exhibit reduced wing/body pigmentation (Åbro 1971, Cordoba-Aguilar et al 2003), poor flight ability/sustainability (Schilder and Marden 2006), and failures during territorial battles (Koskimäki et al 2004).

Gregarines likely suppress host pigment deposition since pigment development is constrained by nutrient absorption (Cordoba-Aguilar et al 2003). Åbro (1971) found that the damselfly, *Pyrrhosoma nymphula*, with dull colors and reduced flight abilities at the end of the season harbored hundreds of gregarines in their midgut. Some specimens with

severely faded pigmentation had intestinal walls perforated and partly dissolved. Cordoba-Aguilar et al (2003) reported sexual behavior modifications common to individuals with reduced pigmentation. In the damselfly, *Calopteryx haemorrhoidalis*, large gregarine infection rates occur in both males and females. Individuals with large gregarine intensities, regardless of gender, exhibit modified mate selection and pre/post copulatory behaviors. Several studies of calopterygid species (see Cordoba-Aguilar et al 2003) have shown that males with more intense wing pigmentation survive and defend territories for longer periods of time, and are preferred by females. Highly infected females tend to accept a mate more rapidly than do uninfected females. Infected males also mate with infected females more frequently than with uninfected females. In both cases, decreased damselfly wing pigmentation apparently indicates a high parasite burden. Cordoba-Aguilar et al (2003) suggest that an uninfected male's intense pigmentation may signal his condition to other males and indicate his ability to resist parasites to females. Additionally, female wing pigmentation may signal her reproductive value and can affect the amount of time she is guarded from intruder males during post-copulatatory ovipositing (Cordoba-Aguilar et al 2003). The bolder the pigmentation, for example, the more protection she gets.

Several studies (Siva-Jothy and Plaistow 1999, Marden and Cobb 2004, Schilder and Marden 2006) have also shown effects of gregarine infection on muscle power output and fat content/distribution in odonates. Siva-Jothy and Plaistow (1999) and Marden and Cobb (2004) both reported decreased fat content in infected individuals whereas Schilder and Marden (2006) reported that infected specimens of *Libellula pulchella* (Anisoptera: Libellulidae) have an impaired lipid catabolism in their muscles. This impaired

catabolism results in an accumulation of lipids in flight muscles and a sole-dependency on carbohydrate-catabolism for flight. In uninfected odonates, a mixture of carbohydrates and lipid substrates provide energy for flight (Schilder and Marden 2006). Such parasite-induced metabolic shifts may impact sustained flight necessary for territorial defense and mating success because lipid catabolism is needed for flight after the first few minutes (Marden and Cobb 2006).

When dragonfly females mate with territory-holders, they consistently mate with physiologically and immunologically superior males (Marden and Cobb 2004) and likely gain genetic benefits (more viable offspring) (Qvarnström and Forsgren 1998). Marden and Cobb (2004) found that males successfully defending territories and the females ovipositing in them had a reduced gregarine burden (mean intensity = 11 trophozoites host^{-1}). In addition, so called "submissive satellite males" were never observed to defend a territory and exhibited elevated gregarine burdens (mean intensity = 30 trophozoites host^{-1}). Gregarine parasites have been shown to alter the physiology and behavior of their odonate hosts and, consequently may play a significant role in determining the overall fitness of the hosts.

Study Objectives

Non-taxonomic odonate-gregarine investigations have focused primarily on damselflies (Åbro 1971, Åbro 1974, Cordoba-Aguilar et al. 2003, Hecker et al. 2002, Koskimäki et al. 2004, Siva-Jothy and Plaistow 1999, Tsubaki and Hooper 2004) with few focusing on dragonflies (Marden and Cobb 2004, Schilder and Marden 2006). Gregarines infections have been considered rare in dragonflies (Clopton 2009). And although gregarines have been considered relatively harmless among their host, recent

damselfly and dragonfly investigations reveal significant fitness cost associated with gregarine parasitism.

Because the distribution of gregarine parasites among dragonfly species is still largely unknown, this research initially identifies dragonfly species common to central Texas that host gregarines, measures the prevalence and intensity of their infection, and analyzes the variability of infection among hosts (i.e., host gender biases, temporal patterns of infection, spatial variation, etc.). A survey of the prevalence and infection intensity among dragonflies is fundamental to our understanding of these parasites and their potentially adverse effects on dragonfly hosts. The following research objectives were addressed: 1) survey dragonfly species common to central Texas for gregarine parasites, 2) measure the gregarine prevalence and intensity among dragonfly host populations at multiple locations through time, and 3) estimate the fitness cost of gregarine parasitism in a common and widespread dragonfly host species, *Erythemis simplicicollis*.

Study Locations

This study was conducted at three locations in McLennan County, Texas: Battle Lake (BL), Tradinghouse Creek Reservoir (TCR), and the Lake Waco Wetland (LWW). BL is a small reservoir (0.32 km^2) with a 3-m maximum depth and supports considerable littoral vegetation and an abundant dragonfly community. TCR is larger (8.14 km^2) with a maximum depth of 13 m (Baird and Tibbs 2005). TCR has little submerged vegetation, and the dragonfly population density is considerably less than at BL (unpublished data). The two reservoirs are 2.5 km apart in eastern McLennan County (Figures 1.2 and 1.3). The third location, LWW, is in western McLennan County and was constructed in 2001

as habitat mitigation for a 2-m pool rise of nearby Waco Lake. The 80-ha wetland receives pumped water from the North Bosque River and routes the flow through five sequential wetland cells before returning it 5-10 days later to the North Bosque River that feeds Waco Lake (Scott et al. 2005).

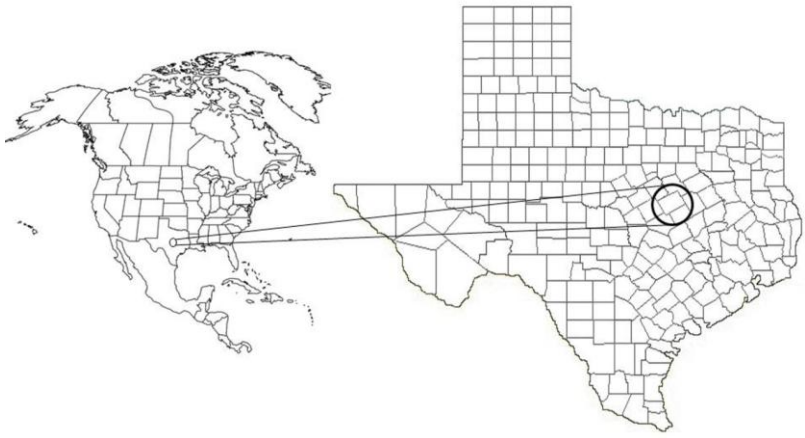

Figure 1.2: The three study sites are located in McLennan County, Texas.

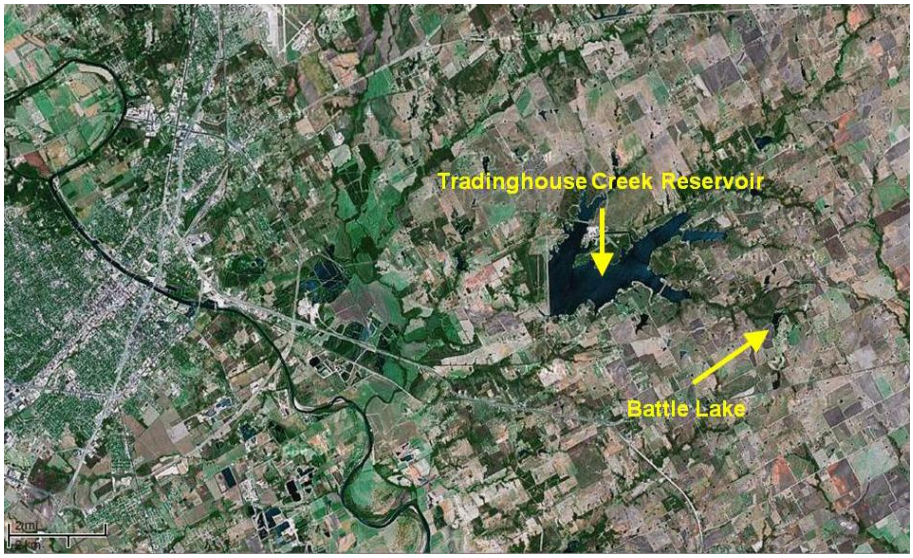

Figure 1.3: Two of the three study locations, Battle Lake and Tradinghouse Creek Reservoir, are 2.5 km apart.

Summary of Chapter Contents

This document is divided into 5 chapters, including the current introductory and background material. Chapter two describes a bidirectional gender bias of parasitism in two common species at BL and TCR, *Brachymesia gravida* and *Erythemis simplicicollis,* and discusses possible factors contributing to the biases. Chapter three presents the host species from BL and TCR and their prevalences/intensities through time. That chapter also describes the distribution of gregarines among hosts and the significance of estimating host age/maturity level. Chapter four describes the hosts from LWW and the lack of seasonality in prevalence and intensity at that location. In chapters three and four, mechanisms are also proposed for the seasonality found at BL and lack of seasonality at LWW. Areas for further research are also discussed in each of those chapters. Finally, chapter five summarizes the significant findings of all three studies.

CHAPTER TWO

Bidirectional Gender Biases of Gregarine Parasitism in Two Coexisting Dragonflies (Anisoptera: Libellulidae)

Abstract

Parasitism affects all taxa and influences individual and population success. Parasitism of adult dragonflies is widespread and frequently includes gregarine (Phylum Apicomplexa) life stages in the gut of the host. This research investigates variation in gregarine parasite prevalence and load in male versus female adults of two species of Libellulidae, *Erythemis simplicicollis* and *Brachymesia gravida*, associated with two central Texas reservoirs in close proximity. Parasite prevalence was biased toward males of *E. simplicicollis* and toward females of *B. gravida*. Results suggest that gender bias in parasite prevalence is influenced by gender behavior and environment more so than by immuno-response differences between genders.

Introduction

Dragonflies process much energy in freshwater ecosystems as predatory naiads as well as adults. These predators carry a burden of gregarine intestinal parasites, but the relationships among parasite prevalence, intensity, and host gender are not well known. Because males and females differ in profound aspects of their biology, we must consider genders separately to understand host-parasite relationships (i.e. modes of transmission, prevalence, load, and effects on fitness) (Zuk and McKean 1996). Morphological and/or physiological sexual dimorphism and sexual behavior differences often lead to

differential exposure and susceptibility to parasitism (Edwards and Smith 2003, Schalk and Forbes 1997, Zuk and McKean 1996).

Gender biases in parasite prevalence and intensity among vertebrates are better documented (Christe et al. 2007, see Zuk and McKean 1996) than for invertebrates. In general, vertebrates exhibit a male bias in susceptibility to parasitism. A primary mechanism for this bias is the negative effect of androgens on the immune response in male vertebrates (Grossman 1985, Roberts et al. 2004, Schalk and Forbes 1997, Zuk and McKean 1996). Gender biases are not often observed among invertebrates because they lack androgens and therefore lack a negative endocrine-immune response (Zuk and McKean 1996). Sheridan et al.'s (2000) review of 61 studies investigating gender biases among invertebrates, for example, reports only nine cases demonstrating a gender host bias in one direction or the other.

Odonates are among the few invertebrates to exhibit gender biases in parasitism (Lajeunese 2007). Hecker et al. (2002) found a female bias in gregarine prevalence in the damselfly, *Enallagma boreale* (Coenagrionidae), and Canales-Lazcano et al. (2005) reported a female bias in gregarine load for *Enallagma praevarum* (Coenagrionidae). Åbro (1996) found a female bias in gregarine intensity for the damselfly, *Calopteryx virgo* (Calopterygidae), and Lajeunesse et al. (2004) found a male bias in prevalence of the ectoparasitic mite, *Limnochares americana*, on two dragonflies species, *Leucorrhinia frigida* and *Nannothemis bella* (Libellulidae). Several studies (Åbro 1974, Andres and Cordero 1998, Cordoba-Aguillar et al. 2006, Lajeunesse 2007), however, report no detectable gender bias in parasitism of odonate hosts.

The purpose of this study was to determine if two common, coexisting libellulid dragonflies, *Erythemis simplicollolis* (Say) and *Brachymesia gravida* (Calvert), have gender biases as hosts for gregarine parasitism, and to discuss potential mechanisms underlying gender biases in odonates.

Materials and Methods

Adult *E. simplicicollis* and *B. gravida* were collected with an aerial net from two reservoirs in McLennan County, Texas, USA from April – November 2007. The reservoirs, Battle Lake (BL), a small reservoir (0.32 km^2) with considerable littoral vegetation, and Tradinghouse Creek Reservoir (TCR), a larger reservoir (8.14 km^2) with little submerged vegetation, are 2.5 km apart. Weekly collections from BL and biweekly collections from TCR occurred between 9:00 am – 12:00 pm along the shorelines of both sites. Netted specimens were placed in glassine envelopes and taken to the lab within two hours of capture. In the lab, their abdomens were separated from the thorax and stored in 70% ethanol.

To quantify parasite prevalence and load, the preserved abdomens were placed ventral-side up on a Styrofoam tray. The abdomens were split longitudinally and pinned to expose the crops and intestines. Gregarines (trophozoites and gamonts) were visible through the intestinal epithelium and counted at 60X magnification.

Parasite prevalences were compared between males and females of each species using contingency χ^2 tests (α=0.05). Gregarine loads were compared between genders of each species using the non-parametric Wilcoxon Rank Sum Test because the parasite load data was not normally distributed (Shapiro-Wilk test, $p<0.001$).

Results

Combined collections at BL and TCR yielded 1,362 adults (*E. simplicicollis*, ♀ N=327, ♂ N=801; *B. gravida*, ♀ N=150, ♂ N=84). Weekly collections at BL where dragonfly populations were more abundant (personal observation) produced larger sample sizes for both species (BL: *E. simplicicollis*, ♀ N=260, ♂ N=760; *B. gravida*, ♀ N=95, ♂ N=56) compared to TCR (*E. simplicicollis*, ♀ N=67, ♂ N=41; *B. gravida*, ♀ N=55, ♂ N=28).

Analysis of combined data for the BL and TCR populations of each species revealed strong gender biases in gregarine prevalence for *B. gravida* (χ^2=12.07, p=0.001) and for *E. simplicicollis* (χ^2=14.81, p<0.001). However, the gender biases were in opposite directions for the two species. Females of *B. gravida* had a significantly greater parasite prevalence (67%) than the males (44%). In contrast, males of *E. simplicicollis* had a greater prevalence (46%) than the females (34%) (Figure 2.1). When parasite prevalences for both host species were analyzed at BL and TCR separately, the female bias for *B. gravida* was consistent at the two sites (BL: χ^2=12.97, p<0.001, TCR: χ^2=5.41, p=0.02), but the male bias for *E. simplicollis* was true only for the BL population (χ^2=5.87, p=0.015) and not the population at TCR (χ^2=1.03, p=0.309) (Table 2.1).
In contrast to significant gender biases for parasite prevalence, mean gregarine intensities did not differ between genders within each species (*B. gravida*: Z = -1.722, p = 0.085; *E. simplicicollis*: Z = -0.235, p = 0.814) (Figure 2.2). Mean gregarine intensities for *B. gravida* and *E. simplicicollis* were 8.82 and 7.73, respectively.

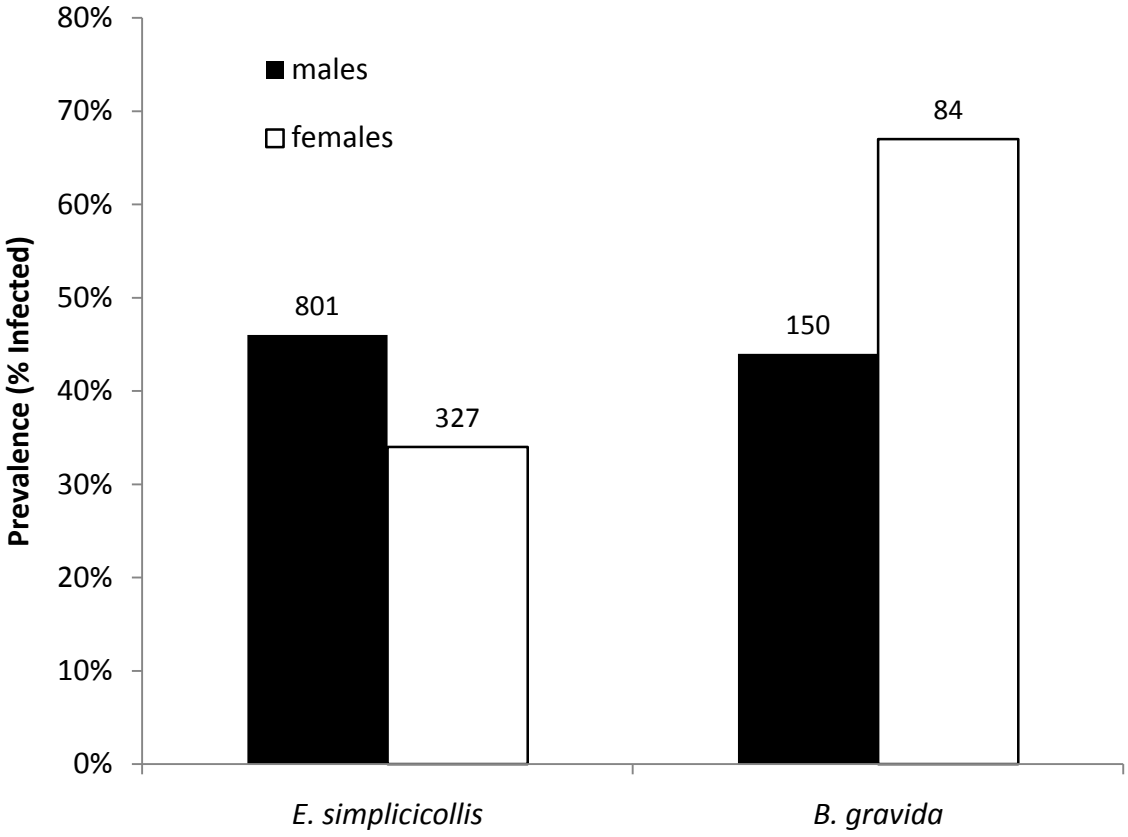

Figure 2.1: Frequency of adult odonates infected with one or more gregarine parasites. Data for *E. simplicicollis* and *B. gravida* are combined samples from two central Texas reservoirs. Numbers above bars represent sample sizes.

Discussion

At Battle Lake and Tradinghouse Creek Reservoir, the female parasitism bias of *B. gravida* was significant, yet prevalence was notably different at both sites (BL: ♀ 86%, ♂ 61%; TCR: ♀ 11%, ♂ 35%). Because these sites differ in size, they likely have different physicochemical properties. Although such properties were not measured in this study, the magnitude of the prevalence differences between the two sites suggests that habitat/environmental factors likely influence the maintenance and transmission of gregarines within dragonfly communities.

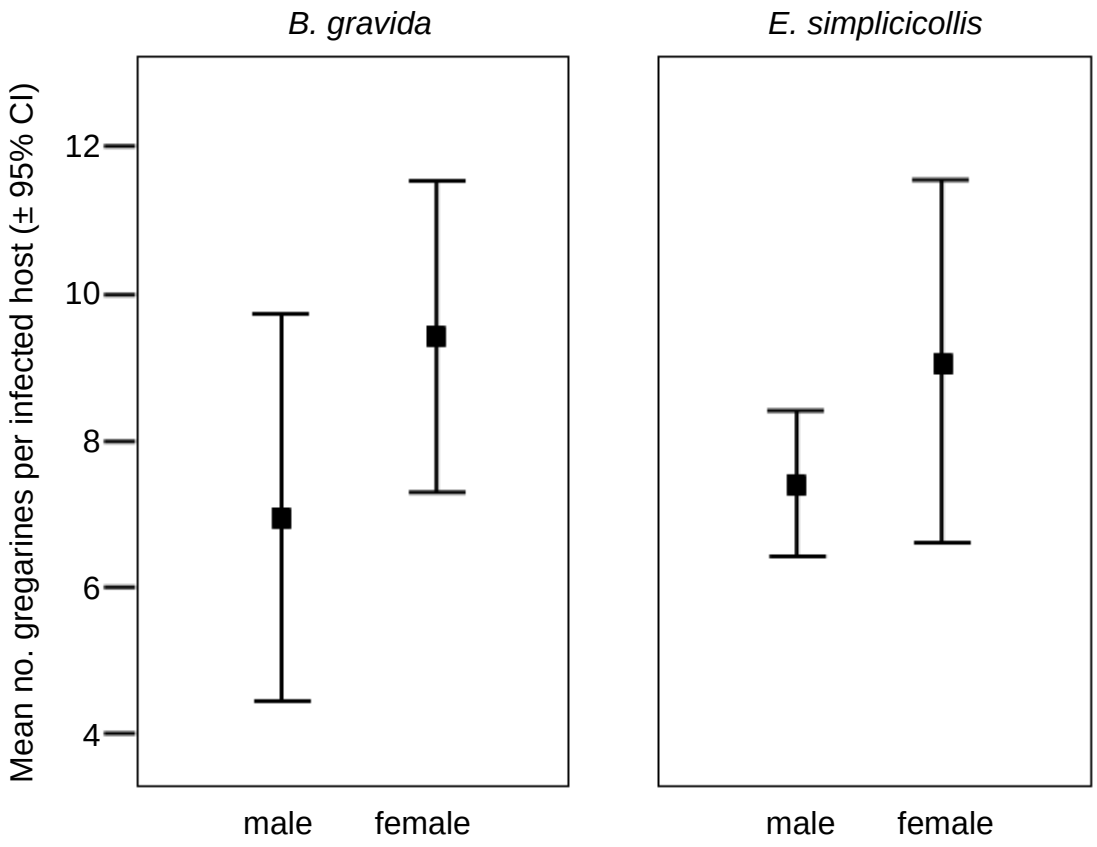

Figure 2.2: Mean gregarine loads for combined samples of infected odonates from two central Texas reservoirs. Error bars are ± 95% confidence intervals.

Male and female animals have different behaviors associated with feeding and reproduction, resulting in differential exposure and susceptibility to parasitism (Christe et al. 2007, Edwards and Smith 2003, Poulin 1996, Schalk and Forbes 1997, Zuk and McKean, 1996). Consequently, parasite success can vary within conspecifics due to host gender differences in encounter rates as well as the effectiveness of their immune responses (Christe et al. 2007, Hecker et al. 2002, Lejeunesse 2007, see Zuk and McKean 1996). Such gender differences often result in differential feeding locations and rates that may increase or decrease the probability of ingesting the transmission stage

Table 2.1: Comparison of gregarine prevalence in the genders of *B. gravida* and *E. simplicicollis*.

Species	Site	Gender	N	Parasite Prevalence	χ^2	p
Brachymesia gravida	BL	Male	56	.61	12.97	<0.001
		Female	95	.86		
	TCR	Male	28	.11	5.41	0.02
		Female	55	.35		
	Combined	Male	84	.44	12.07	0.001
		Female	150	.67		
Erythemis simplicicollis	BL	Male	760	.49	5.87	0.015
		Female	260	.40		
	TCR	Male	41	.05	1.03	0.309
		Female	67	.10		
	Combined	Male	801	.46	14.81	<0.001
		Female	327	.34		

and/or shift energy allocation away from immunity towards reproduction (Zuk and McKean 1996).

Variation in immune responses between genders may underlie host gender biases in parasitism. Most studies of mammals (see Christe et al. 2007, Zuk and McKean 1996) report a male bias in parasite prevalence and load compared to females. This bias is usually attributed to the negative influence of steroid hormones (e.g. testosterone) on the immune response. Insects, however, do not produce testosterone and, consequently lack this endocrine-immune interaction. Therefore male and female insects typically do not differ in parasite prevalence or load due to immunocompetence (Sheridan et al. 2000). Although I found a gender bias in both odonate species studied, the biases were in opposing directions, and parasite intensities did not differ between males and females for both species (Figure 2.2). If the gender biases had been in the same direction for both species or if parasite intensities had differed, then it would indicate a potential immunocompetence difference between odonate genders and result in a universal gender bias of parasitism, i.e. a male or female bias. This was not the case, however. My results suggest that immunocompetence does not differ between genders of these two odonate species, or is specifically ineffective against gregarine parasites for either host gender. Cordoba-Aguilar et al.'s (2006) study may support the latter. They found no gender bias in gregarine prevalence or intensity in two damselflies (*Hetaerina americana* and *Argia tezpi*), but based on two parameters commonly used to estimate an insect's immune system, phenyloxidase and hydrolytic lysosomal enzyme concentrations (Cordoba-Aguilar et al. 2006, Schwarzenbach and Ward 2006), females were more immunocompentent than males.

Another explanation for host gender biases associated with parasitism is variation in host exposure to the infective parasite stage. If both genders of a species share habitats for foraging and have similar immune responses, then parasite prevalence and intensity patterns should be similar between genders (Canales-Lazcana et al. 2005). However, gender-specific behavior may expose one gender to the parasite's infective stages more so than the other gender. Tinsley (1989) found that flukes more heavily parasitize male spadefoot toads because males spend most of their breeding season in water, whereas females visit the water only briefly to lay eggs. Such behavioral differences in odonate species could result in the bidirectional gender biases found in this study. Although general behavior has been described for *E. simplicicollis* (May and Baird 2002, McVey 1981, Paulson 1966), little is known about *B. gravida*. Only one study (Paulson 1966) briefly describes the behavior of the latter species, and includes little gender-specific information. Without adequate behavioral descriptions for both species, it's difficult to clarify if the bidirectional gender biases in these two species result from differential gender exposure to the parasites.

A final question elicited by host gender biases considers mechanisms of parasite preference (an innate and active selection of a specific host to maximize fitness). In most cases of gender bias, parasites exhibit some degree of preference, as in the ectoparastic mites parasitizing many odonate species. Larval mites attach phoretically to the final odonate instar in the water. As the odonate emerges, the larval mite transfers to the teneral and feeds on body fluids (Rolff et al. 2001). After feeding, the mite detaches, falls back into the water, and completes its development (Grant and Samways 2007). Because these mites depend on aquatic habitats for life cycle completion, it's

advantageous to attach to a host that either stays around water or returns to it for reproduction. Several insect studies (see Edwards and Smith 2003) report greater mite parasitism on female hosts because females usually return to water for ovipositing. McLachlan's (1999) report that mites on male chironomids often move to females during copulation supports this hypothesis.

Whether gregarine parasites prefer a particular host gender is not clear. Gregarine preference may not be possible because the oocysts of these intestinal parasites are ingested indiscriminately by a potential host (Clopton et al. 1992). The parasite would best complete its life cycle in the gender that spends the most time near water. But selection of that host gender would only be realized if the sporozoites in the ingested oocyst fail to excyst in an unpreferred host and instead pass through the digestive tract, return to the environment, and remain viable. Clopton et al. (1992) reported that some gregarine species are specific not only to a host species, but also to the host's life cycle stage. Excystation assays by Smith and Cook (2008) show that gregarine excystation is species-specific in cockroaches. Although no studies quantify the concentration/availability of oocysts in the aquatic habitat, or the success rate of parasite transmission for gregarines, I assume that failure to excyst in an unpreferred gender would be a risky strategy for the parasite. Its ingestion into a second potential host of the preferred gender may be improbable. In addition, the absence of a gregarine intensity difference between the genders of both species indicate that gregarine parasites lack the ability to discriminate between the genders of their hosts, or simply have no preference.

The environment, gender-specific host behavior, host immunity, and parasite preference all regulate parasite intensity and prevalence (Schalk and Forbes 1997, Zuk

and McKean 1996). The contrasting gender biases found in this study do not fully distinguish among these factors. They do, however, bring focus to some paths for research investigating factors associated with the occurrence and maintenance of gregarine parasites in odonate communities.

Acknowledgments

I thank David Cummings for assistance in the field and lab.

CHAPTER THREE

Patterns of Gregarine Parasitism in Dragonflies: Host, Habitat, and Seasonality

Abstract

Gregarines are ubiquitous protozoan parasites that infect arthropods worldwide. More than 1,600 gregarine species have been described, but only a small percentage of invertebrates have been surveyed for these apicomplexan parasites. Adult dragonfly populations were surveyed for gregarines at two reservoirs in Texas, USA for two years. Gregarine prevalence and intensity were compared intraspecifically between host genders and reservoirs, among wing loads, and through time. Of the 29 dragonfly species collected, 41% hosted gregarines. Nine of these dragonfly species were previously undocumented as hosts. Among the commonly collected hosts, prevalence ranged from 18% to 52%. Parasites were aggregated among hosts and had a median intensity of five parasites per host. Gregarines were found only in hosts exceeding a minimum wing load, indicating that gregarines are likely not transferred from the naiad to adult during emergence. Prevalence and intensity increased during both years, suggesting that gregarine oocyst viability parallels increasing host population densities and may be short-lived. Prevalence and intensity also differed between dragonfly populations at two reservoirs. Regression analyses revealed that host species, host gender, month, and year were significant explanatory variables related to gregarine prevalence and intensity. Abundant information on odonate distributions, diversity, and mating activities makes dragonfly-gregarine systems excellent avenues for ecological, evolutionary, and

parasitological research. Our results emphasize the importance of considering season, hosts, and habitat when studying gregarine-dragonfly ecology.

Introduction

Dragonflies host several major parasitic groups, including water mites, tapeworms, flukes, roundworms, horsehair worms, and gregarines (Corbet 1999, Forbes and Robb 2008). Gregarines (phylum Apicomplexa) are among the most ubiquitous and diverse protozoan parasites (Schreurs and Janovy 2008, Smith and Clopton 2003) infecting a variety of invertebrates, particularly annelids, nematodes, echinoderms, molluscs, and insects (Bush et al. 2001, Clopton 2002, Manwell 1961, Roberts and Janovy 2005, Smith and Clopton 2003). More than 1,600 gregarine species have been described, but only a small percentage of invertebrates have been surveyed for these apicomplexan parasites (Roberts and Janovy 2005). Among insect hosts, orthopterans and odonates (particularly zygopterans) are the most commonly infected (Åbro 1974, Corbet 1999). Currently, nine gregarine genera are known to parasitize odonates (Clopton et al. 1993, Clopton 1995, Clopton 2004, Hays et al. 2007, Percival et al. 1995, Richardson and Janovy 1990, Sarkar 1997).

Odonate-infecting gregarines are obligate monoxenous parasites (Bush et al. 2001, Omoto et al. 2004). Dragonflies ingest infective oocysts during feeding and/or drinking. Ingested oocysts excyst and release sporozoites that attach to the host's intestinal epithelium as trophozoites. After absorbing nutrients and maturing, trophozoites detach as gamonts. Two gamonts of opposite mating types fuse to form stable mating pairs. Once fused, the gamonts encyst to form a gametocyst, which passes out with the host's feces. Gametogenesis and fertilization occur within the gametocyst,

which eventually ruptures to release hundreds of infective oocysts into the environment (Roberts and Janovy 2005, Zuk 1987).

The wealth of information on odonate distributions, diversity, and mating activities makes dragonfly-gregarine systems excellent avenues for ecological, evolutionary, and parasitological research (Forbes and Robb 2008). Critical to this research is describing the poorly-known distribution of gregarine infections among dragonfly species, within populations, and through time. This research (1) identifies dragonfly species that host gregarine parasites, (2) estimates the prevalence and intensity of infection in these hosts, and (3) investigates the occurrence of parasitism through time and between sampling locations. Parasitic terms used follow Bush et al. (1997).

Materials and Methods

Study Sites

Dragonfly populations were sampled primarily from two adjacent study areas, Battle Lake (BL) and Tradinghouse Creek Reservoir (TCR). Both systems are in McLennan County, Texas, and are 2.5 km apart. BL is a small reservoir (0.32 km^2) with a 3-m maximum depth and supports considerable littoral vegetation and an abundant dragonfly community. TCR is larger (8.14 km^2) with a maximum depth of 13 m (Baird and Tibbs 2005). TCR has little submerged vegetation, and the dragonfly population density is considerably less than at BL (unpublished data).

Sampling & Dissections

Adult dragonfly populations were sampled approximately weekly at BL, biweekly from TCR, and irregularly at the additional locations from March 2007-November 2008.

Individuals were netted within 15 m of the shorelines, taken to the lab, killed using ethyl acetate, and identified (Abbott 2005). Dragonflies collected in 2008 were weighed to the nearest 0.1 mg. A regression equation was developed to correct for weight loss after removal from the kill jar (presumably due to dessication): $y=0.000032x - 0.000157$, where x is minutes after removal from the kill jar, and y is weight lost. After an individual was weighed, its abdomen was separated from the thorax and stored in 70% ethanol.

Weight generally indicates adult maturity because maturing individuals feed and gain weight with time (Corbet 1999). However, weight alone is an unreliable measure of maturity because it is confounded by overall body size which varies significantly among and within species (Bried and Ervin 2007). Wing load (mg body wt X wing surface area^{-1}) better indicates maturity because incorporation of wing area values adjusts weight values for body size (Grabow and Rüppell 1995). To determine wing surface area, hindwing lengths were measured and regressed to calculate total area according to equations we developed for *Brachymesia gravida*, *Celithemis eponina*, *Erythemis simplicicollis*, and *Pachydiplax longipennis*.

Each species had a wing load threshold below which parasitism was not detected. In this study, adults with wing loads exceeding the threshold were considered mature, i.e. potential hosts, while those below the threshold were considered immature. To determine the wing load thresholds associated with parasitized individuals for each species, wing loads and parasite counts were plotted. The minimum wing load, i.e. wing load threshold, for each species and gender was determined and identified those dragonfly adults mature enough to host visible parasites.

To determine if gregarines are transferred with emerging naiads to the adult, late-instar naiads were collected from BL in 2007 and reared to emergence in aerated 38-L aquaria. The half-filled rearing aquaria had test-tube racks extending above the water level to provide a suitable substrate for emergence. Emerged tenerals (n=73) were preserved and examined for parasites.

Parasite Identification

Several live dragonflies were dissected for the purpose of collecting parasites for identification. After abdomens were opened and parasites (trophozoites and gamonts) were observed, the contents of the infected intestinal section were smeared on a coverslip. The coverslip was placed in warm AFA (alcohol formalin-acetic acid) fixative for 5-7 minutes and stored in 70% ethanol for staining using the protocol of Clopton (2006). Parasites were identified by Dr. Tamara Cook, Sam Houston State University.

Statistical Analyses

Parasite aggregation (*k*) was estimated for all host populations combined (Elliot 1977) . When *k* is large (>8), the distribution is random; as it approaches zero, the distribution is highly aggregated (Poulin 2006).

Differences in parasite prevalence (the percentage of hosts infected) between genders of the four most abundant dragonfly species and between the two study areas, BL and TCR, were tested using contingency χ^2 tests (α=0.05). Differences in intensity (the number of parasites in an infected individual) between genders and among wing loads of infected versus uninfected individuals were compared using the nonparametric Wilcoxon

Rank Sum Test because data for parasite intensity and wing load was not normally distributed (Shapiro-Wilk test, $P<0.001$).

To describe variation in parasite prevalence in dragonflies at BL over time (regardless of maturity), parasite prevalence was calculated for each month through 2007 and 2008 for the four most abundant host species. Freedman's test detected departures from a uniform occurrence throughout the year, and Edwards' test estimated the date for the annual peak in prevalence (Abramson 2005, Vezzani and Wisnivesky 2006). Both tests were run using WINIPEPI software (Abramson 2004). Logistic and Poisson regression models ($\alpha=0.05$) were developed for parasite prevalence and intensity, respectively, to identify variables that significantly influenced prevalence and intensity (Boisier et al. 2002).

Results

Hosts

Of the 29 dragonfly species collected (n=5,994 specimens), 12 species (41%) hosted gregarines (Table 3.1). Infected dragonflies were collected from April-November of both years. Species that were collected but had no gregarine stages visible at 60X included members of Libellulidae: *Celithemis elisa* (n=4), *Dythemis velox* (2), *Erythrodiplax umbrata* (1), *Ladona deplanata* (3), *Libellula croceipennis* (1), *Macrodiplax balteata* (2), *Orthemis ferruginea* (3), *Pantala flavescens* (9), *Pantala hymenaea* (14), *Plathemis lydia* (14), *Sympetrum corruptum* (12); and Gomphidae: *Aphylla angustifolia* (4), *Dromogomphus spoliatus* (4), *Gomphus graslinellus* (1), *Gomphus hybridus* (1), *Gomphus militaris* (2), *Phyllogomphoides stigmatus* (5), and *Stylurus intricatus* (5).

The distribution of the parasite intensity for all host species combined was highly aggregated (k=0.0003) with few infected adults exceeding 50 parasites (Figure 3.1). The median intensity was five with an interquartile range (IQR) of 2-12.75 trophozoites and gamonts per host. The maximum intensity of 201 parasites occurred in one female of *E. simplicicollis*. Gametocysts occurred in 1.8% of all adults collected. Gregarines were tentatively identified as *Actinocephalus* spp. and *Geneiorhynchus* spp. (Eugregarinorida: Actinocephalidae).

The most abundant and frequent host species collected at BL and TCR during both years were *E. simplicicollis* (BL=2,182, TCR=255), *P. longipennis* (BL=1,569; TCR=58), *C. eponina* (BL=899, TCR=100), and *B. gravida* (BL=538, TCR=167). Data from these four libellulid species were used for location comparisons and seasonal analyses at BL.

Gender biases in parasite prevalence were detected in *E. simplicicollis* (males=53%, females=45%; χ^2_1=13.93, P<0.001) and *B. gravida* (males=45%, females=74%; χ^2_1 =60.28, P<0.001) when all individuals during both years and locations were combined. No gender difference for prevalence was detected in *C. eponina* or *P. longipennis*. Likewise, significant differences in parasite intensity between genders (reported as the median intensity with IQR) also occurred for *E. simplicicollis* (median$_{♂}$=6.0, IQR=2.0-14.0; median$_{♀}$=9, IQR=3.0-21.3; Z= -4.773, P<0.001) and *B. gravida* (median$_{♂}$=3.0, IQR= 1.0-5.0; median$_{♀}$=5.0, IQR=2.0-11.0; Z= -3.214, P=0.001). No gender differences in intensities were detected for *C. eponina* and *P. longipennis*.

In 2008, adult dragonfly wing loads were calculated to estimate maturity. When mature male and female hosts (those exceeding the wing load thresholds, see Figure 3.2

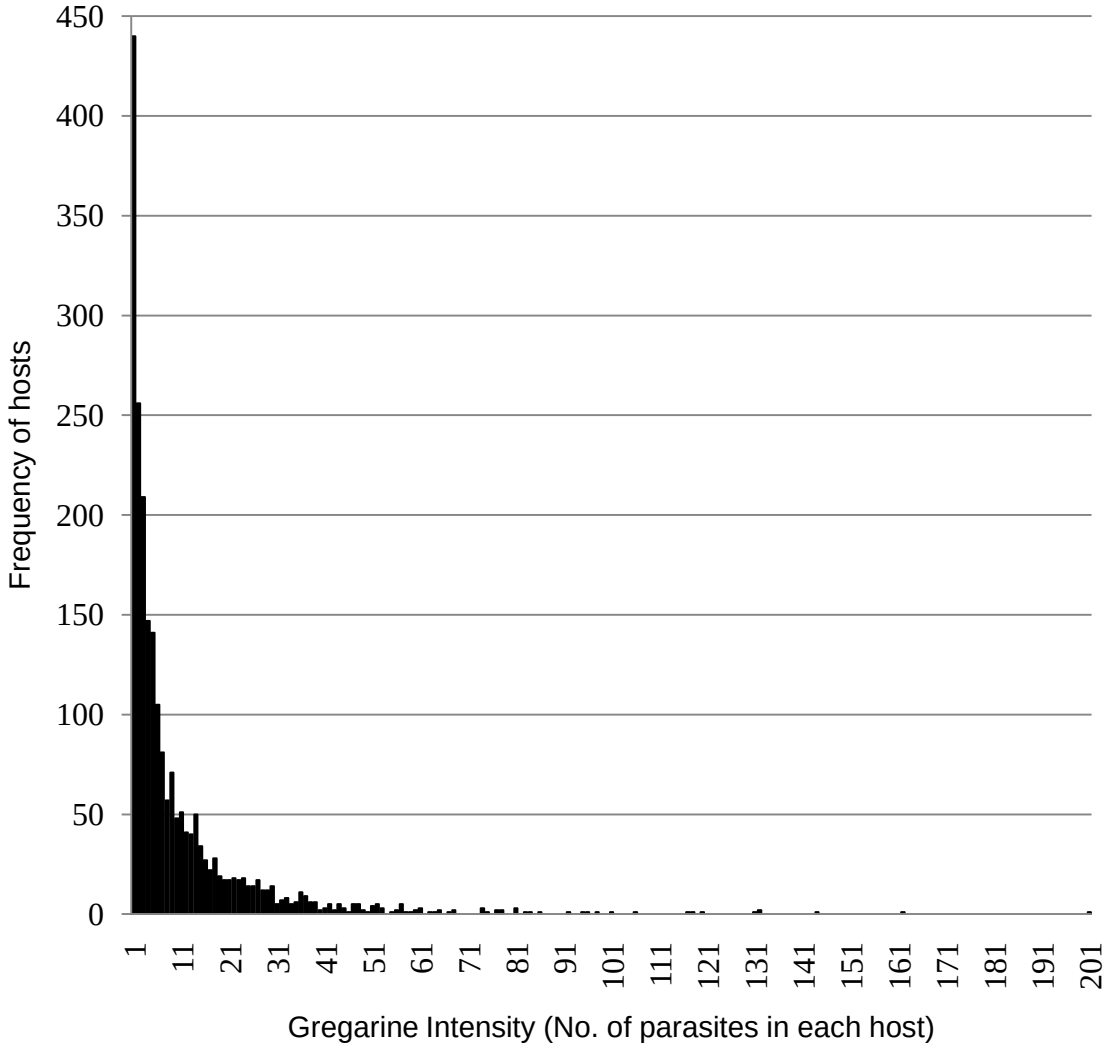

Figure 3.1: Intensity of gregarine parasites in all dragonfly species.

Table 3.1: Dragonfly species infected with gregarine parasites from all collections (2007-2008): total number of dragonflies captured (n), prevalence (%) of gregarines, median intensity (Int_{med}), and the maximum intensity (Int_{max}).

Dragonfly Assemblage	n	Prevalence	Int_{med}	Int_{max}
All locations				
Anax junius	36	2%	5.0	-
males	31	3%	5.0	-
females	5	0%	-	-
Brachymesia gravida	712	52%	4.0	131
males	258	46%	3.0	96
females	454	56%	5.0	131
Celithemis eponina	876	18%	3.0	42
males	467	17%	2.0	27
females	409	19%	3.0	42
Dythemis fugax	47	19%	1.0	8
males	37	21%	1.0	8
females	10	10%	1.0	1
Epitheca princeps	5	20%	9.0	9
males	3	33%	9.0	9
females	2	0%	-	-
Erythemis simplicicollis	2460	51%	6.0	201
males	1565	52%	6.0	162
females	895	47%	9.0	201
Libellula luctuosa	316	32%	4.0	47
males	238	33%	4.0	47
females	78	29%	3.0	29
Pachydiplax longipennis	1196	22%	3.0	75
males	1125	22%	3.0	75
females	71	19%	4.0	19
Perithemis tenera	93	10%	1.0	8
males	64	8%	1.0	8
females	29	14%	1.5	4
Sympetrum ambiguum	4	25%	20	20
males	3	33%	20	20
females	1	0%	-	-
Sympetrum ambiguum	4	25%	20	20
males	3	33%	20	20
females	1	0%	-	-

Dragonfly Assemblage	n	Prevalence	Int_{med}	Int_{max}
All locations (cont.)				
Tramea lacerata	38	55	1.5	2
males	35	6%	1.5	2
females	3	0%	-	-
Tramea onusta	16	6%	4	4
males	14	7%	4	4
females	2	0%	-	-
Battle Lake Reservoir (BL)				
Anax junius	19	5%	5	5
males	17	6%	5	5
females	2	0%	-	-
Brachymesia gravida	541	60%	4.5	131
males	193	54%	3.0	96
females	348	64%	5.0	131
Celithemis eponina	944	17%	3.0	42
males	501	18%	3.0	27
females	443	16%	3.0	42
Dythemis fugax	21	23%	1.0	8
males	14	33%	1.0	8
females	7	0%	-	-
Erythemis simplicicollis	2200	53%	7.0	201
males	1460	55%	6.0	162
females	740	49%	10.0	201
Epitheca princeps	2	50%	14	14
males	1	0%	-	-
females	1	100%	14	14
Libelulla luctuosa	186	39%	5.0	47
males	145	42%	5.0	47
females	41	33%	4.0	29
Pachidiplax longipennis	1075	24%	3.0	57
males	1028	24%	3.0	57
females	47	24%	8.0	19
Perithemis tenera	24	13%	1.0	1
males	18	11%	1.0	1
females	6	17%	1.0	1
Tramea lacerata	36	6%	1.0	2
males	32	6%	1.5	2
females	3	0%	-	-

Dragonfly Assemblage	n	Prevalence	Int_{med}	Int_{max}
Tradinghouse Creek Reservoir (TCR)				
Brachymesia gravida	160	24%	2.0	17
males	54	13%	1.0	5
females	106	29%	3.0	17
Celithemis eponina	121	20%	3.0	30
males	62	8%	1.0	5
females	59	32%	3.0	30
Dythemis fugax	21	14%	1.0	7
males	18	11%	4.0	7
females	3	33%	1.0	1
Epitheca princeps	2	50%	9.0	9
males	1	100%	9.0	9
females	1	0.%	-	-
Erythemis simplicicollis	255	26%	4.0	29
males	104	21%	3.5	29
females	151	30%	5.0	22
Libellula luctuosa	99	25%	3.0	20
males	68	25%	2.0	20
females	31	26%	3.0	6
Pachydiplax longipennis	58	9%	2.0	2
males	45	9%	1.5	2
females	13	8%	2.0	2
Perithemis tenera	61	8%	4.0	8
males	41	7%	4.0	8
females	20	10%	2.5	4

and Table 3.2) were analyzed, no gender biases in prevalence were detected (*E. simplicicollis*: prevalence$_{♂}$ = 70%, prevalence$_{♀}$=67%, χ^2_1 =1.08, *P*=0.30; *B. gravida:* prevalence$_{♂}$=61%, prevalence$_{♀}$=68%, χ^2_1=2.25, *P*=0.13).

None of the reared tenerals contained gregarine trophozoites, gamonts, or gametocysts. These tenerals were *Celithemis eponina* (9 females, 22 males), *Celithemis fasciata* (3 females), *Dythemis fugax* (3 males), *Erythemis simplicicollis* (4 males, 1

female), *Pachydiplax longipennis* (9 males), *Perithemis tenera* (11 females, 2 males), and *Tramea lacerata* (3 females, 3 males).

For the four most abundant hosts, infected individuals had heavier and narrower ranges of wing loads than uninfected individuals when mature and immature individuals were combined (Figure 3.3). Median wing loads (mg cm^{-2} with IQR) for infected vs. uninfected males, respectively, were *B. gravida*: 21.1 (19.8-22.4) vs. 18.7 (13.2-21.7); *C. eponina*: 15.5 (14.8-16.3) vs. 12.2 (10.3-15.4); *E. simplicicollis*: 24.1 (22.7-25.9) vs. 23.1 (17.8-25.2); and *P. longipennis*: 20.8 (18.9-22.4) vs. 20.5 (18.8-22.4). Infected males had significantly higher wing loads (each $P<0.001$) with the exception of *P. longipennis* ($P=0.80$). Infected females had significantly higher wing loads than the uninfected (each $P < 0.001$): *B. gravida*: 24.3 (22.7-26.2) vs. 21.7 (13.8-24.7); *C. eponina*: 16.8 (15.5-17.8) vs. 13.7 (9.5-16.9); *E. simplicicollis*: 29.2 (27.3-31.1) vs. 16.3 (13.8-26.6); and *P. longipennis*: 21.5 (17.7-24.9) vs. 11.1 (10.4-17.7). When only mature hosts were considered, no differences were detected between the wing loads of infected and uninfected genders of these four species (Table 3.2).

Study Areas

Parasite prevalences and intensities were greater in BL populations than in TCR populations. BL males had a significantly greater parasite prevalence than TCR males for all four species: *B. gravida* ($P< 0.001$), *C. eponina* ($P=0.02$), *E. simplicicollis* ($P<0.001$), and *P. longipennis* ($P=0.02$). BL females also had significantly greater parasite prevelances than TCR females: *B. gravida* ($P<0.001$) and *E. simplicicollis* ($P<0.001$). No significant differences were found between parasite prevalences of BL and TCR females of *C. eponina* and *P. longipennis* (Fig 3.4). Median parasite intensities

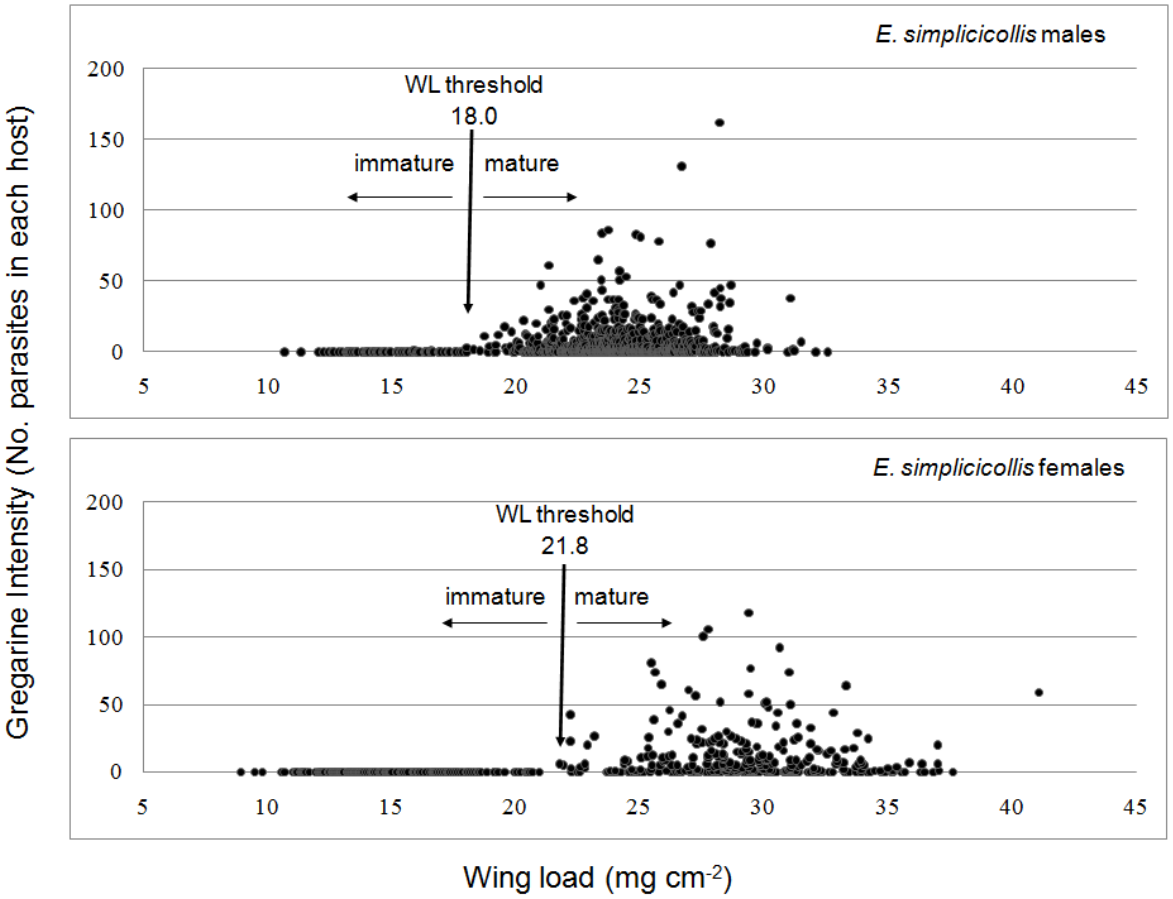

Figure 3.2: Distribution of gregarine intensity among wing loads (mg cm^{-2}) of male (top) and female (bottom) *E. simplicicollis*. WL threshold represents the minimum wing load of an infected adult below which parasitism was never detected. Adults below the threshold were designated immature and those exceeding the threshold were designated mature enough for parasitism to be visually apparent. See Table 2 for wing load thresholds for other abundant host species.

Table 3.2:. Minimum wing loads (WL thresholds) for infection for four abundant dragonfly species and median wing loads with interquartile ranges (IQR) of infected vs. uninfected individuals.

Species	WL threshold	median $WL_{infected}$	median $WL_{uninfected}$
Brachymesia gravida			
Males	17.3	21.1 (19.9-22.4)	21.5 (19.8-23.5)
Females	16.9	24.4 (22.8-26.3)	24.4 (22.6-26.1)
Celithemis eponina			
Males	13.9	15.6 (15.2-16.2)	15.8 (14.9-16.8)
Females	14.4	16.9 (15.8-18.1)	17.0 (16.2-18.1)
Erythemis simplicicollis			
Males	18.0	24.1 (22.7-25.9)	24.1 (22.7-25.7)
Females	21.8	29.1 (27.3-31.1)	29.5 (26.6-31.8)
Pachydiplax longipennis			
Males	15.1	20.7 (18.9-22.4)	20.6 (18.9-22.4)
Females	16.5	21.5 (17.7-24.9)	22.6 (18.9-23.7)

were significantly greater in BL populations for *E.simplicicollis* ($P_{♂}$=0.01, $P_{♀} < 0.001$) and *B. gravida* ($P_{♂}$=0.02, $P_{♀}$=0.002), but did not differ for *C. eponina* or *P. longipennis* (Figure 3.4).

Seasonality

Parasite prevalence for each of the four most abundant species showed significant seasonal variation at BL for combined immature and mature hosts (Figure3.5) and in mature hosts (Table 3.3) (Freedman's test, $P<0.01$). The peak dates for prevalence as indicated by Edwards' test (Abramson 2004) were between late July and early September.

Logistic regression analyses revealed that the significant explanatory variables related to the probability of being parasitized at BL were host gender, host species, month, and year ($P<0.001$). Likewise, the Poisson regression models identified the same

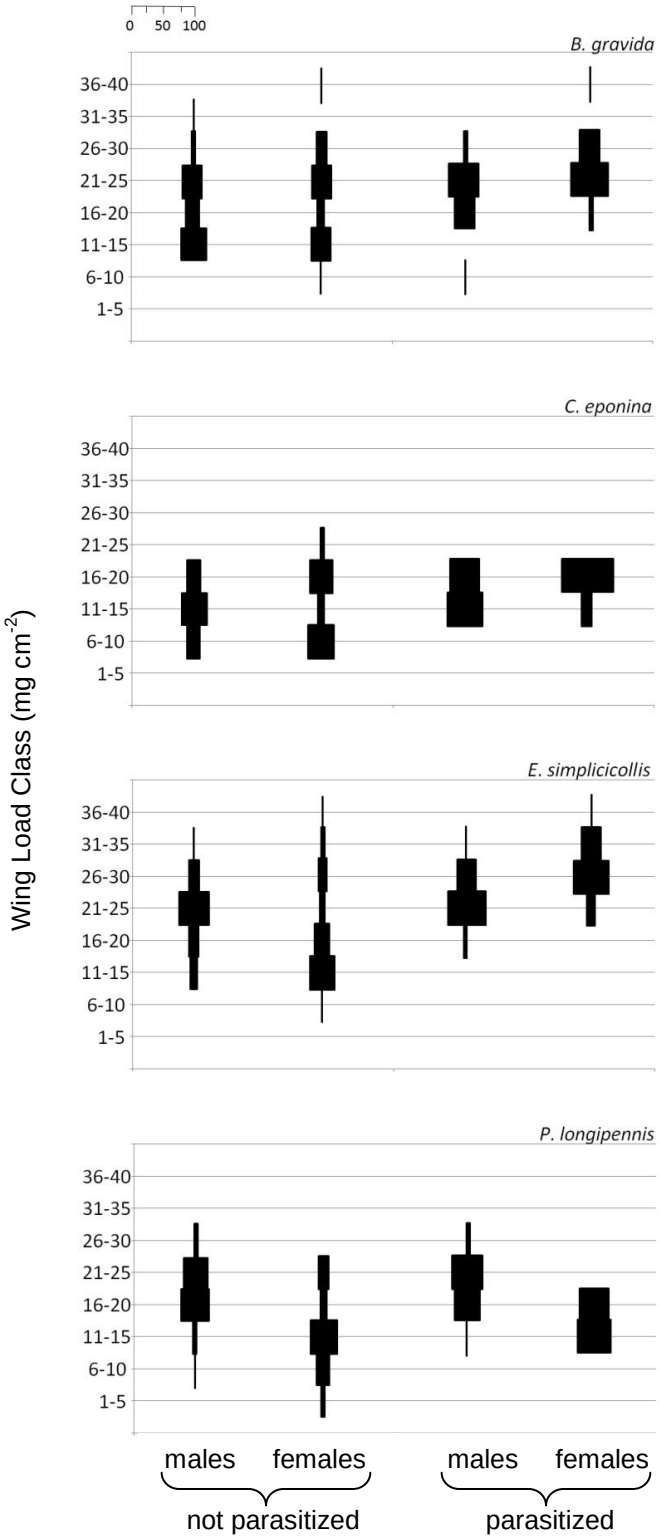

Figure 3.3: Distributions of wing loads (mg cm^{-2}) for infected and uninfected host species. The width of each bar represents the percentage of hosts in that wing load class.

variables as significant for intensity variation (host gender $P<0.005$; host species, month, and year $P<0.0001$).

Discussion

In this paper, I report the occurrence of gregarines in nine previously undocumented dragonfly host species. Of the confirmed host species (Table 3.1), only the naiad of *Anax junius* and adults of *B. gravida* and *E. simplicicollis* were previously reported as hosts (Clopton et al. 2007, Locklin and Vodopich 2009, Chapter 2). Moreover, the odonate species surveyed in this paper that lacked gregarines should be considered as non-hosts only tentatively since the number of individuals examined was relatively small (Jovani and Tella 2006). Notably, all species surveyed with collections greater than 14 individuals hosted a parasite in at least one adult.

Gregarines are aggregated in their host populations, i.e. most dragonfly individuals harbor low numbers of parasites while a few harbor many (Figure 3.1), and followed a negative binomial distribution (Wilson et al. 2002). Aggregation is by far the predominant pattern across natural host-parasite systems (Poulin 2006). Aggregated distributions may reveal population and evolutionary dynamics of a parasite and its host. Such distributions result from the degree and timing of exposure of host individuals to parasitic infective stages and to differences in a host's susceptibility and sensitivity to the infectious agent (Wilson et al. 2002). Wilson et al. (2002) identified three major implications of aggregated distributions. First, to best estimate parasite prevalences and intensities in populations with aggregated parasite distributions, many hosts should be examined (Jovani and Tella 2006). Second, aggregated distributions present problems for data analyses that assume normality. Such data must either be transformed or

analyzed with nonparametric tests. Finally, aggregation suggests that the impact of parasites is likely intensity-dependent. Individuals with the highest parasite intensity, i.e. those at the tail of the distribution (see Figure 3.1), are relatively few in number but are subject to the greatest impact (Wilson et al. 2002). Few hosts have high parasite counts because eugregarines lack proliferation phases in the host (Rodriguez et al. 2007). It follows that eugregarine infection often has no apparent impact on fitness or obvious effect on the host (see Rodriguez et al. 2007). Low parasite intensity in most hosts may reduce detection of negative impacts of eugregarines infections. For example, a decrease in fat absorption by damselflies was only detected at high eugregarine intensities (Siva-Jothy and Plaistow 1999). Similarly, damselfly alimentary canals develop lesions when gregarine numbers exceed 100, allowing pathogenic bacteria into the hemocoel via a ruptured midgut (Åbro 1974). This low impact associated with low parasite numbers contributes to ambiguity classifying some gregarine species as parasites or commensals.

Parasite prevalence and intensity vary among dragonfly species and sometimes between genders (Åbro 1996, Canales-Lazcano et al. 2005, Hecker et al. 2002, Lajeunesse et al. 2004, Lejeunesse 2007) because each species and gender differs in their life history, physiology, and behavior (Zuk and McKean 1996). Specific differences may increase or decrease the exposure and ingestion of gregarine oocysts or alter the tolerance to excysted and attached trophozoites. Locklin and Vodopich (2009) (Chapter 2) reported gender biases in gregarine prevalence for *E. simplicicollis* (male bias) and *B. gravida* (female bias) from BL and TCR in 2007, but those biases were demonstrable in 2008 only when immature and mature individuals were considered together. In 2008 when maturity was considered, analysis revealed that parasite prevalence was not biased

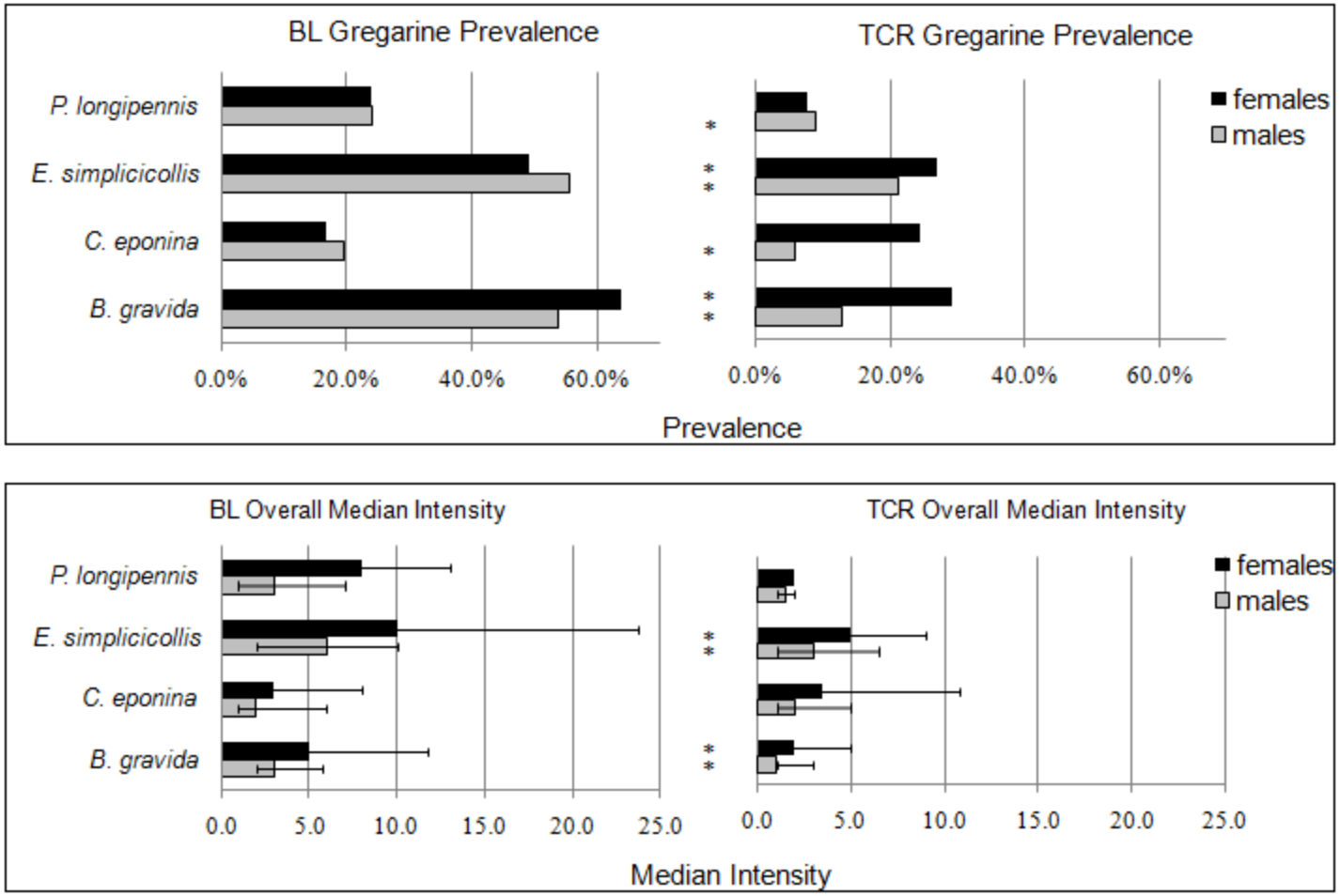

Figure 3.4: Gregarine parasite prevalence (top) and median intensity (bottom) at Battle Lake (BL) and Tradinghouse Creek Reservoir (TCR). Prevalence and median intensities are based on all specimens of each species caught. * indicates significance at $P<0.05$. Error bars represent the interquartile ranges (IQR)

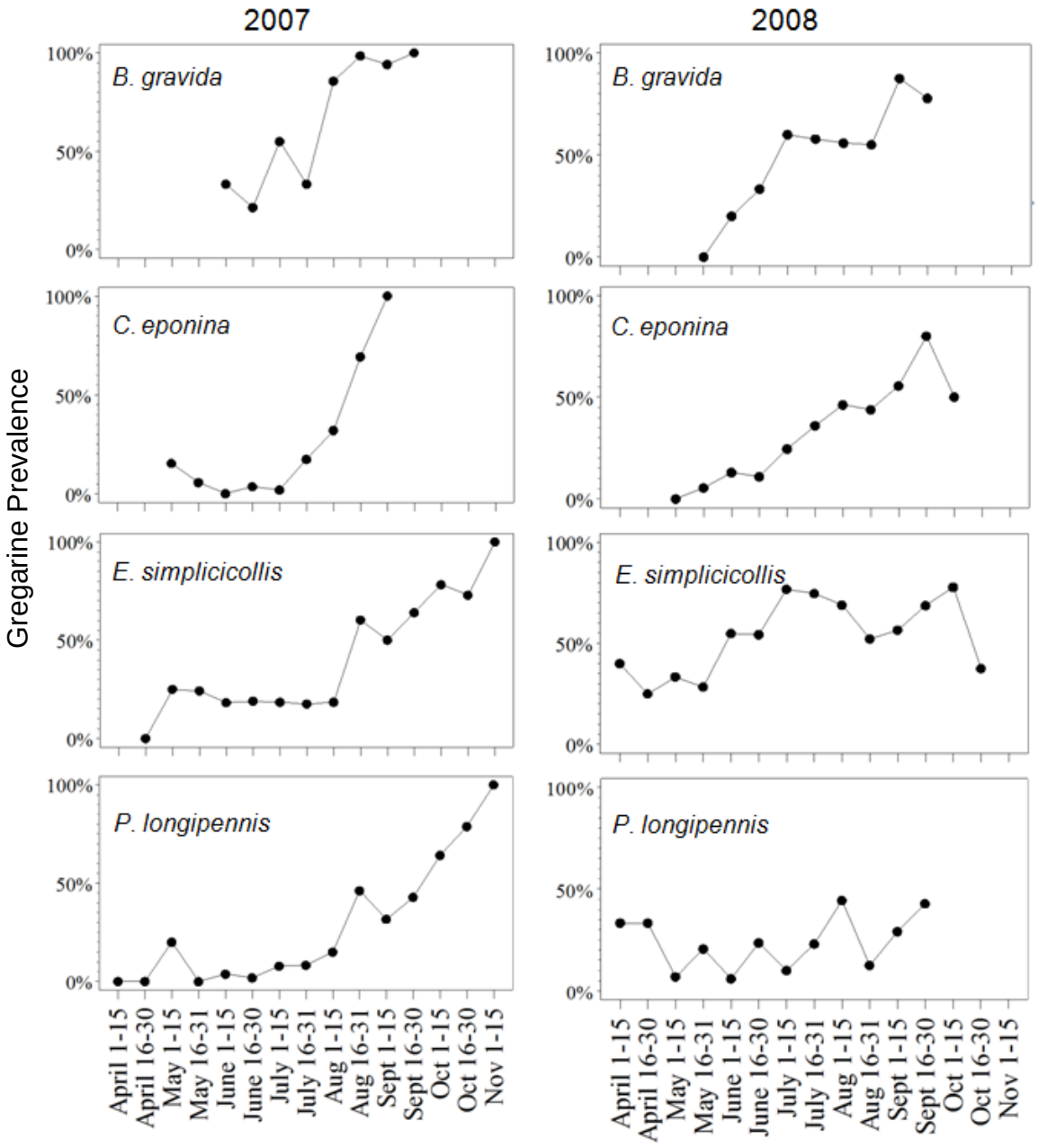

Figure 3.5: Seasonal patterns of gregarine prevalence in selected dragonfly hosts collected at Battle Lake (BL) in 2007 and 2008. Data for November are not presented for some species due to low sample size.

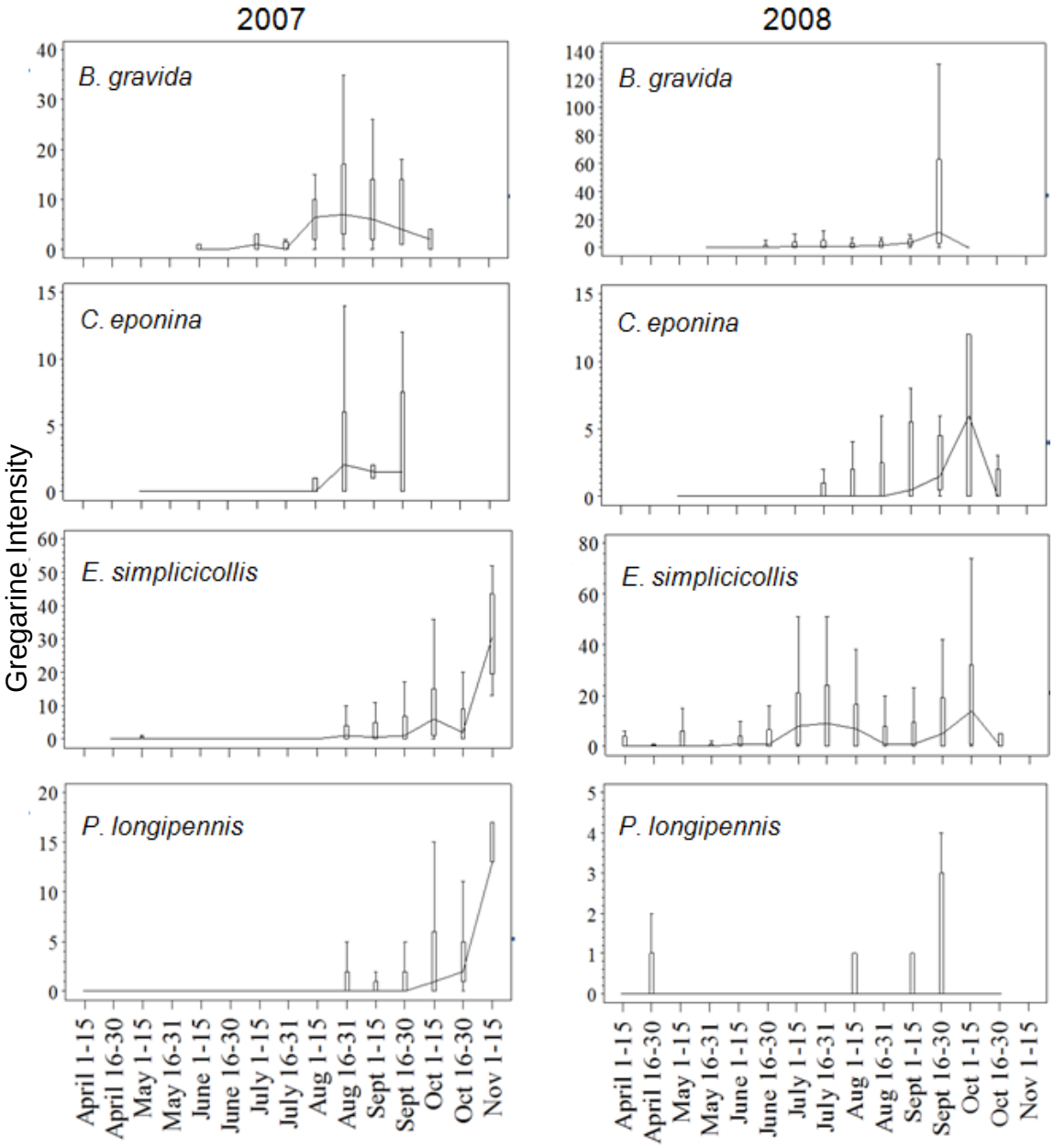

Figure 3.6: Seasonal patterns of gregarine intensity in selected dragonfly hosts collected at Battle Lake in 2007 and 2008: median (solid black line), interquartile range (boxed), and range (enclosed by lines) of intensity.

Table 3.3: Prevalence and sample size (n) among dragonfly adults exceeding wing load thresholds for four abundant dragonfly species from Battle Lake, April-October, 2008. Data for November are excluded due to low sample sizes.

Species	April	May	June	July	Aug	Sept	Oct
Brachymesia gravida							
Prevalence	-	0%	49%	74%	81%	67%	-
n	-	2	39	158	21	3	-
Celithemis eponina							
Prevalence	-	19%	15%	37%	59%	40%	-
n	-	32	72	70	17	5	-
Erythemis simplicicollis							
Prevalence	32%	50%	68%	87%	88%	69%	68%
n	28	96	114	192	82	233	24
Pachydiplax longipennis							
Prevalence	-	33%	17%	19%	20%	16%	67%
n	-	21	59	26	30	25	6

between genders of mature *E. simplicicollis* and *B. gravida*. The 2007 biases were likely due to considering all individuals of *E. simplicicollis* and *B. gravida*, not just mature individuals (i.e. those exceeding wing load thresholds). This finding emphasizes the importance of adequately estimating maturity/age of hosts when analyzing parasite distributions in populations. Unfortunately dragonfly age is difficult to assess, but some measure of relative maturity, such as wing load, should be applied in future gregarine surveys.

The absence of gregarines in reared tenerals suggests that gregarine parasites are not transferred from the naiad to adult stage during emergence or are stage-specific for their host's life cycle. Because the cuticle (intima) in the foregut and hindgut is shed during ecdysis (Triplehorn and Johnson 2005), trophozoites may be eliminated during that process. Emergence from naiad to adult could rid the host of its gregarine parasites. Whether the same gregarine species infect dragonfly naiads as well as adults is unknown. Gregarines infecting dragonflies may be stage-specific with distinct niches corresponding to naidal and adult stages of the hosts. Gregarines infecting *Tenebrio molitor* (Coleoptera) were reported to be stage-specific, i.e. four gregarine species infect larvae and a fifth species infects adults (Clopton et al. 1992). More gregarine-odonate surveys and taxonomic work are clearly needed.

Gregarine-infected dragonflies had higher wing loads than uninfected hosts when immature adults are included (Figure 3.3). However, because tenerals are not infected and parasitism is not apparent until after a period of maturation, I compared wing loads of mature infected with mature uninfected individuals. The distribution of wing loads of infected individuals did not significantly differ from those of uninfected individuals

(Table 3.2), suggesting that parasitism does not affect wing load. Gregarine parasitism appears to neither increase nor decrease weight of the host.

Habitat-specific environmental conditions can affect the transmission of parasites through host populations (Amano et al. 2008, Cáceres et al. 2006, Halmetoja et al. 2000, Lafferty and Kuris 2005, Morley 2007). I did not measure environmental parameters at either location, but the degree of variation in parasite prevalences and intensities found between BL and TCR populations (Figure 3.4) is surprising since the lakes are close to one another. Environmental parameters (i.e. degrees of lake stratification, water depth, temperature, etc.) that might affect the distribution, availability, and viability of infective oocysts could contribute to the degree of parasitism differences between populations. Therefore, infection level may be a function of the environment as well as species-specific physiologies and behaviors.

Host population densities may also contribute to variation in levels of parasitism at different locations. Hosts at high densities may be at greater risk of acquiring parasites due to (1) increased contact rates between hosts, and/or (2) an increased concentration of infectious life stages released by hosts. The latter increases the opportunity for uninfected hosts to encounter infectious material (Lindsey et al. 2009, Schmid-Hempel 1998, Steinhaus 1958). Vezzani and Wisnivesky (2006) reported that gregarine prevalence differed between conspecific mosquito populations in Argentina and suggested that high prevalence depended on host abundance. In this gregarine-odonate system, the large number of infective oocysts shed by hosts at BL should mirror high host density. Similarly parasite prevalence and intensity should positively correlate with host

density. Dense host populations may explain the high level of gregarine parasitism at BL compared to TCR.

The influence of host density on parasite intensity may underlie two antagonistic hypotheses describing host investment in resistance. The density-dependent prophylaxis hypothesis (Wilson and Reeson 1998) predicts that as host densities increase and, consequently, are more exposed to infective stages of the parasites, energy allocation towards parasite resistance (immunological and/or behavioral) will increase. Thus, greater parasite resistance (prophylaxis) counters the risk of parasite transmission at high host population densities. Conversely, the second hypothesis predicts that dragonflies living in high densities compete intensely for resources (mates, food, territories, etc.). This intense competition leads to physiological and/or nutritional stress increasing their susceptibility to parasitism. Less crowded populations can invest more energy towards resistance than those in more crowded conditions because less crowded populations are likely in better physical condition and can better resist parasites. Data from dragonfly populations at the two locations support the latter hypothesis, known as the crowding-and-stress hypothesis (Steinhaus 1958). However, experimental work and more comparisons, especially among sites with varying degrees of host densities, are needed.

Gregarine parasitism exhibited strong seasonality at BL. Two possible explanations for low parasite levels at the beginning of the season and high levels towards the end are oocyst viability and/or oocyst accessibility to hosts. Adult dragonflies were present from April-November of both years, but adults flying from September-November had highest probabilities of infection (Figure 3.5) and carried more

parasites (Figure 3.6). This pattern suggests that dragonfly exposure to viable and accessible gregarine oocysts increased with the season.

Although the longevity of viable gregarine oocysts infecting odonates is unknown, the seasonal results suggest that oocyst viability is likely short-lived. I speculate that during the flight season, infection increased as infected hosts released viable oocysts into the environment that were then ingested by more hosts. As the number of infected hosts increased and continued to release viable oocysts, host ingestion of these infective stages increased and resulted in increasing levels of gregarine prevalence and intensity and more release of oocysts. Towards the end of the season when host populations declined, this positive feedback cycle ended. When adult dragonflies were absent (December-March), only oocysts were present at BL (assuming that naidal and adult dragonflies are parasitized by different gregarine species). Colonization of the parasites in host populations the following season appears to depend on viable oocysts that survived the winter. Because prevalence and intensities remained low for many weeks after adult dragonflies emerged, most oocysts for ingestion may have been non-viable. Only oocysts shed late in the previous flight season (i.e. October or November) may have remained viable in the spring; those shed earlier in the season did not. In this scenario, only when newly infected hosts began releasing and thus increasing the concentration of viable oocysts did parasite prevalence and intensity increase to initiate the positive feedback cycle.

Another possible explanation for seasonality in prevalence and intensity is variation in oocyst accessibility to the host. Oocysts are ingested as hosts feed on insects carrying the oocysts on their bodies (Åbro 1976) and/or drinking oocyst-contaminated

water. Seasonal differences in the vertical or horizontal distributions of oocysts in the water column will likely affect host infection because accessibility of oocysts may change. For example, oocysts concentrations in surface waters may increase after rainfall as oocysts in the watershed wash into the lake (Muchiri et al. 2009). Seasonal emergence patterns of aquatic insects carrying oocysts may also affect prevalence and intensity in predatory hosts. As prey insects harboring oocysts emerge, oocysts accessibility to dragonflies may increase and result in an increase of gregarine parasitism. Temperature regimes of the lake may also cause unequal oocyst distributions in the water column. In a summer-stratified lake, oocyst concentrations may increase in the epilimnion as infected hosts release oocysts and uninfected adult dragonflies encounter them as they drink water. Much of this remains speculative because the distribution of gregarine oocysts in water columns has not been well studied. Development of molecular techniques for detection of gregarines, as done for *Cryptosporodium* (Muchiri et al. 2009), will facilitate investigations on oocyst distributions in the environment.

Variation in gregarine infection of odonate populations offers many opportunities for research. Because this system for parasitism is influenced by host, habitat, and time, its analysis reflects broad principles of population, ecological, and evolutionary research. However, taxonomic and systematic work is needed for odonate-infecting gregarines. The poorly-known taxonomy of gregarine fauna in dragonflies hinders analysis of their distributions among hosts and their impact on dragonfly fitness. We know little, for example, about host specificity in this system. Does a gregarine species infect one or multiple host species? Similarly, do dragonfly species serve as hosts to one or more gregarine species? These specific questions remain to be addressed, and our study

emphasizes the importance of season, hosts, and habitats for understanding gregarine and dragonfly ecology.

Acknowledgments

I thank Dave Cummings, Josh Huckabee, Sal LaMastra, Justin Clanton, Victoria Prescott, Isis Domingues, Edgar Alvarez, and Tamara Strohm for assistance in the lab and field. Terry Austin and Christie Finch assisted with graphs and Tamara Cook identified the gregarines. This research was funded by a Texas Academy of Science research grant and a Temple College Faculty Leave Development grant.

CHAPTER FOUR

Eugregarine Parasitism of *Erythemis simplicicollis* (Anisoptera: Libellulidae) at a Constructed Wetland: A Fitness Cost to Females?

Abstract

Eugregarine parasites infect a wide variety of invertebrates. Some authors suggest that eugregarines are rather harmless, but recent studies suggest otherwise. Among odonate-eugregarine investigations, damselflies have been more frequently studied than dragonflies. We surveyed adult dragonfly populations for eugregarines at a constructed, flow-through wetland system and assessed the fitness cost of infection in a common and widespread dragonfly host species, *Erythemis simplicicollis* (Libellulidae). Populations were sampled weekly through the flight season. Host fitness parameters measured included wing load, egg size, clutch size, and total egg count. Of the 22 host species surveyed, eight hosted eugregarines and two of these dragonfly species were previously undocumented as hosts. While eugregarine parasitism has been shown to exhibit seasonality previously, parasite prevalence and intensity in *E. simplicicollis* in this study showed no seasonal trend. The fitness parameters measured were not correlated with the presence or intensity of eugregarines. These findings suggest that either eugregarines do not affect wing loading and egg production in *E. simplicicollis*, or that virulence depends on parasite intensity and/or the specific eugregarine species infecting the hosts.

Introduction

Dragonflies and damselflies are excellent models for studying biological processes prominent in ecological, evolutionary, and behavioral studies (see Córdoba-Aguilar and Cordero-Rivera 2005). They also host a variety of ecto- and endoparasites, most of which influence their hosts' fitness (Bonn et al. 1996, Canales-Lazcano et al. 2005, Córdoba-Aguilar 2002, Córdoba-Aguilar et al. 2003, Marden and Cobb 2004, Reinhardt 1996, Rolff et al. 2000). Eugregarine-odonate investigations have focused primarily on damselfly hosts (Åbro 1971, Åbro 1976, Canales-Lazcano et al. 2005, Clopton 2004, Cordoba-Aguilar 2002, Cordoba-Aguilar et al. 2003, Hecker et al. 2002, Siva-Jothy 2000, Siva-Jothy and Plaistow 1999, Tsubaki and Hooper 2004,) with relatively few studies on dragonfly hosts (Clopton et al. 2007, Locklin and Vodopich 2009, Locklin and Vodopich 2010, Marden and Cobb 2004, Schilder and Marden 2006).

Nine genera of eugregarines (Apicomplexa: Eugregarinorida: Actinocephalidae) have been identified as odonate parasites: *Actinocephalus*, *Calyxocephalus, Domadracunculus, Geneiorhynchus, Hoplorhynchus*, *Mukundaella, Nubenocephalus, Prismatospora*, and *Steganorhynchus* (Clopton et al. 1993, Clopton 1995, Clopton 2004, Hays et al. 2007, Percival et al. 1995, Richardon and Janovy 1990, Sarkar 1997). All are monoxenous eugregarines that infect their hosts when odonates ingest oocyst-contaminated water and/or insect prey phoretically carrying oocysts on/in their bodies (Åbro 1976). Excysted sporozoites attach to the odonate's intestinal epithelium as trophozoites, absorb nutrients, mature, and detach as gamonts. Two gamonts fuse to form a gametocyst that passes out of the host with feces. Within the gametocyst, hundreds of gametes form and fuse to produce the infective oocysts. To complete the life

cycle, oocysts are then released into the environment as the gametocyst ruptures (Bush et al. 2001, Roberts and Janovy 2005).

Eugregarine parasites have been historically viewed as relatively harmless (Bush et al. 2001), but some studies have shown that the cost of eugregarine infections involves effects on fecundity and mortality of invertebrates (see Smith and CLOPTON 2003). Among odonate studies, eugregarines have been shown to reduce longevity (Canales-Lazcano et al. 2005, Córdoba-Aguilar et al. 2003, Hecker et al. 2002, Tsubaki and Hooper 2004), reduce fecundity (Canales-Lazcano et al. 2005, Córdoba-Aguilar et al. 2003;), influence mating success (Córdoba-Aguilar et al. 2003), impair flight-muscle performance (Schilder and Marden 2006), hinder the ability to maintain territories (Córdoba-Aguilar 2002, Marden and Cobb 2004), reduce fat content (Siva-Jothy and Plaistow 1999), and impair fat oxidation in flight muscles (Marden and Cobb 2004).

This study was designed to (1) survey dragonfly populations for eugregarine parasitism, (2) determine the prevalence and intensity patterns through a flight season at a constructed wetland system, and (3) investigate impacts of eugregarine parasitism on fitness parameters including wing load, egg size, total egg count, and clutch size in a common and widespread host, *Erythemis simplicicollis* (Libellulidae).

Materials and Methods

This study was conducted at the Lake Waco Wetland (LWW), TX, USA (31º60’88N, 97º30’69W). The wetland was constructed in 2001 as habitat mitigation for a 2-m pool rise of nearby Waco Lake. The 80-ha wetland receives pumped water from the North Bosque River and routes the flow through five sequential wetland cells before returning it 5-10 days later to the river that feeds Waco Lake (Scott et al 2005).

Adult dragonfly populations were sampled weekly from May-October 2009 (=flight season). Individuals were netted within 15 m of the shorelines, taken to the lab, killed with ethyl acetate, identified (Abbott 2005), dorsally scanned at 600 dpi (Mitchell and Laswell 2000), and stored in 70% ethanol. To survey for parasites and determine their prevalence (percentage of individuals infected) and intensity (number of parasites per infected individual), preserved abdomens were placed ventral-side up on a Styrofoam tray and dissected. The abdomens were split longitudinally and pinned to expose the crops and intestines. Parasites (trophozoites and gamonts) that were visible through the intestinal epithelium were counted.

I calculated monthly eugregarine prevalences and intensities in *Erythemis simplicicollis* through the flight season and investigated potential impacts on parameters relating to species fitness, i.e. changes in wing load, egg size, total egg count, and clutch size. I define clutch size as the number of eggs laid (Watanabe and Matsu'ura 2006) during induced oviposition (see methods below). Total egg count is the sum of eggs released during induced oviposition and the eggs retained in the abdomen. Egg viability was not tested. In adult dragonflies, eugregarines are not visually detectable in recently emerged tenerals (Locklin and Vodopich 2010, Chapter 3). Only mature host individuals are candidates for answering questions about parasite prevalence and intensity. I used wing loads (mg body wt x wing surface area^{-1}) as a surrogate for maturity of *E. simplicicollis*. Minimum wing load values for parasitized *E. simplicicollis* (♂ = 18.0 mg cm^{-2}; ♀ = 21.8 mg cm^{-2}) were used to characterize mature adults (Locklin and Vodopich 2010, Chapter 3). To calculate wing loads, total wing surface area of each *E. simplicicollis* was estimated by measuring the right hind wing length (mm) from the

second axillary sclerite to the wing tip using Adobe Photoshop and regressing total wing surface area, *y*, using the following equations:

♂ $y = 0.470x - 5.94$ (r^2=0.92, 18 d.f., p <0.001)

♀ $y = 0.450x - 4.66$ (r^2=0.83, 23 d.f., p <0.001),

where *x* is hind wing length (mm).

To assess measures of fitness, females were captured in the field and eggs were immediately collected by inducing oviposition (Susa and Watanabe 2007). The tip of each female's abdomen was repeatedly dipped vertically into a 7-mL vial of wetland water once per second until she stopped releasing eggs. If no eggs were released initially, then dipping continued for three minutes to ensure that she had no eggs to release or that she was unwilling. The females were preserved. The released eggs were preserved in 50% ethanol and later counted at 60X. Eggs retained in abdomens were also counted after dissection. The females caught for analysis of clutch size and total egg count were captured mid-morning presumably before daily ovipositing began.

To measure egg sizes, females were induced to oviposit and the lengths and widths of ten randomly selected eggs from each female were measured (mm) using an ocular micrometer mounted in a compound microscope. Egg circumference, *C*, was calculated using a formula for ellipse perimeter:

$$C = \pi[1.5(a + b) - ((a \times b)^{½})]$$

where a = 0.5 x length and b = 0.5 x width (Schenk and Söndgerath 2005).

Results

Twenty-two dragonfly species (n = 1,378) were collected at the wetland, and eight species were parasitized by eugregarines (Table 4.1). Three of the unparasitized species

collected (Table 4.2), *Anax junius* (Aeshnidae), *Epitheca princeps* (Cordulliidae), and *Tramea lacerata* (Libellulidae), have been previously reported to host eugregarines (Clopton et al. 2007, Locklin and Vodopich 2010, Chapter 3). Euregarines were tentatively identified as members of the genera *Actinocephalus* and *Geneiorhynchus* (Eugregarinorida: Actinocephalidae).

Monthly eugregarine prevalences in mature *E. simplicicollis* showed no seasonal trend through the flight season (Freedman's test, $V_N = 0.07$, p = NS) (Figure 4.1). Eugregarine intensities were not normally distributed among hosts (Shapiro-Wilk test, $p < 0.001$), therefore nonparametric analyses were used to assess intensity data. Monthly median intensities ranged from 1.0 – 3.5 eugregarines in *E. simplicicollis* (Figure 4.1) and the maximum intensity of 60 eugregarines occurred in a male *E. simplicicollis*.

Wing loads among mature individuals of *E. simplicicollis* were not affected by the presence/absence or intensity of eugregarines. Median male wing loads (mg cm^{-2} with IQR) of infected versus uninfected individuals, respectively, were 25.6 (24.6 – 27.3) and 25.3 (23.8 – 27.0) and in females were 30.9 (28.6 – 33.6) and 29.9 (27.1 – 32.4). No difference was detected between median wing loads of infected versus uninfected males (Wilcoxon Rank Sum Test, Z = -1.36, p = 0.173) or females (Z = -2.36, p = 0.18). Likewise, wing load did not correlate with eugregarine intensity in males (N = 189, Spearman's correlation, $r_s = 0.37$, p = 0.79) or females (N = 288, $r_s = 0.01$, p = 0.99) (Figure 4.2).

Egg size, total egg count, and clutch size of *E. simplicicollis* were not correlated with the presence/absence or intensity of eugregarines. Of the females captured for egg analyses, 88% were gravid and 57% of these gravid females released one or more eggs

Table 4.1: Dragonfly species (all members of Libellulidae) infected with eugregarine parasites at the Lake Waco Wetland. * indicates newly reported host. N = sample size, Prev = gregarine prevalence, Med Int = median gregarine intensity, IQR = interquartile range of intensity, Max Int = maximum gregarine intensity

Species	N	Prev (%)	Med Int	IQR	Max Int
Brachymesia gravida	1				
Males	1	100	11	–	11
Females	0	–	–	–	–
Celithemis eponina	67				
Males	34	9	2	1-9	9
Females	33	3	3	–	3
Erythemis simplicicollis	881				
Males	350	27	2	1-5	60
Females	531	32	2	1-4	55
*Libellula incesta**	3				
Males	1	0	–	–	–
Females	2	50	1	–	1
Libellula luctuosa	11				
Males	7	14	1	–	1
Females	4	0	–	–	–
*Pantala flavescens**	52				
Males	20	0	–	–	–
Females	32	3	1	–	1
Pachydiplax longipennis	134				
Males	108	12	2	1-2	4
Females	26	12	1	1-2	2
Perithemis tenera	64				
Males	41	10	2	1-6	7
Females	23	22	3	1-4	4

Table 4.2: Dragonfly species collected at the Lake Waco Wetland that did not host eugregarine parasites. * indicates a previously reported host species

Family	Species	n
Aeshnidae	*Anax junius**	27
	Males	24
	Females	3
Corduliidae	*Epitheca princeps**	1
	Males	1
	Females	0
Gomphidae	*Arigomphus submedianus*	9
	Males	5
	Females	4
	Dromogomphus spoliatus	10
	Males	5
	Females	5
	Gomphus militaris	18
	Males	13
	Females	5
	Stylurus plagiatus	2
	Males	2
	Females	0
Libellulidae	*Dythemis nigrescens*	4
	Males	4
	Females	0
	Libellula comanche	1
	Males	1
	Females	0
	Libellula vibrans	1
	Males	1
	Females	0
	Orthemis ferriginea	2
	Males	1
	Females	1
	Pantala hymenaea	26
	Males	13
	Females	13
	Plathemis lydia	45
	Males	21
	Females	24
	*Tramea lacerata**	17
	Males	12
	Females	5
	Tramea onusta	2
	Males	2
	Females	0

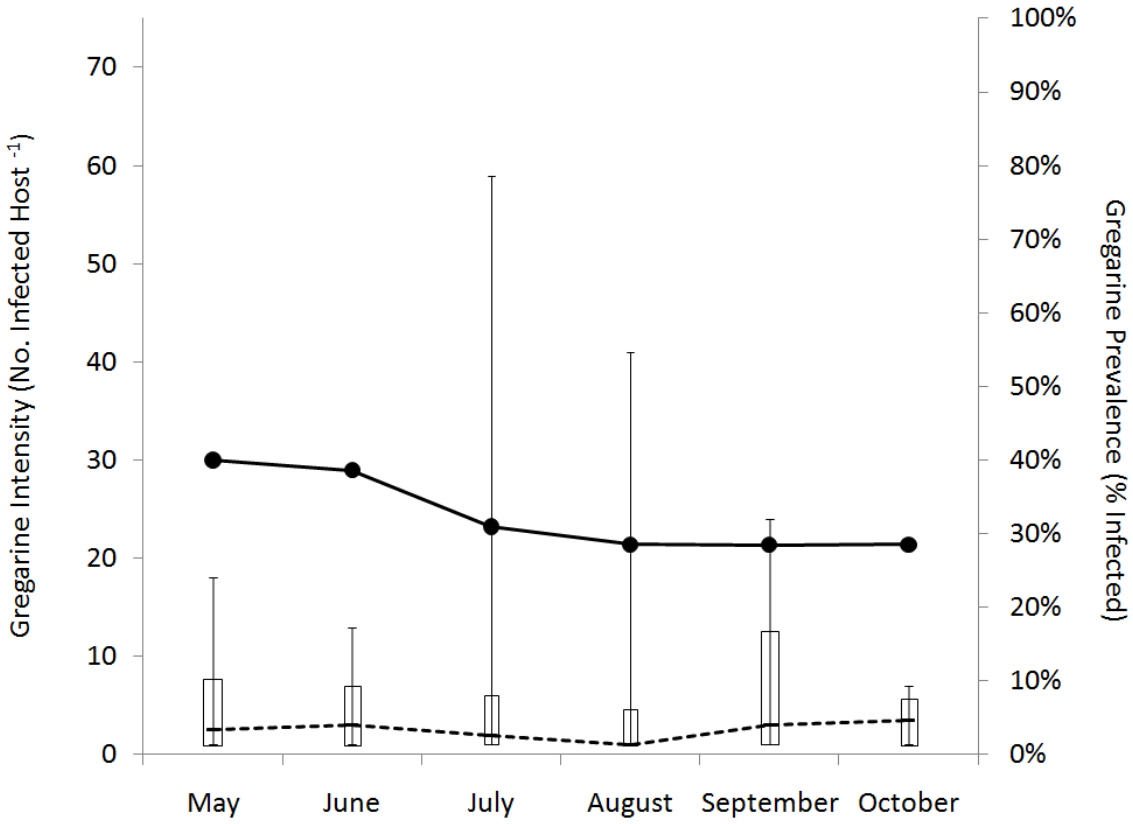

Figure 4.1: Median gregarine intensity (dashed line) and gregarine prevalence (solid line) in *E. simplicicollis* through the odonate flight season at the Lake Waco Wetland, Texas, USA, 2009. Intensity data include interquartile range (boxed) and range (extended bars).

into the glass vials. Egg sizes for female *E. simplicicollis* ranged from 1.2 – 1.4 mm, but the presence of eugregarines had no effect on mean egg size. Mean egg sizes from infected versus uninfected females did not differ (t = 1.41, p = 0.17, N = 24), and egg size did not correlate with the intensity of infection (N = 24, r_s= -0.19, p = 0.37) (Figure 4.3). Total egg counts ranged from 0 – 1,611 eggs female^{-1}. Mean numbers of total eggs from infected versus uninfected females were not significantly different (t = -1.63, p = 0.11), and eugregarine intensity did not correlate with the total number of eggs (N = 84, r_s= 0.143, p = 0.193) (Figure 4.4). Clutch size data (Figure 4.5) included only females having eggs available to release. Mean clutch size was 199 eggs female^{-1} (range = 0 – 967). The presence/absence of eugregarines had no significant effect on whether or not a female released eggs (χ_1^2 = 0.168, p = 0.682). No difference was found between the mean clutch sizes of infected versus uninfected females (t = -0.741, p = 0.461). Clutch sizes did not correlate with eugregarine intensity (N = 74, r_s = 0.09, p = 0.44) (Figure 4.5).

Discussion

Of the eight parasitized dragonfly species collected at the wetland (Table 4.1), two (*Libellula incesta* and *Pantala flavescens*, Libellulidae) are reported as hosts for the first time in this paper, and six were reported previously to host eugregarines (Locklin and Vodopich 2009, Locklin and Vodopich 2010, Chapters 2 and 3). Those odonate species surveyed in Locklin and Vodopich (2010) (Chapter 3) and those in this paper that lacked eugregarines should be considered as non-hosts only tentatively because the number of individuals examined was relatively small (Table 4.2).

The lack of seasonal variation in parasite prevalence and intensity at LWW may be associated with water residence time. This wetland's continual flow and short

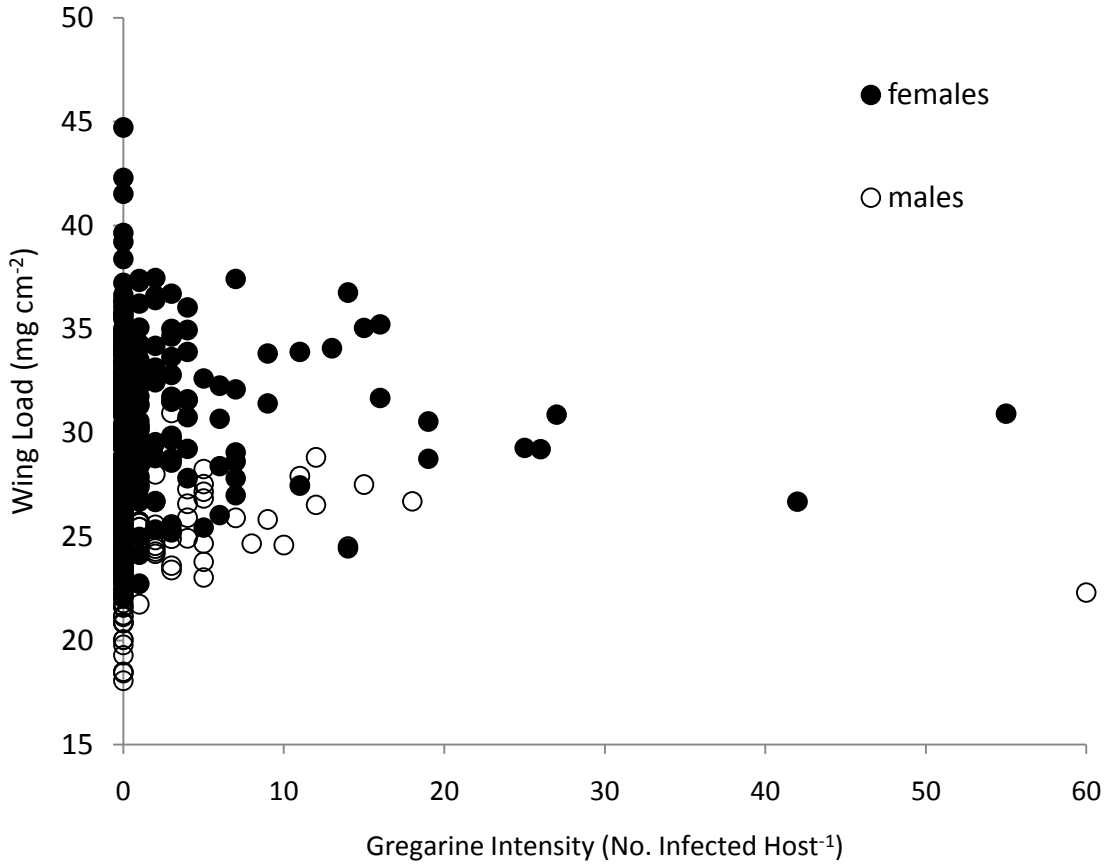

Figure 4.2: Gregarine intensity vs. wing loads of males (open symbols) and females (closed symbols) of *E. simplicicollis*. No correlation was detected in males (N = 189, r_s = 0.37, p = 0.79) or females (N = 288, r_s = 0.01, p = 0.99).

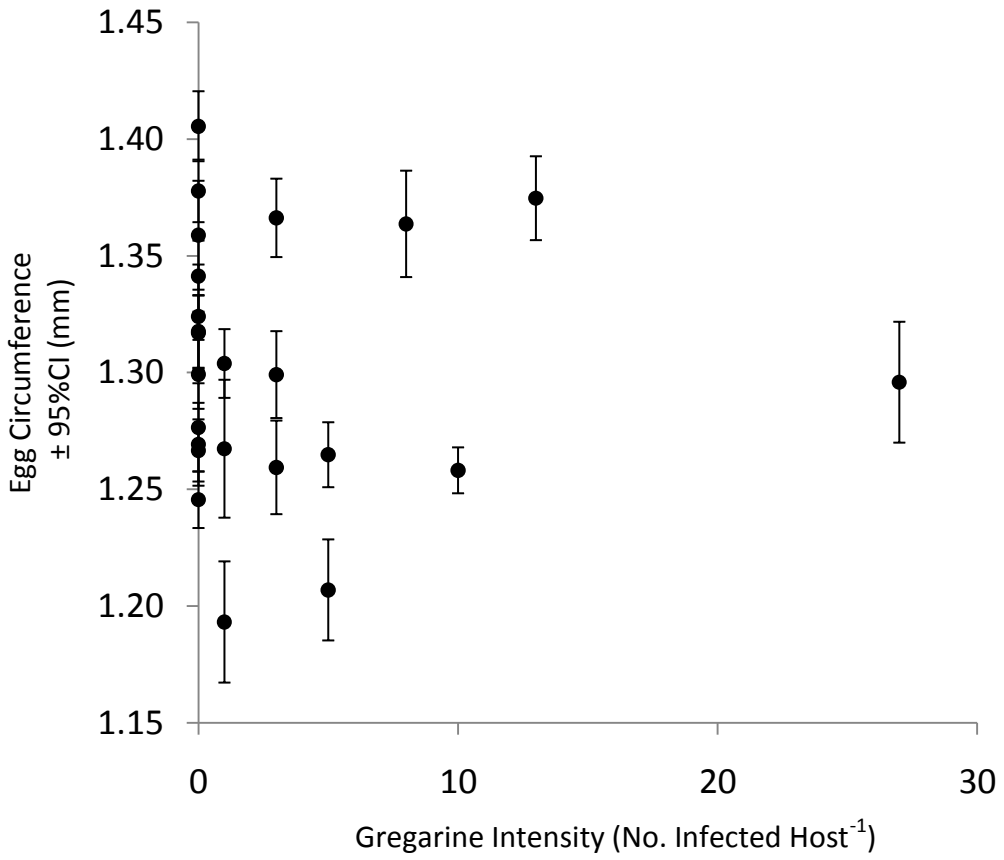

Figure 4.3: Gregarine intensity vs. egg circumference. Each data point represents the mean circumference (mm) of ten eggs from a female; error bars represent 95% CI. No correlation was detected (N = 24, r_s = -0.19, p = 0.37).

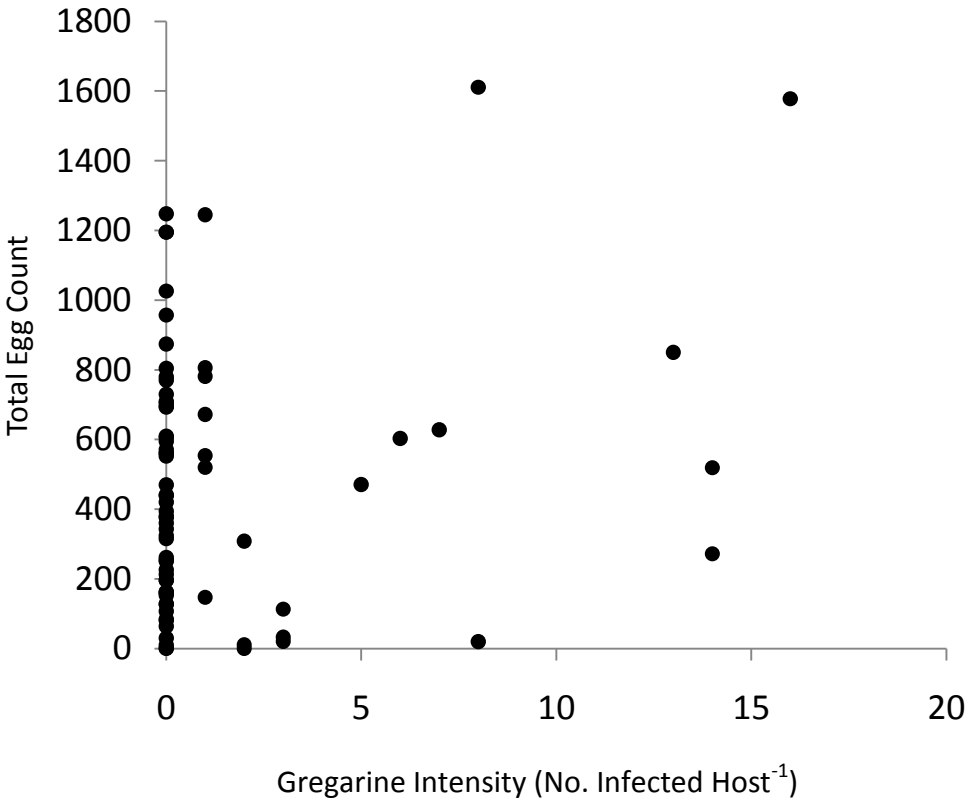

Figure 4.4: Gregarine intensity vs. total egg count. Total egg count includes all eggs released during induced oviposition plus retained eggs found during abdominal dissections. No correlation was detected (N = 84, r_s = 0.14, p = 0.19).

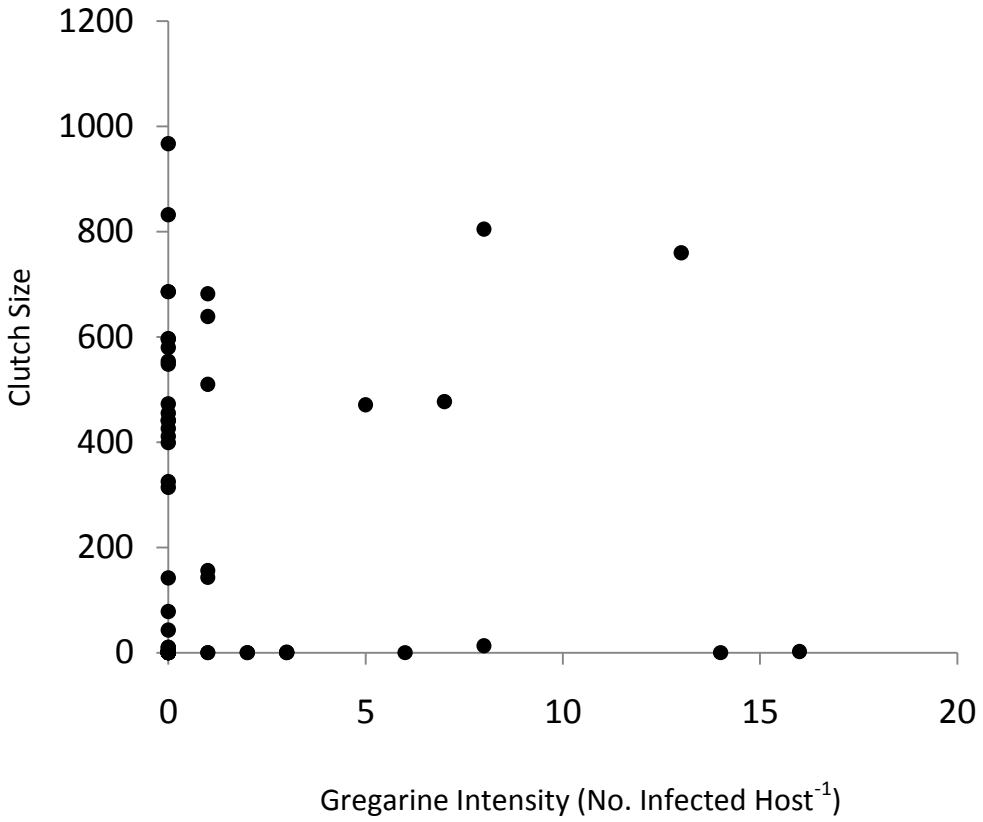

Figure 4.5: Gregarine intensity vs. clutch size. Clutch size data include only females with eggs to oviposit. No correlation was detected (N = 74, r_s = 0.09, p = 0.44).

residence time (5 – 10 days) likely dampen seasonal variation in oocyst density through time. Locklin and Vodopich (2010) (Chapter 3) found that eugregarine prevalence and intensity increased during the dragonfly flight season of each of two subsequent years in dragonfly populations at Battle Lake, a small nearby reservoir with a longer water residence time. In that study, I speculated that viable eugregarine oocyst concentrations and/or availability to hosts increased during the flight season and resulted in higher levels of infection towards the end of the season. In the current study, however, I detected no significant seasonality in eugregarine prevalence or intensity (Figure 4.1). I propose that oocysts shed by infected hosts are constantly being washed through the wetland into the nearby North Bosque River because of the wetland's brief water residence time. Consequently, a reduced but stable concentration of eugregarine oocysts is likely and would result in the relatively unchanged levels of eugregarine infections found in this study.

Local physical, chemical, and biological processes may also influence the distribution and longevity of viable eugregarine oocysts and foster the steady prevalence and intensity found at LWW compared to Battle Lake. Because the morphometry and flow regimes differ between the two aquatic systems, abiotic conditions (e.g. water temperature, dissolved oxygen levels, etc.) in each also likely differ (Kvarnäs 2001). The influence of these conditions on oocyst viability warrants further research. In an analogous system, much research has sought to detect and evaluate oocyst viability of the closely-related *Crypotosporidium parvum* (e.g., Call et al. 2001, Kato et al. 2002, Pokorny et al. 2002). Unfortunately, the degree to which eugregarine oocysts are

encountered by dragonflies in aquatic and/or terrestrial environments remains uncertain, and the factors influencing oocyst viability and longevity are still unknown.

Commonly, digestive-tract parasites reduce the host's ability to absorb nutrients and to accumulate and appropriately distribute fat (Siva-Jothy and Plaistow 1999). During maturation from teneral to reproducing adult, odonates acquire color and mass critical for mate selection, and build fat reserves for sustained flight and egg production (Córdoba-Aguilar and Cordero-Rivera 2005). Males distribute most of their fat in the thorax (Anholt et al 1991) to support sustained flight and maintain territories (Marden and Waage 1990, Plaistow and Siva-Jothy 1996). The initial moments of flight depend on carbohydrate oxidation and soon transition to lipid oxidation if flight is sustained (Schilder and Marden 2006). Siva-Jothy and Plaistow (1999) found that eugregarines reduced fat content in prereproductive males of the damselfly, *Calopteryx splendus xanthostoma* (Zygoptera: Calopterygidae). Schilder and Marden (2006) reported that eugregarines impaired fatty acid oxidation by flight muscles in infected males of the dragonfly, *Libellula pulchella* (Anisoptera: Libellulidae). Decreased fat content and/or impaired fat oxidation in infected odonates likely reduce male fitness because they hinder sustained flight.

Female odonates distribute more fat in the abdomen presumably for egg production (Anholt et al. 1991). If parasitized females have less fat content and/or an impaired fat metabolism, then I hypothesized that egg sizes, clutch sizes, and/or the number of eggs produced would be less among infected females than uninfected females. Córdoba-Aguilar et al. (2003) found a negative correlation between eugregarine intensity and egg production in the damselfly, *Calopteryx haemorrhoidalis*. Canales-Lazcano et

al. (2005) also found that eugregarine infection reduced egg numbers in female damselflies of *Enallagma praevarum*. However, I detected no correlation between parasite infection and the dragonfly egg parameters measured.

Parasites often have life history strategies that damage or impair the morphology and/or physiology of their host. But quantifying the fitness costs of such damage is difficult because the magnitude of host cost is determined by the damage a parasite causes (Siva-Jothy 1999). The lack of detectable virulence described in this study may be due to 1) relatively low eugregarine intensities in the population, and/or 2) variation of virulence associated with individual gregarine species.

The level of virulence a host experiences may depend on parasite intensity. Eugregarine (order Eugregarinorida) and neogregarine (order Neogregarinorida) infections, for example, manifest dramatically different impacts on their hosts – eugregarines generally do little harm (Rodriguez et al. 2007) whereas neogregarine infections often significantly impact their hosts negatively (Altizer and Oberhauser 1999, Bradley and Altizer 2005, Lindsey et al. 2009, Lord 2006). The difference in impact may stem from neogregarines proliferating vegetatively in the host while eugregarines do not. Specifically, neogregarines undergo multiple asexual divisions (merogony) after entering host's cells and the resulting merozoites spread and infect other tissues in that host. Eugregarine intensity, however, depends entirely on the number of oocysts ingested because they lack a vegetative reproductive stage. Consequently, eugregarine intensities tend to be lower than neogregarine intensities. Rodriguez et al (2007) suggested that unless parasite intensities exceed some threshold number, fitness impacts in the host may be negligible. Åbro (1974) reported that eugregarine intensities greater than 100 caused

lesions in the alimentary canals of infected damselflies which may have permitted entry of pathogens into the haemocoel. Unfortunately for our efforts to detect fitness cost, intensities were low in females of *E. simplicicollis* (median = 2, max = 55). Analyzing individuals with more intense infections may show that eugregarines can affect fitness of *E. simplicicollis* females with respect to egg production. However, Canales-Lazcano et al (2005) and Siva-Jothy and Plaistow (1999) found that eugregarines were associated with reduced egg numbers and fat content, respectively, in damselflies with relatively low intensities. This suggests that significant fitness costs do not depend exclusively on eugregarine numbers, at least for some odonate host species. Moreover, if intensity relates directly to fitness costs of *E. simplicicollis*, then most individuals will not show signs of parasitemia due to the nature of eugregarine reproduction (no merogony) and their aggregated distribution (i.e. a negative binomial distribution) across dragonfly populations. Most infected odonates have low parasite intensities (Locklin and Vodopich 2010, Chapter 3). As a consequence, detecting effects of intense eugregarine infections in natural dragonfly populations may prove difficult because relatively few hosts have high intensities.

Parasite virulence may also depend on the parasite species infecting a host. For example, *Entamoeba histolytica* and *E. dispar* are protozoan parasites that infect humans. These closely-related congeners are difficult to morphologically differentiate (Clark et al. 2006), but their virulence levels are significantly different. The former kills up to 100,000 people annually whereas the latter is a commensal (Diamond and Clark 1993; Roberts and Janovy 2005). Our understanding of species-level diversity among the eugregarine fauna infecting Odonata is progressing. Several species have been described

from nine eugregarine genera identified in odonates, although none of the adult dragonfly species that we surveyed in the current study or in Locklin and Vodopich (2010) (Chapter 3) were hosts identified in those descriptions. However, if odonate-infecting eugregarines exhibit strong host species- and/or stage-specificity, then many new eugregarine species await description. Furthermore, if virulence varies among eugregarine species and their host species, then conclusions on the fitness costs (or lack thereof) in this host may be strengthened when more is known about the specific eugregarine fauna infecting *E. simplicicollis*.

Acknowledgments

We thank Joshua Huckabee, Sal LaMastra, Tamara Strohm, Victoria. Prescott, and Austin Cunningham for assistance in the lab and field, and Tamara Cook for identifying the gregarines. This research was funded by a Texas Academy of Science research grant and a Temple College Faculty Leave Development grant.

CHAPTER FIVE

Conclusion

Although gregarine parasites have been considered rare in dragonflies (Clopton 2009), surveys at three different locations in central Texas found that gregarine parasitism of dragonflies can be common and intense. Of the 37 dragonfly species surveyed (N=7,264 individuals), 14 species hosted gregarines. Prior to this work, the naiad of *Anax junius* (Aeshnidae) was the only dragonfly reported in the literature to host gregarines (Clopton et al. 2007).

The dragonfly-gregarine system is highly variable. Poisson and logistic regression models revealed that dragonfly species, host gender, month, and year were significant explanatory variables associated with gregarine parasite prevalence and intensity. In addition, parasite infection significantly differed between habitat locations.

Within a host population, parasites were only found in individuals exceeding a wing load threshold. This has two major implications. First, parasites likely are not transferred from naiad to adult during emergence. Second, gregarine surveys should consider this maturity-bias and use some type of maturity surrogate (such as wing loads) because including all adults in a population, regardless of maturity, may bias analyses and lead to inappropriate conclusions regarding patterns of parasitism in host populations (i.e. gender biases). Gregarines were also highly aggregated among hosts. Consequently, most infected individuals will harbor few parasites.

Strong seasonality in prevalence and intensity was apparent at Battle Lake but not at Lake Waco Wetland. Two factors likely influence this pattern: host density and

environment. First, host density (estimated to be greater at Battle Lake compared to Lake Waco Wetland) likely increases parasite abundance and transmission rates through host populations. Potential hosts at high densities may be at greater risk of acquiring parasites due to an increased concentration of infectious life stages (oocysts) released by infected hosts. This increases the opportunity for uninfected hosts to encounter infectious material. Second, the environment likely plays a significant role in the seasonal increase of parasitism at Battle Lake and not Lake Waco Wetland. Because Lake Waco Wetland is a flow-through system, oocysts released by infected host are likely flushed through it resulting in reduced but stable infection rates at the wetland. Oocysts released at Battle Lake, in contrast, remain in the system and have a higher probability of being ingested by a potential host.

The fitness parameters measured in this study were not affected by the presence/absence of gregarines in their hosts, and parasite intensities did not correlate with the fitness parameters measured for dragonflies at Lake Waco Wetland. Some authors suggest that the cost of infection depends on parasite intensity, but no such relationship along the intensity gradient was detected in this study. This may be due to the reduced scale of infection intensities found in dragonflies of the wetland, i.e. intensities were relatively low at the wetland compared to Battle Lake. Greater gregarine intensities may in fact result in a greater fitness cost for hosts, but most parasitized individuals in the population should not show measurable fitness costs because gregarines are aggregated among their dragonfly hosts.

Gregarine parasites infect a wide variety of invertebrates. Most studies investigating odonate-gregarine interactions have focused on damselflies. However,

dragonflies can also be frequently infected. The results of this research indicate much variability in this host-parasite system (among and within host populations across time and locations). Consequently, this system has great potential for research opportunities. The three studies herein emphasize the importance of season, hosts, and habitats for understanding gregarine and dragonfly ecology.

APPENDICES

APPENDIX A

Peer-Review Publications Derived From This Research

Chapter Two

Locklin, J. L. and D. S. Vodopich. 2009. Bidirectional gender biases of gregarine parasitism in two coexisting dragonflies (Anisoptera: Libellulidae). Odonatologica 38: 133-140.

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Chapter Three

Locklin, J. L. and D. S. Vodopich. 2010. Patterns of gregarine parasitism in dragonflies: host, habitat, and seasonality. Parasitology Research *in press.*

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Chapter Four

Locklin, J. L. and D. S. Vodopich. *In-Review*. Eugregarine parasitism of *Erythemis simplicicollis* (Anisoptera: Libellulidae) at a constructed wetland: a fitness cost to females? Odonatologica.

APPENDIX B

Table B.1: Battle Lake Water Temperature Data. Data collected hourly from 1 Feb – 31 Dec, 2008 (HOBO® U22 Water Temp Pro v2) and is summarized by month.

Month	Mean (Cº)	Min (Cº)	Max (Cº)	Range (Cº)
Feb	13.5	9.0	16.3	7.3
Mar	16.3	8.9	21.6	12.7
Apr	21.8	16.6	27.2	10.6
May	25.7	20.9	31.9	11.0
Jun	29.0	26.5	32.7	6.2
Jul	30.5	26.1	34.6	8.5
Aug	29.4	25.5	34.4	8.9
Sept	25.4	18.0	31.5	13.5
Oct	21.0	13.5	26.7	13.2
Nov	16.3	10.7	22.4	11.7
Dec	10.7	5.4	16.4	11.0

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