

**EUGREGARINE PARASITISM
OF *ERYTHEMIS SIMPLICICOLLIS* (SAY)
AT A CONSTRUCTED WETLAND:
A FITNESS COST TO FEMALES?
(ANISOPTERA: LIBELLULIDAE)**

J.L. LOCKLIN* and D.S. VODOPICH

Department of Biology, Baylor University, One Bear Place 97388, Waco, TX 76798, United States

Received May 28, 2010 / Revised and Accepted September 13, 2010

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, *Zygoptera* have been more frequently studied than *Anisoptera*. Adult dragonfly populations were surveyed for eugregarines at a constructed, flow-through wetland system and the fitness cost of infection was assessed in a common and widespread dragonfly host sp., *E. simplicicollis*. Populations were sampled weekly throughout the flight season. Host fitness parameters measured included wing load, egg size, clutch size, and total egg count. Of the 22 host spp. surveyed, 8 hosted eugregarines and 2 of these odon. spp. were previously undocumented as hosts. While eugregarine parasitism has been shown to exhibit seasonality, 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 spp. infecting the hosts.

INTRODUCTION

Dragonflies host a variety of ecto- and endoparasites, most of which influence their hosts' fitness (BONN et al., 1996; REINHARDT, 1996; ROLFF et al., 2000; CORDOBA-AGUILAR, 2002; CORDOBA-AGUILAR et al., 2003; MARDEN & COBB, 2004; CANALES-LAZCANO et al., 2005). Eugregarine-odonate investigations have focused primarily on damselfly hosts (ÁBRO, 1971,

*Current address: Department of Biology, Temple College, MBS, Temple, TX 76504, United States;
– jason.locklin@templejc.edu

1976; SIVA-JOTHY & PLAISTOW, 1999; SIVA-JOTHY, 2000; CORDOBA-AGUILAR, 2002; HECKER et al., 2002; CORDOBA-AGUILAR et al., 2003; CLOPTON, 2004; TSUBAKI & HOOPER, 2004; CANALES-LAZCANO et al., 2005) with relatively few studies on dragonfly hosts (MARDEN & COBB, 2004; SCHILDER & MARDEN, 2006; CLOPTON et al., 2007; LOCKLIN & VODOPICH, 2009; 2010).

Nine genera of eugregarines (Apicomplexa: Eugregarinorida: Actinocephaliidae) have been identified as odonate parasites: *Actinocephalus*, *Calyxocephalus*, *Domadracunculus*, *Geneiorhynchus*, *Hoplorhynchus*, *Mukundaella*, *Nubenocephalus*, *Prismatospora*, and *Steganorhynchus* (RICHARDSON & JANOVY, 1990; CLOPTON et al., 1993; CLOPTON, 1995; PERCIVAL et al., 1995; SARKAR, 1997; CLOPTON, 2004; HAYS et al., 2007). 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 & 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 & CLOPTON, 2003). Among odonate studies, eugregarines have been shown to reduce longevity (HECKER et al., 2002; CORDOBA-AGUILAR et al., 2003; CANALES-LAZCANO et al., 2005; TSUBAKI & HOOPER, 2004), reduce fecundity (CORDOBA-AGUILAR et al., 2003; CANALES-LAZCANO et al., 2005), influence mating success (CORDOBA-AGUILAR et al., 2003), impair flight-muscle performance (SCHILDER & MARDEN, 2006), hinder the ability to maintain territories (MARDEN & COBB, 2004; CORDOBA-AGUILAR, 2002), reduce fat content (SIVA-JOTHY & PLAISTOW, 1999), and impair fat oxidation in flight muscles (MARDEN & COBB, 2004).

The present study was designed to (1) survey dragonfly populations for eugregarine parasitism, (2) determine the prevalence and intensity patterns throughout 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).

METHODS

This study was conducted at the Lake Waco Wetland (LWW), TX, USA (31°60'88N, 97°30'69W). 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). All collected individuals were netted within 15 m of the shorelines, taken to the laboratory, killed with ethyl acetate, identified (ABBOTT, 2005), and stored in 70% ethanol. Prior to preservation, specimens of *E. simplicicollis* were dorsally scanned at 600 dpi (MITCHELL & LASWELL, 2000) and weighed to the nearest 0.1 mg. Weight loss due to desiccation in the laboratory was corrected following LOCKLIN & VODOPICH (2010). 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.

We calculated monthly eugregarine prevalences and intensities in *E. simplicicollis* throughout the flight season and investigated potential impacts on parameters relating to species fitness, i.e. differences in wing load, egg size, total egg count, and clutch size. We define clutch size as the number of eggs laid (WATANABE & MATSUURA, 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 teneral (LOCKLIN & VODOPICH, 2010). Only mature host individuals are candidates for answering questions about parasite prevalence and intensity. Because wing loads (mg body wt x wing surface area⁻¹) were not affected by eugregarines (see below), we used wing loads 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 signify mature adults (LOCKLIN & VODOPICH, 2010).

To calculate wing loads, total wing surface area of each *E. simplicicollis* was estimated by measuring the right hindwing 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:

$$\delta \quad y = 0.470x - 5.94 \quad (r^2=0.92, 18 \text{ d.f.}, p < 0.001)$$

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

where x is hindwing length (mm).

To collect eggs, females were captured and induced to oviposit in the field (SUSA & 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 widths and lengths of ten randomly selected eggs from 24 females were measured (mm) using an ocular micrometer mounted in a compound microscope. Egg circumference, C , was calculated using a formula for ellipse perimeter:

$$C = \pi[1.5(a + b) - ((a \times b)^{0.5})]$$

where $a = 0.5 \times \text{length}$ and $b = 0.5 \times \text{width}$ (SCHENK & SÖNDGERATH, 2005). The eggs from the other females 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.

RESULTS

Specimens of twenty-two anisopteran species ($n = 1,378$) were collected at the wetland, eight of which were parasitized by eugregarines (Tab I). Four of the unparasitized species collected (Tab II), *Anax junius* (Aeshnidae), *Epithea princeps* (Corduliidae), *Tramea lacerata* (Libellulidae), and *T. onusta* (Libellulidae) have been previously reported to host eugregarines (CLOPTON et al., 2007; LOCKLIN & VODOPICH, 2010). Eugregarines 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, $V_N = 0.07$, $p = \text{NS}$) (Fig. 1).

Table I
Dragonfly species (all members of Libellulidae) infected with eugregarine parasites at the Lake Waco Wetland; – IQR = interquartile range – [* indicates newly reported host]

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

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* (Fig. 1) and the maximum intensity of 60 eugregarines occurred in a male *E. simplicicollis*.

Wing loads among mature *E. simplicicollis* individuals were not related to the presence/absence or intensity of eugregarines. Median 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) in males and 30.9 (28.6 – 33.6) and 29.9 (27.1 – 32.4) in females. No difference was detected between median wing loads of infected versus uninfected in either 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 either males ($N = 189$, Spearman's correlation, $r_s = 0.37$, $p = 0.79$) or females ($N = 288$, $r_s = 0.01$, $p = 0.99$) (Fig. 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 into the glass vials. Egg sizes (circumference) 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 ($N = 24$, $r_s = -0.19$, $p = 0.37$) (Fig. 3). Total egg counts ranged from 0 – 1,611 eggs female⁻¹. Mean numbers of total eggs from infected versus

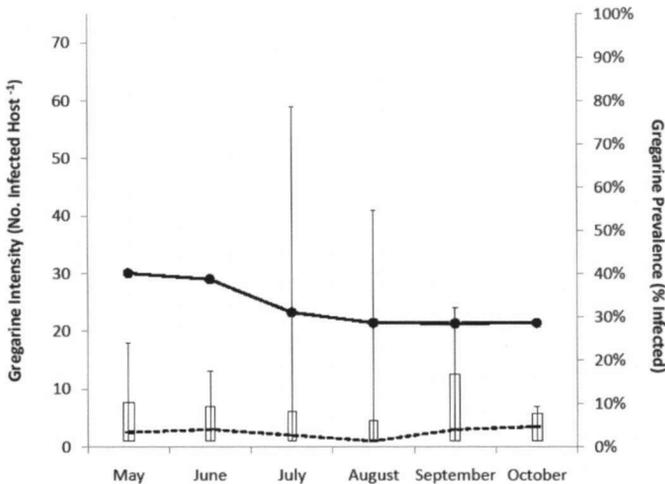


Fig. 1. Median gregarine intensity (dashed line) and gregarine prevalence (solid line) in *E. simplicicollis* throughout the odonate flight season at the Lake Waco Wetland, Texas, USA, 2009. Intensity data include interquartile range (boxes) and range (extended bars).

uninfected females were not significantly different ($t = -1.63$, $p = 0.11$), and eugregarine intensity did not correlate with the total number of eggs ($N = 84$, $r_s = 0.143$, $p = 0.193$) (Fig. 3). Clutch size data (Fig. 3) 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 ($\chi^2_1 = 0.168$, $p = 0.682$). 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 ($N = 74$, $r_s = 0.09$, $p = 0.44$) (Fig. 3).

DISCUSSION

Of the eight parasitized dragonfly species collected at the wetland (Tab. I), 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 & VODOPICH, 2009; 2010). Those odonate species surveyed in LOCKLIN & VODOPICH (2010) 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 (Tab. II).

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 residence time (5-10 days) is likely to dampen seasonal variation in oocyst density through time. LOCKLIN & VODOPICH (2010) found that eugregarine prevalence and intensity increased during the dragonfly flight season of each of

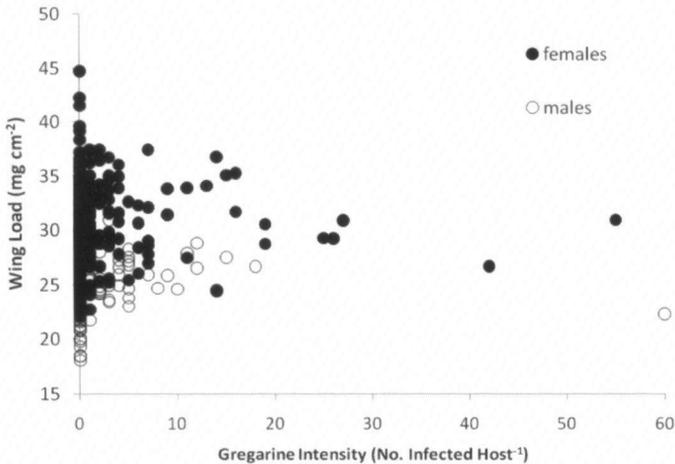


Fig. 2. Wing loads of males (open symbols) and females (closed symbols) *E. simplicicollis* versus gregarine intensity. No correlation was detected in males ($N = 189$, $r_s = 0.37$, $p = 0.79$) or females ($N = 288$, $r_s = 0.01$, $p = 0.99$).

two subsequent years in dragonfly populations at Battle Lake, a small nearby reservoir with a longer water residence time. In that study, we 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, we detected no significant seasonality in eugregarine prevalence or intensity (Fig. 1). We 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 are also likely to 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 *Cryptosporidium 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 & 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 & CORDERO-RIVERA, 2005). Males distribute most of their fat in the thorax (ANHOLT et al., 1991) to support sustained flight and maintain territories (MARDEN & WAAGE, 1990; PLAISTOW & SIVA-JOTHY, 1996). The initial moments of flight depend on carbohydrate oxidation and then transition to lipid oxidation if flight is sustained (SCHILDER & MARDEN, 2006). SIVA-JOTHY & PLAISTOW (1999) found that eugregarines reduced fat content in pre-reproductive *Calopteryx splendens xanthostoma* males (Zygoptera: Calopterygidae). SCHILDER & MARDEN (2006) reported that eugregarines impaired fatty acid oxidation by flight muscles in infected *Libellula pulchella* males (Anisoptera: Libellulidae). Decreased fat content and/or impaired fat oxidation in infected odonates are likely to 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 we hypothesized that egg sizes, clutch sizes,

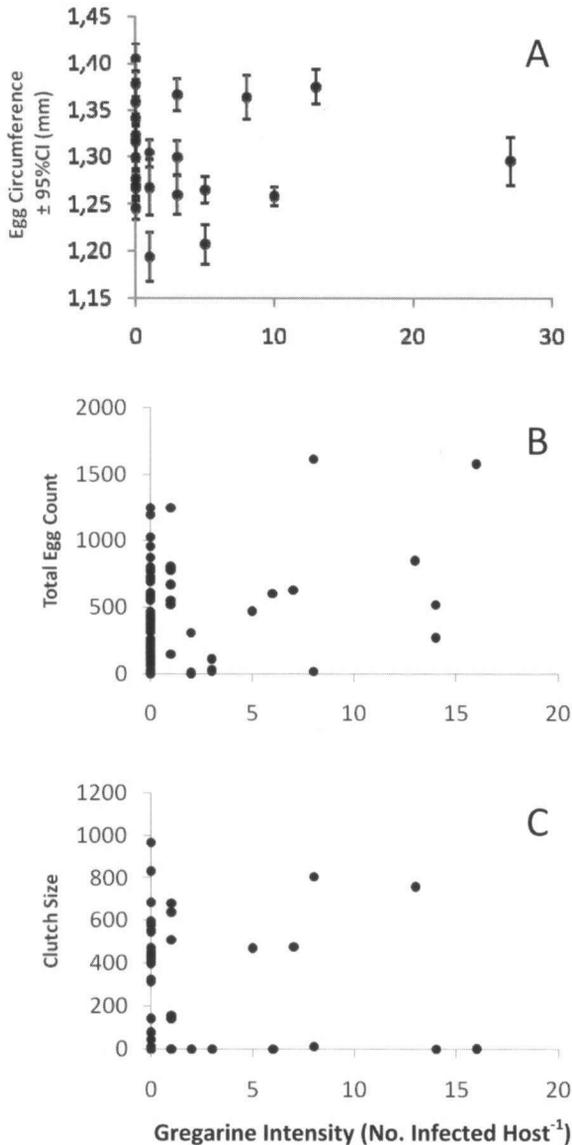


Fig. 3. Egg circumference (A), total egg count (B), and clutch size (C) of *E. simplicicollis* versus gregarine intensity. A: Each data point represents the mean circumference (mm) of ten eggs from a female; error bars represent 95% CI. No correlation was detected ($N = 24$, $r_s = -0.19$, $p = 0.37$). B: Total egg count includes all eggs released during induced oviposition plus retained eggs found during abdominal dissections. No correlation was detected ($N = 84$, $r_s = 0.14$, $p = 0.19$). C: Clutch size data include only females with eggs to oviposit. No correlation was detected ($N = 74$, $r_s = 0.09$, $p = 0.44$).

and/or the number of eggs produced would be less among infected females than uninfected females. CORDOBA-AGUILAR et al., (2003) found a negative correlation between eugregarine intensity and egg production in *Calopteryx haemorrhoidalis*. CANALES-LAZCANO et al. (2005) also found that eugregarine infection reduced egg numbers in female *Enallagma praevarum*. However, we detected no correlation between parasite intensity 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 & PLAISTOW, 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 & OBERHAUSER, 1999; LORD, 2006; BRADLEY & ALTIZER, 2005; LINDSEY et al., 2009). 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 Zygoptera which may have permitted entry of pathogens into the haemocoel. Unfortunately for our efforts to detect fitness costs, intensities were low in *E. simplicicollis* females (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 & 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 parasitism due to the nature of eugregarine reproduction (no merogony) and their aggregated distribution (i.e. a negative bi-

Table II
Dragonfly species collected at the Lake Waco Wetland that did not host eugregarine parasites – [* indicates a previously reported host species]

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

nomial distribution) across dragonfly populations. Most infected odonates have low parasite intensities (LOCKLIN & VODOPICH, 2010). 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 differentiate morphologically (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 & CLARK, 1993; ROBERTS & JANOBY, 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 & VODOPICH (2010) 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*.

ACKNOWLEDGEMENTS

We thank JOSHUA HUCKABEE, SAL LAMASTRA, VICTORIA PRESCOTT, TAMARA STROHM and AUSTIN CUNNINGHAM for assistance in the laboratory 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.

REFERENCES

- ABBOTT, J.C., 2005. *Dragonflies and damselflies of Texas and the south-central United States*. Princeton Univ. Press, Princeton.
- ÅBRO, A., 1971. Gregarines: their effect on damselflies (Odonata: Zygoptera). *Entomologia scand.* 2: 294-300.
- ÅBRO, A., 1974. The gregarine infection in different species of Odonata from the same habitat. *Zool. Scripta* 3: 111-120.
- ÅBRO, A., 1976. The mode of gregarine infection in Zygoptera (Odonata). *Zool. Scripta* 5: 265-275.
- ALTIZER, S.M. & K. OBERHAUSER, 1999. Effects of the protozoan parasite *Ophryocystis elektroscirrha* on the fitness of monarch butterflies (*Danaus plexippus*). *J. Invert. Pathol.* 74: 76-88.
- ANHOLT, B.R., J. MARDEN & D. JENKINS, 1991. Patterns of mass gain and sexual dimorphism in adult dragonflies. *Can. J. Zool.* 69: 1156-1163.
- BONN, A., M. GASSE, J. ROLFF & A. MARTENS, 1996. Increased fluctuating asymmetry in the damselfly *Coenagrion puella* is correlated with ectoparasitic water mites: implications for fluctuating asymmetry theory. *Oecologia* 108: 596-598.
- BRADELY, C.A. & S. ALTIZER, 2005. Parasites hinder monarch butterfly flight: implication for disease spread in migratory hosts. *Ecol. Lett.* 8: 290-300.
- BUSH, A.O., J.C. FERNANDEZ, G.W. ESCH & J.R. SEED, 2001. *Parasitism: the diversity and ecology of animal parasites*. Cambridge Univ. Press, Cambridge.
- CALL, J.L., M. ARROWOOD, L. XIE, K. HANCOCK & V.C. TSANG, 2001. Immunoassay for viable *Cryptosporidium parvum* oocysts in turbid environmental water samples. *J. Parasitol.* 87: 203-210.
- CANALES-LAZCANO, J., J. CONTRERAS-GARDUNO, & A. CORDOBA-AGUILAR, 2005. Fitness-related attributes and gregarine burden in a non-territorial damselfly *Enallagma praevarum* Hagen (Zygoptera: Coenagrionidae). *Odonatologica* 34: 123-130.
- CLARK, C.G., F. KAFFASHIAN, B. TAWARI, J.J. WINDSOR, A. TWIGG-FLESNER, M.C.G. DAVIES-MOREL, J. BLESSMANN, F. EBERT, B. PESCHEL, A.L. VAN, C.J. JACKSON, L. MACFARLANE & E. TANNICH, 2006. New insights into the phylogeny of Entamoeba species provided by analysis of four new small subunit rRNA genes. *Int. J. syst. evol. Microbiol.* 56: 2235-2239.
- CLOPTON, R.E., 1995. *Domadracunculus janovyi* n. gen., n. sp. (Apicomplexa: Actinocephalidae) from adults of *Ischnura verticalis* (Odonata: Zygoptera) in Texas. *J. Parasitol.* 81: 256-260.
- CLOPTON, R.E., 2004. *Calyxocephalus karyopera* g. nov., sp. nov. (Eugregarinorida: Actinocephalidae: Actinocephalinae) from the ebony jewelwing damselfly *Calopteryx maculata* (Zygoptera: Calopterygidae) in southeast Nebraska: implications for mechanical prey-vector stabilization of exogenous gregarine development. *Comp. Parasitol.* 71: 141-153.

- CLOPTON, R.E., T.J. COOK & J.L. COOK, 2007. Revision of *Geneiorhynchus* Schneider, 1875 (Apicomplexa: Eugregarinida: Actinocephalidae: Acanthosporinae) with recognition of four new species of *Geneiorhynchus* and description of *Geneiorhynchus manifestus* n. sp. parasitizing naiads of the Green Darner, *Anax junius* (Odonata: Aeshnidae) in the Texas Big Thicket. *Comp. Parasitol.* 74: 273-285.
- CLOPTON, R.E., T.J. PERCIVAL & J. JANOVY, Jr, 1993. *Nubenocephalus nebraskensis* n. gen., n. sp. (Apicomplexa: Actinocephalidae) described from *Argia bipunctulata* (Odonata: Zygoptera). *J. Parasitol.* 79: 533-537.
- CORDOBA-AGUILAR, A., 2002. Wing pigmentation in territorial male damselflies, *Calopteryx haemorrhoidalis*: a possible relation to sexual selection. *Anim. Behav.* 63: 759-766.
- CORDOBA-AGUILAR, A. & A. CORDERO-RIVERA, 2005. Evolution and ecology of Calopterygidae (Zygoptera: Odonata): status of knowledge and research perspectives. *Neotrop. Ent.* 34: 861-879.
- CÓRDOBA-AGUILAR, A., J.C. SALAMANCA-OCANA & M. LOPEZARAIZA, 2003. Female reproductive decisions and parasite burden in a calopterygid damselfly (Insecta: Odonata). *Anim. Behav.* 66: 81-87.
- DIAMOND, L.S. & C.G. CLARK, 1993. A redescription of *Entamoeba histolytica* Schaudinn, 1903 (Emended Walker, 1911) separating it from *Entamoeba dispar* Brumpt, 1925. *J. Eukaryot. Microbiol.* 40:340-344.
- HAYS, J.J., R.E. CLOPTON, T.J. COOK & J.L. COOK, 2007. Revision of the genus *Nubenocephalus* and description of *Nubenocephalus secundus* n. sp. (Apicomplexa: Actinocephalidae) parasitizing adults of *Argia sedula* (Odonata: Zygoptera: Coenagrionidae) in the primitive Texas Big Thicket, U.S.A. *Comp. Parasitol.* 74: 286-293.
- HECKER, K.R., M.R. FORBES & N.J. LEONARD, 2002. Parasitism of damselflies (*Enallagma boreale*) by gregarines: sex biases and relations to adult survivorship. *Can. J. Zool.* 80: 162-168.
- KATO, S., M.B. JENKINS, E.A. FOGARTY & D.D. BOWMAN, 2002. Effects of freeze-thaw events on the viability of *Cryptosporidium parvum* oocysts in soil. *J. Parasitol.* 88: 718-722.
- KVARNÄS, H., 2001. Morphometry and hydrology of the four large lakes of Sweden. *Ambio* 30: 467-474.
- LINDSEY, E., M. MUDRESH, D. VARUN, K. OBERHAUSER & S. ALTIZER, 2009. Crowding and disease: effects of host density on response to infection in a butterfly-parasite interaction. *Ecol. Ent.* 34: 551-561.
- LOCKLIN, J.L. & D.S. VODOPICH, 2009. Bidirectional gender biases of gregarine parasitism in two coexisting dragonflies (Anisoptera: Libellulidae). *Odonatologica* 38: 133-140.
- LOCKLIN, J.L. & D.S. VODOPICH, 2010. Patterns of gregarine parasitism in dragonflies: host, habitat, and seasonality. *Parasitol. Res.* 107: 75-87.
- LORD, J.C., 2006. Interaction of *Mattesia oryzaephili* (Neogregarinorida: Lipotrophidae) with *Cephalonomia* spp. (Hymenoptera: Bethyloidea) and their hosts *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae). *Biol. Control* 37: 167-172.
- MARDEN, J.H. & J.R. COBB, 2004. Territorial and mating success of dragonflies that vary in muscle power output and presence of gregarine gut parasites. *Anim. Behav.* 68: 657-665.
- MARDEN, J.H. & J.K. WAAGE, 1990. Escalated damselfly territorial contests are energetic wars of attrition. *Anim. Behav.* 39: 954-959.
- MITCHELL, F.L. & J.L. LASWELL, 2000. Digital dragonflies. *Am. Ent.* 46: 110-115.
- PERCIVAL, T.J., R.E. CLOPTON & J. JANOVY, Jr, 1995. *Hoplorhynchus acanthatholius* n. sp. and *Steganorhynchus dunwoodii* n. gen., n. sp. (Apicomplexa: Eugregarinorida) described from coenagrionid damselflies. *J. Eukar. Microbiol.* 42: 406-410.
- PLAISTOW S. & M.T. SIVA-JOTHY, 1996. Energetic constraints and male mate securing tactics

- in the damselfly *Calopteryx splendens xanthostoma* (Charpentier). *Proc. R. Soc. Lond. (B)* 263: 1233-1238.
- POKORNY, N.J., S.C. WEIR, R.A. CARRENO, J.T. TREVORS & H. LEE, 2002. Influence of temperature on *Cryptosporidium parvum* oocyst infectivity in river water samples as detected by tissue culture assay. *J. Parasitol.* 88: 641-643.
- REINHARDT, K., 1996. Negative effects of *Arrenurus* water mites of the flight distance of the damselfly, *Nehalennia speciosa* (Odonata: Coenagrionidae). *Aqua. Insects* 18: 233-240.
- RICHARDSON, S. & J. JANOVY, Jr, 1990. *Actinocephalus carrilynnae* n. sp. (Apicomplexa: Eugregarinorida) from the Blue Damselfly, *Enallagma civile* (Hagen). *J. Eukar. Microbiol.* 37: 567-570.
- ROBERTS, L.S. & J. JANOVY, Jr, 2005. *Foundations of parasitology*. [7th edn] McGraw-Hill, Dubuque, Iowa.
- RODRIGUEZ, Y., C.K. OMOTO & R. GOMULKIEWICZ, 2007. Individual and population effects of eugregarine, *Gregarina niphandrodes* (Eugregarinida: Gregarinidae), on *Tenebrio molitor* (Coleoptera: Tenebrionidae). *Environ. Ent.* 36: 689-693.
- ROLFF, J., H. ANTVOGEL & I. SCHRIMPF, 2000. No correlation between ectoparasitism and male mating success in a damselfly: why parasite behavior matters. *J. Insect Behav.* 13: 563-571.
- SARKAR, N.K., 1997. Observations of three new and one known species of cephaline gregarines (Apicomplexa: Sporozoa: Eugregarinida: Septatina) from the odonates of Mahananda Forest, West Bengal, India. *Arch. Protistenk.* 148: 209-213.
- SCHENK, K. & D. SONDEGERATH, 2005. Influence of egg size differences within egg clutches on larval parameters in nine libellulid species (Odonata). *Ecol. Ent.* 30: 456-463.
- SCHILDER, R.J. & J.H. MARDEN, 2006. Metabolic syndrome and obesity in an insect. *Proc. natl. Acad. Sci. USA* 103: 18805-18809.
- SCOTT, J.T., R.D. DOYLE & C.T. FILSTRUP, 2005. Periphyton nutrient limitation and nitrogen fixation potential along a wetland nutrient-depletion gradient. *Wetlands* 25: 439-448.
- SIVA-JOTHY, M.T., 2000. A mechanistic link between parasite resistance and expression of a sexually selected trait in a damselfly. *Proc. R. Soc. Lond. [B]* 267: 2523-2527.
- SIVA-JOTHY, M.T. & S.J. PLAISTOW, 1999. A fitness cost of eugregarine parasitism in a damselfly. *Ecol. Ent.* 24: 465-470.
- SMITH, A.J. & R.E. CLOPTON, 2003. Efficacy of oral metronidazole and potassium sorbate against two gregarine parasites, *Protomagalhaensia granulosa* and *Gregarina cubensis* (Apicomplexa: Eugregarinida), infecting the death's head cockroach, *Blaberus discoidalis*. *Comp. Parasitol.* 70: 196-199.
- SUSA, K. & M. WATANABE, 2007. Egg production in *Sympetrum infuscatum* (Selys) females living in a forest-paddy field complex (Anisoptera: Libellulidae). *Odonatologica* 36: 159-170.
- TSUBAKI, Y. & R.E. HOOPER, 2004. Effects of eugregarine parasites on adult longevity in the polymorphic damselfly *Mnais costalis* Selys. *Ecol. Ent.* 29: 361-366.
- WATANABE, M. & S. MATSU'URA, 2006. Fecundity and oviposition in Mortonagrion hirosei Asahina, *M. selenion* (Ris), *Ischnura asiatica* (Brauer) and *I. senegalensis* (Rambur), coexisting in estuarine landscapes of the warm temperate zone of Japan (Zygoptera: Coenagrionidae). *Odonatologica* 35: 159-166.