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**TESTING FOR SEX BIASES AND MORPH BIASES IN PARASITISM OF
ZYGOPTERANS (ODONATA) BY GREGARINES (EUGREGARINIDAE)**

by

KERRY ROBIN HECKER

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of
Master of Science

Department of Biology

Carleton University

Ottawa, Ontario

September 1999

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**Testing for sex biases and morph biases in parasitism of Zygopterans (Odonata) by
Gregarines (Eugregarinidae)**

Submitted by Kerry Hecker
in partial fulfillment of the requirements for
the degree of Master of Science

Chair

Supervisor

ABSTRACT

I studied gregarine parasites of damselflies, Enallagma boreale (Selys) to elucidate causes and consequences of sex biases in parasitism of adult hosts. I found some evidence that adult females had higher prevalence and intensity of infection than males. Both sexes showed a positive correlation between number of gregarines and longevity under conditions of food stress. This may be because food ingested with the infective cysts is more beneficial than the parasites are harmful.

I also studied Nehalennia irene damselflies and their gregarine parasites, predicting that female morphs would differ in measures of parasitism, therefore balancing the advantages accrued to one morph at high population densities. I found that female morphs did not differ in measures of parasitism. There was no significant difference in prevalence between the sexes across five separate ponds, although females had more gregarines than males. I found no correlation between the number of gregarines and longevity of hosts.

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Nothing is rich but the inexhaustible wealth of nature.
She shows us only surfaces, but she is
a million fathoms deep.

-Ralph Waldo Emerson

CHAPTER 1

General Introduction

1.1 Background and rationale for study

Parasitism is an ecological association between species in which one, the parasite, lives on or in the body of the other, the host. Anderson and May (1978) require three conditions for an animal species to be parasitic: utilization of the host as a habitat; nutritional dependence upon the host, and; causing “harm” to its host. The definition is further narrowed by excluding species that kill their hosts as a prerequisite for successful development (these are called parasitoids). The parasite’s degree of dependence on the host varies widely, from species that spend their entire life cycle within hosts (such as malarial protozoa), to species with a short parasitic phase and a longer free-living stage (such as mosquitoes).

The importance of the host to the parasite is intuitive; the host is the parasite's habitat for the length of time they coexist (Marquardt and Demaree 1985; Toft et al. 1991). The influence of parasites on the host, however, has been underestimated in the past, as only direct influences on the host were considered. Early studies show that parasites can affect host fitness through direct effects on reproductive output (Minchella and Loverde 1981) and by reducing survival of the host (Anderson and May 1978). Several indirect parasite effects include reduction in host resilience (Festa-Bianchet 1989, Arnqvist and Mäki 1990, Sheldon and Verhulst 1996), alteration of host appearance (Hamilton and Zuk 1982, Møller 1988), and alteration of host behaviour (Poulin 1994).

Many hosts try to negate the effects of parasites through immune response (Sheldon and Verhulst 1996) or behaviour (Toft et al. 1991). Parasites that exert sublethal effects, or weaken their host only under certain detrimental conditions, may have a subtle and largely unrecognized influence on host populations (Levin and Pimental 1981), or the evolution of host life histories (Hochberg et al. 1992; Forbes 1993). The challenge to researchers is to identify factors that explain the variability seen in parasite-host associations: from inevitable host death as a result of infection, to a nearly symbiotic association (Anderson and May 1978).

Many factors have been deemed important in understanding the persistence of parasites in host populations and the degree to which parasites cause harm. One of these is mode of transmission. Transmission of parasites can occur in many ways, though the methods are classified into two major categories: direct and indirect transmission. Direct transmission occurs when it is achieved by physical contact between hosts of the same species or by transmission stages of the parasite which pass into the habitat of the host and are picked up by inhalation, ingestion, or direct penetration of the host's body (Anderson and May 1981). Indirect transmission involves passing of parasites from one host to the next via one or more intermediate host species (Anderson and May 1981). Parasites with direct transmission are thought to evolve to low virulence because they often use the host as a vector (Ewald 1988), but this is by no means a general rule.

Researchers also have concentrated on the extent to which individual hosts within populations vary in either their susceptibility, immune responsiveness, or in the fitness costs they pay when parasitised (Hamilton and Zuk 1982). All of these factors can influence the transmission and reproductive success of parasites. There are three basic

ways in which a host-parasite relationship can vary among hosts: first, the hosts may differ in susceptibility to the parasite, through either a higher exposure or encounter rate, or a higher rate of retention; second, hosts may have different responses to the parasite, which can be behavioural, physiological, or immunological; third, parasites may be more costly to certain hosts. In this thesis, I examine the extent to which the sexes and different morphs of damselflies (Odonata: Coenagrionidae) may differ in their exposure to gut protozoans that show direct transmission via ingestion, and how these hosts may differ in costs faced when parasitised by these protozoans (Apicomplexa: Eugregarinidae). Odonatan hosts do not show gross histological responses to these parasites, as seen for other parasites, such as water mites (Forbes et al. in press).

1.2 Specific objectives

My main objective is to examine the extent to which individuals within species vary in aspects of parasitism. I am particularly interested in two facets: sex and morph biases in both measures and costs of parasitism. In some species, the sexes are as morphologically distinct from one another as are individuals from different species (Alcock 1993). In some species of Coenagrionid damselflies, adult males and females differ greatly in habitat use and foraging behaviour (Anholt 1992), which could expose them differentially to parasites. Additionally, much recent work has focused on sex biases in immune responses, whereby males appear less capable of withstanding parasite infections than females (Schalk and Forbes 1997). Studies of sex biases in immunity have largely been restricted to vertebrates. Yet it is important to keep in mind that if

sexes differ in measures of parasitism, then either differential exposure or differential immunity or both could account for these patterns. Sex biases in parasitism by gregarines for a species of univoltine damselfly, Enallagma boreale (Selys), is the topic of Chapter 2.

In many species, distinct genetic morphs are known to exist; yet whether parasites are important in explaining persistence of two or more morphs within populations still remains largely untested. Losey et al. (1997) demonstrated differential selection on the ed and green morphs of the pea aphid, Acyrtosiphon pisum; a parasitoid prefers the green morphs, while a predator preferentially kills the red morphs. They conclude that density-dependent predation by different types of enemies on different morphs is sufficient to maintain the colour polymorphism in the population.

Many damselflies show distinctive colour and/or pattern polymorphisms which occur solely in the females, yet the factors maintaining these polymorphisms are debated. I further examine sex biases in parasitism by gregarines for another univoltine Coenagrionid damselfly, Nehalennia irene Hagen in Chapter 3. In this chapter, I also explore whether female morphs show different degrees of parasitism, or whether they are differentially affected by parasites. This work builds on previous work (Forbes et al. 1995, 1997) which shows that one morph appears to increase in relative abundance at high-density sites, but still remains the minority morph. This particular morph appears better able to avoid costly male harassment which is likely to be more frequent and more intense at high density sites; however, it does not become the majority morph (Forbes et al. 1995). Parasites with direct transmission can be more prevalent at high population densities (Arnqvist and Mäki 1990); if the one morph is more susceptible to gregarines, then its ability to increase in relative abundance may be thwarted.

Finally, in the Appendix to this thesis, I report on parasitism measures for males and two female morphs of the New Zealand endemic damselfly, Xanthocnemis zealandica. Unlike the other two species, little is known about sex or morph differences in behaviour, but information on parasitism by gregarines will enable future comparative work on relative susceptibility of species to their parasites and virulence of those parasites.

1.3 Measures of parasitism used in this thesis

Measures of parasitism at the population level are useful for studies that extend the principles of natural selection over generations, to the evolution of both host and parasite populations. Two measures of parasitism used throughout this thesis are prevalence and intensity (*sensu lato* Bush et al. 1997). Prevalence is the number of hosts infected with 1 or more individuals of a particular parasite species (or taxonomic group) divided by the number of hosts examined for that purpose. This measurement is commonly expressed as a percentage when used descriptively, and as a proportion when incorporated into mathematical models (Bush et al. 1997). Intensity of infection is the number of individuals of a particular parasite species (or taxon) in a single infected host. Mean intensity is the average intensity of a particular species of parasite among the infected members of a particular host species, meaning that uninfected members of the host species are excluded from this statistic (Bush et al. 1997). Prevalence is often used to compare exposure of hosts to infective stages of parasites, while intensity is often examined to determine what effects, if any, parasites have on hosts.

CHAPTER 2

Parasitism of Enallagma boreale (Hagen) damselflies by gregarines: seasonal patterns, sex biases, and relations to adult survivorship.

2.1 Introduction

The degree to which parasites harm individual hosts is important for assessing their impact on host populations and, ultimately, ecological communities (Minchella and Scott 1991). Of particular interest is the degree to which individuals within a species vary in their susceptibility to, or their defense against, parasites, and how this may affect the host population. For example, in models of macroparasite-host interactions, only female hosts are considered. The impact of parasites on female fecundity and survivorship, relative to aggregation of parasites on individual hosts, determines (at least theoretically) the impact of parasites upon host populations (Anderson and May 1981).

For this reasons, researchers have become interested in differential susceptibility and/or immune responses of males and females to parasitic infections. Sex differences in immune function and exposure to parasites are well documented for vertebrates and may explain sex differences in prevalence or intensity of parasitism (Poulin 1996; Raga et al., 1997; Schalk and Forbes 1997). For invertebrates, sex differences in parasitism are also

known (Zuk and McKean 1996; Wedekind and Jakobsen 1998); however, their causes are less well understood. Here, I determined whether sex differences exist in either prevalence or intensity of infection by gut protozoans for the damselfly, Enallagma boreale (Selys). I also examined whether infections by these gregarines are more costly to either sex.

I examined this parasite-host association for several reasons. First, although the taxonomy of gregarines is not well understood, keys to gregarine parasites of damselflies are under construction (R. Clopton pers. comm., Clopton in press). Second, the basic ecology and life cycle of parasitic gregarines for odonate insects is fairly well understood. Gregarines appear to be ingested in their infective stage, along with insect prey, by foraging damselflies and dragonflies. Gregarine oocysts (the infective stages) have been found attached to the legs of chironomid midges (Åbro, 1974); such insects can be completely macerated by damselflies before they are ingested as a bolus (within which the oocysts are embedded). Third, male and female damselflies differ in their foraging ecology. For many Coenagrionid damselflies, and for E. boreale in particular, males are “time minimizers” spending only enough time foraging away from ponds to ensure they have energy for daily mate searching (Anholt 1992). In comparison, females are “energy maximizers” and will spend more time feeding to mature their clutches of eggs (Richardson and Baker 1997). Their foraging is usually done away from the natal pond, perhaps to avoid harassment from mate-searching males. It is this difference in foraging ecology that is responsible for biases in operational sex ratios at ponds, with male-searching males outnumbering receptive females (e.g., Fincke 1994b). Based on this sex difference in foraging ecology, I predicted that females should be more heavily

parasitized by gregarines than same-aged males, simply because females ingest more food (in fact, heavy newly-emerged males may even lose weight over the pre-reproductive period, Anholt 1992).

I had four main objectives. First, I examined whether larval males and females differ in their degree of parasitism by gregarines. Sex differences in habitat use and foraging ecology are not evident for larval damselflies, although at least one study on another Coenagrionid has indicated that males and females may differ in their developmental time (Baker et al. 1992). I expected that any differences in parasitism of adult sexes would be due to differences in foraging ecology of males and females. However, I first had to examine whether gregarine parasitism existed for larval E. boreale and, if present, whether the larval sexes showed equal parasitism. Related to this objective, I examined whether larval damselflies might lose any resident gregarines during eclosion to adults. Thus, I was principally interested in whether pre-reproductive adult damselflies, called teneral, start their adult life with few or no gregarines and acquire them through time, since this is the basis for expecting the adult sexes to differ in prevalence or intensity measures.

My main prediction was that adult males and females of the same or similar age would differ in their gregarine numbers, for reasons outlined above. As with other parasitic infections, gregarine infections are expected to change over the lifetime of individual hosts. In fact, age-related profiles of parasitism are well-documented for many parasite-host associations in general (Scott 1988) and for insect-gregarine associations in particular (Zuk 1988). Thus, researchers have to be sure that hosts included in samples for comparisons are the same or similar age. Otherwise, any sex biases may be obscured

by age variation within samples of hosts. However, obtaining accurate ages of damselflies is difficult. The mark-recapture method cannot be used because recapture success is low for some species like E. boreale, (M.R. Forbes, unpublished data). Counting daily growth rings based on cuticular sections is used to age some insects, including odonates (Neville 1967, Zuk 1988); however, I found this method is prone to error after the pre-reproductive period for E. boreale. One way to ensure reduced age variation, for at least some samples, is by surveying ponds at the start of the flight season when the first mature damselflies return to ponds and are approximately the same age. Another indirect method is to sample only those individuals that have near to fully-engorged Arrenurus spp. mites, since these adults are approximately the same age (around 14 days old, Forbes 1991a). I employed both of these latter methods to control indirectly for age variation within at least some samples.

My third objective was to compare variation in prevalence and intensity of gregarines across two sites and two sampling periods. I reasoned that I might find seasonal and site variation for gregarines as reported by others (Åbro 1987). Specifically, I wanted to sample a site with mid-to-high prevalence; if prevalence of gut protozoans is low, then sex biases may not be observed unless very large samples were collected. In relation to my second objective, I expected to find sex biases in parasitism only when age of hosts could be controlled for, and when enough infective stages were present that accumulation of parasites for individual hosts was likely.

Finally, I examined whether gregarines affected adult males differently than adult females, by bringing damselflies (with unknown infection status) into the lab and subjecting them to food shortages. Food shortages often occur at my study location

during a series of cool rainy days when damselflies are forced to roost and forgo feeding (cf. Leung and Forbes 1997). Here, I made no specific prediction. Damselfly males might survive less well with a given number of gregarines simply because they have fed less than females. Previous experiments with a related species (Enallagma ebrium (Hagen)) showed that males survived less well than females under semi-natural conditions (Forbes and Leung, 1995). On the other hand, females are also committing much of their energy to eggs and may be less able to survive long periods without food.

2.2 Methods

2.2.1 Study species

E. boreale is a common and widespread damselfly in the family Coenagrionidae that typically inhabits beaver ponds and freshwater marshes (Forbes 1991c). The life cycle of Enallagma damselflies is univoltine. The mating system of E. boreale, like that of many other Enallagma species, is defined as scramble mate competition (Fincke 1994b). Although females show a suite of male rejection behaviours, these typically follow oviposition (which occurs underwater in the absence of the male) (Fincke 1994b, M.R. Forbes, pers. obs.). After the female has laid her eggs, she resurfaces and soon flies away from the breeding pond, often chased by single males at the pond margin. This indiscriminate sexual activity of males appears to be one reason females forage away from the pond's edge.

Gregarines in this study are Hoplorhynchus spp. (R. Clopton, pers. comm.).

These are septate gregarines (Eugregarinidae) with one damselfly host in their life cycle (Figure 2.1). The infective oocytes are ingested by the host, and once in the midgut, they split to produce eight sporozoites per oocyte (Åbro 1976). The sporozoites then attach to the gut wall as trophozoites, and absorb digesta. After growing in size for some time, trophozoites detach from the gut epithelium, and develop into gamonts that move about freely in the gut. Gamonts proceed to find a “mate”, and undergo syzygy, after which the two attached individuals are enclosed in a protective covering, and become a gametocyst. Approximately two weeks after ingestion of the oocysts, the gametocyst is excreted (the actual amount of time is temperature dependent; Åbro 1974). Each gametocyst undergoes development and eventually releases many oocysts; in some species, development of oocysts follows an obligatory period of cool temperatures (Åbro 1976). The process through which gregarine oocysts become attached to the tarsal setae of small flying insects is unknown. However, the prey of damselflies are apparently the carriers of the gregarine infective cysts (Åbro 1976).

2.2.2 Study sites

I chose two study sites, because I expected that spatial variation in parasitism may exist and because sex biases may only be detectable when the prevalence of parasitism was high. In another study of gut protozoans in insects, Arnqvist (1992) showed that prevalence related positively to host population densities. More specifically, Åbro (1974) showed both spatial and temporal variation in gregarine numbers within damselfly hosts, as did Taylor and Merriam (1996).

Figure 2.1 - Combined life cycle of damselfly and gregarine. Beginning in the upper left-hand corner and continuing clockwise. First, the damselfly ingests an infective oocyst. The oocyst bursts its envelope to release sporozoites. Sporozoites attach themselves to the cells of the gut wall, using their epimerite; they are now called trophozoites. The trophozoites proceed to absorb digesta from the gut lumen of the damselfly, until the gregarine is mature and releases its epimerite. The gregarine is now a free-moving gamont. A male and female gamont join in syzygy, their method of sexual reproduction. The now fused gregarines are enclosed in a protective coating; at this stage, they are known as a gametocyst. The gametocyst is excreted along with feces from the damselfly. While in the outside environment, the gametocyst undergoes a process of maturation which may include freezing and thawing cycles. The matured gametocyst bursts its protective coating and releases a small number of infective oocysts.

My two study sites were Jack's Marsh and Upper Dowsley pond (hereafter JM and UD, respectively). Both sites are flooded beaver ponds located less than 10 km away from the Queen's University Biological Station, near Chaffeys Lock, Ontario, Canada (44° 34' N; 79° 15' W). Both support dense populations of E. boreale (in UD, M.R. Forbes estimated close to 10,000 damselflies emerging in one day, unpubl. data). JM is roughly oval in shape and ca. 1 ha. in size, whereas UD is < 1 ha. and approximately rounded in shape. The ponds at both sites have grassy margins with sedges and are bordered by mixed deciduous forests. Emergent vegetation at both sites is largely cattails (Typha spp.) and sedges (Carex spp), although many dead trees exist in JM and serve as emergence sites for E. boreale. Other descriptions of sites are presented in Forbes (1991c).

2.2.3 Collections and dissections

Larvae and adults were collected from 16 May - 12 June, 1997, and from 6 May - 23 May, 1998 (Table 2.1). Final-instar larvae (with wingpads extending to the fourth abdominal segment) were collected using an aquatic D-frame net. Adults, both pre-reproductive (tenerals) and mature adults, were caught in aerial insect nets. Adults were classified as teneral if the wings had a rainbow sheen, and the body was soft and gray to brown in colour (Walker, 1953). In comparison, reproductively mature adults had clear, hardened wings and brightly coloured, firm bodies. Over all samples, 62 larvae, 204 tenerals and 520 adults were collected (Table 2.1).

After collection, each damselfly was sexed. Following decapitation, their

complete gut was removed by grasping the posterior abdominal segment with forceps and gently pulling. The gut was then preserved in sugared ethanol (1L H₂O : 1L 95% EtOH : 40g sucrose) and stored individually in an Eppendorf microcentrifuge tube for subsequent dissection. Using a dissecting microscope, the gut was located and then dissected with 00 insect pins. The pins were used to break open the gut wall and separate gregarines from the gut and digesta. A drop of 2.5 % pharmaceutical iodine placed on the dissected gut dyed the gregarines a unique shade of dark red, increasing their contrast with the gut for accurate enumeration. The gregarine trophozoites, gamonts, and oocytes were counted. For comparison of the effects of parasites on adult sexes, 31 males and 28 females were collected on 2 June, 1997. Individuals were placed singly in plastic cups containing 2 ml of water beneath window screen mesh, and a wooden dowel for a perch. Each cup was covered with perforated aluminum foil. The cups were placed in a dark, cool room (between 14.0°C and 19.5°C) until the individuals died. As mentioned, these conditions simulated a natural period of food deprivation resulting from prolonged cool, cloudy weather (Forbes and Baker 1991). To ascertain the time of death, cups were checked every 12 hours for dead or moribund individuals. Any dead individuals were recorded and subsequently dissected in order to count their gregarines. I measured forewings of damselflies as an index of body size (Forbes 1991b).

2.2.4 Statistics

Gregarine prevalence was compared between male and female larvae and adults using 2 x 2 Chi - square tests. To allow direct comparisons between samples, I also calculated 95%

Table 2.1. Sampling schedules and numbers of damselflies collected for examining parasitism by gregarines. JM and UD refer to the two study sites: Jack's marsh and Upper Dowsley, respectively. Number of individuals captured is reported in the order (male, female).

Year	Site	Day(s)	Numbers of male and female		
			larvae	teneral	adults
1997	JM	16-20 May	30, 32		
		30 May		43, 37	
		2 June		16, 45	42, 36
		11 June			37, 50
	UD	2 June		24, 39	52, 61
		12 June			50, 40
1998	JM	19-23 May			88, 64
TOTALS			30, 32	83, 121	269, 251

confidence limits for prevalence of infections, following Snedecor and Cochran (1980). I applied $\ln$ transformation to gregarine numbers to approximate normal distributions for intensity data and to allow parametric tests, because untransformed data on intensity of infection do not conform to normal distributions (using Shapiro-Wilks's tests). The $\ln$ -transformed data were normally distributed. I then used unpaired t - tests or 2 - way ANOVA to compare mean intensity between sexes of larvae or sexes of adults, for each site individually. For the survivorship experiment, I performed linear regression with the number of days survived versus $\ln$ (number of gregarines), and used one-way ANCOVA to compare the effect of gregarines on survivorship between the sexes, controlling for wing length as a covariate (Zar 1996).

2.3 Results

2.3.1 General observations

Damselflies containing gregarines did not appear to be unhealthy, or damaged. Once the damselfly gut had been removed, gregarines often could be seen through the gut wall.

The parasites were most often in the mid-gut, but for a few individuals, gregarines were found in the hindgut, and for 1 host, they were found in the foregut. Occasional presence of gregarines in the hindgut and foregut may be an artifact of processing of individuals.

Within the hosts, gregarines were at one or all of the various stages in their life cycle (see above). Trophozoites were normally distributed in size, and ranged from 0.3 mm to 2.5 mm ($n = 91$).

2.3.2 Prevalence and intensity of parasitism for larvae and newly-eclosed adults

Larvae at JM had a high prevalence of gregarine infection overall ($91.9 \pm 6.7\%$ infected). I found no significant difference between larval males and females in either prevalence (Table 2.2) or intensity of infection (Table 2.3). On average, male and female larvae had approximately 12 and 15 gregarines, respectively. Results from recently eclosed (or teneral) damselflies were illuminating. They showed a low overall prevalence of infection by gregarines ($20.0 \pm 5.5\%$) as compared to larvae. Male and female tenerals did not differ in prevalence or intensity of infection at JM or UD (Table 2.2).

2.3.3 Prevalence and intensity of parasitism for mature adults

Over all samples, mature damselflies had a high prevalence of infection ($64.4 \pm 4.9\%$); females were more likely to be parasitized than males once all samples were combined ($73.3 \pm 6.3\%$ versus $55.2 \pm 7.2\%$ respectively, $\chi^2 = 13.0$, $p < 0.001$). However, this aggregate statistic is somewhat misleading. The 95% confidence limits around prevalence for males and females did not overlap in only one of four site-by-date samples in 1997; namely, females had significantly higher prevalence of gregarines than males only for the first sample (2 June) at UD (Table 2.4). There also were no sex differences in intensity of parasitism by gregarines for mature adults in any site-by-date comparison (Table 2.5). This same result was obtained when a 2-way ANOVA was performed for each site, with sex and date as main effects and ln-transformed numbers of gregarines (for infected individuals) as the dependent variable. Sex did not account for significant variation in transformed numbers of gregarines at JM ($F_{(1,82)} = 0.00012$, $p > 0.1$), and

Table 2.2. Comparison of prevalence of gregarine infection in E. boreale larvae and teneral. JM and UD refer to the study sites Jack's marsh and Upper Dowsley, respectively. Prevalence at UD was so low for teneral males as to preclude statistical tests of intensity. Prevalence is reported $\pm$ 95% confidence intervals for each sample, following Snedecor and Cochran (1980).

Stage	Site	Sex	N	Prevalence (%)	χ^2 - value	p - value
larval	JM	male	30	93.3 $\pm$ 9.1	0.15	0.69
	JM	female	32	90.6 $\pm$ 10.2		
teneral	JM	male	59	23.7 $\pm$ 10.7	0.01	0.94
	JM	female	82	23.2 $\pm$ 9.1		
	UD	male	24	4.2 $\pm$ 7.8	1.89	0.17
	UD	female	39	15.4 $\pm$ 11.2		

Table 2.3. Comparison of intensity of gregarine infection in *E. boreale* larvae and teneral. JM and UD refer to the study sites Jack's marsh and Upper Dowsley, respectively. Intensity in the one parasitized male teneral was within the 95% CI of intensity of female tenerals. BT mean refers to the back-transformed mean.

Stage	Site	Sex	N	ln(intensity) $\pm$ SE	BT mean	t-value	p-value
larval	JM	male	28	2.25 $\pm$ 0.17	9.5	-0.30	0.76
	JM	female	29	2.32 $\pm$ 0.18	10.3		
teneral	JM	male	14	1.39 $\pm$ 0.14	4.0	0.089	0.93
	JM	female	19	1.37 $\pm$ 0.20	13.9		
	UD	male	1	1.09 $\pm$ N/A	93.0	N/A	N/A
	UD	female	6	1.36 $\pm$ 0.34	83.9		

N/A signifies that there were < 2 individuals in this category, therefore meaningful comparison was impossible

neither did date ($F_{(1,82)} = 2.56, p > 0.1$). The interaction term was not significant ($F_{(1,82)} = 0.0064, p > 0.1$). At UD, however, date was significant ($F_{(1,147)} = 7.20, p < 0.01$), although neither sex ($F_{(1,147)} = 0.12, p > 0.1$) nor the interaction between sex and date were significant ($F_{(1,147)} = 0.51, p > 0.1$). A Tukey's honest significant difference test for unequal N (Zar 1996) showed that damselflies from the first UD sample (June 2) had more parasites than those from the second sample (June 12; $p > 0.01$).

I controlled for age within my sample when I examined prevalence of gregarines for males and females at JM in 1998. I only compared males and females that still retained their A. drepaniphorus Cook mites, as a way of approximating age of hosts. In that sample, prevalence of gregarine infection in females ($71.4 \pm 11\%$) was higher than, but not significantly different from that, of similar-age males ($58.6 \pm 10\%$) ($\chi^2 = 2.8, p = 0.096$). The ln transformed data were close to normal (Shapiro-Wilk's $W = 0.93, p < 0.0001$), so parametric 1-way ANOVA was used to analyse these data for intensity of infection. The ANOVA had the main effect of sex, and dependent variable of ln(number of gregarines). There was no significant difference between males and females in mean intensity (mean numbers of gregarines ($\pm$ S.E.) for males = 10.1 ± 2.7 ; females = 9.3 ± 1.4 ; $F = 0.56, p > 0.1$).

2.3.4 Survivorship

Intensity data, for individuals used in the survivorship experiment, were not normally distributed. However, ln-transformed gregarine numbers were normally distributed (Shapiro-Wilk's $W = 0.9, p < 0.08$), so parametric regression analyses were used. Males

Table 2.4. Comparison of prevalence of gregarine infection between E. boreale adult males and females. JM and UD refer to the study sites Jack's marsh and Upper Dowsley, respectively. Prevalence is reported $\pm$ 95% confidence intervals for each sample, following Snedecor and Cochran (1980).

Site	Date	Sex	N	Prevalence (%)	χ^2	p-value
JM	2 June, 1997	male	42	14.3 $\pm$ 10.5	0.01	0.93
		female	36	13.9 $\pm$ 11.3		
	11 June, 1997	male	37	78.4 $\pm$ 13.3	3.32	0.069
		female	50	92.0 $\pm$ 7.52		
UD	2 June, 1997	male	52	48.1 $\pm$ 13.6	17.9	0.000
		female	61	85.2 $\pm$ 8.9		
	12 June, 1997	male	50	80.0 $\pm$ 11.1	0.38	0.54
		female	40	85.0 $\pm$ 11.1		
JM	19-23 May, 1998	male	88	58.6 $\pm$ 10.0	2.8	0.096
		female	64	71.4 $\pm$ 11.0		

Table 2.5. Comparison of intensity of gregarine infection between E. boreale adult males and females. JM and UD refer to the study sites Jack's marsh and Upper Dowsley, respectively. BT mean refers to the back-transformed mean.

Site	Date	Sex	N	ln(intensity)±SE	BT mean	t-value	p-value
JM	2 June, 1997	male	6	1.65±0.44	5.2	-0.043	0.97
		female	5	1.67±0.26	5.3		
	11 June, 1997	male	29	2.21±0.20	9.1	0.42	0.67
		female	46	2.17±0.15	8.3		
UD	2 June, 1997	male	25	2.18±0.15	8.8	-0.24	0.81
		female	52	2.24±0.14	9.4		
	12 June, 1997	male	40	1.89±0.16	6.6	0.79	0.43
		female	34	1.72±0.12	5.6		
JM	19-23 May, 1998	male	52	2.31±0.14	10	2.8	0.80
		female	46	2.23±0.12	9.3		

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showed a significant and positive simple linear relationship between gregarine number and days survived ($r^2 = 0.20$, $F_{(1, 29)} = 7.3$, $p < 0.01$) (Figure 2.2). Females did not show a significant linear relationship between these variables ($r^2 = 0.089$, $F_{(1, 26)} = 2.6$, $p > 0.1$), however, the second-order polynomial relationship between days survived and number of gregarines was significant ($r^2 = 0.22$, $F_{(1, 26)} = 3.4$, $p < 0.05$) (Figure 2.2). Females, on average, survived for a longer period than males (females, 8.2 ± 0.3 days; males, 5.8 ± 0.4 ; $t = -5.02$, $df = 57$, $p < 0.000005$). A one-way ANCOVA was performed, with sex as the main effect, time survived as the dependent variable, and $\ln$ number of gregarines as covariate. I found that the relationship between number of gregarines and time survived was not significantly different for males and females (test for parallelism, $F = 0.69$, $p > 0.1$).

2.4 Discussion

My data confirm that larval sexes show equal parasitism by gregarines; this may be due to little difference in their foraging ecology. In fact, sex of odonate larvae is often not considered to be important in studies of effects of predators or population density on foraging rates and individual condition (Johnson 1991, but see Baker et al, 1992 for an exception). However, high prevalence and intensities of infection in larvae was somewhat unexpected. Åbro (1974, 1987, 1990) and others (Taylor and Merriam 1996) have not reported gregarines from larval damselflies, although gregarines have been reported and described, without intensities or prevalence, from calopterygid and other odonate larvae (Schneider 1875; Geus 1969). Secondly, I found that newly-emerged

Figure 2.2 Relationship between time survived and intensity of gregarine infection.
Females (upper panel) and males (lower panel) did not have significantly different
relationships

adult damselflies have low levels of infection, indicating that the majority of parasites are lost sometime around eclosion. This may be a result of the gregarines completing their life cycle and being excreted as infective cysts (approximately two-week life cycle; Åbro, 1976); however, it may also be due to the shedding of the gut wall of the fore- and hind- gut along with the gut contents during eclosion. Metamorphosis has not been proposed previously as a mechanism for reducing parasite numbers.

Mature adult damselflies showed seasonal changes in prevalence and intensity of infection; both increased over time. This is an expected trend, at the individual level. Gregarine cysts are ingested along with food, and they remain in the gut. As an individual damselfly eats more, it will ingest progressively more cysts, increasing the number of internal parasites (Åbro, 1976). As the season progresses, the proportion of older damselflies also may increase, thus prevalence and intensity of infection within the population increase. As mentioned, I attempted to control for age variation within samples using two approaches for approximating ages of damselflies. First, I collected at some sites at the very beginning of the flight season when the first matures arrived at the pond. I also collected E. boreale parasitized by near to fully engorged A. drepaniphorus. These hosts may be classified as relatively young since there has not been enough time for mites to detach.

I hypothesized that differences in the feeding ecology of adult males and females would cause the females to have more parasites than the males. My results partially supported this idea. Males and females differed in prevalence of gregarine infection at UD on the first sampling date, but did not differ at the later sampling date, or on either date at JM. The prevalence of infection at JM on the first sampling date was probably too

low to conduct an adequate test using these sample sizes. I suspect that the lack of difference in the later sampling dates was due to the high variability of prevalence and intensity of infection which would occur in a sample of individuals of differing ages. In the second year, I compared males and females that still retained their A. drepaniphorus mites (as already indicated, this controls for approximate age). In that year, I found that females had a higher prevalence of gregarines; this result was not significant, but noteworthy ($0.05 < p < 0.1$). Intensity of infection did not differ between males and females in that sample, as found for the early sample at UD in the previous year.

My results, while seemingly variable, can be explained in the following manner. I found evidence of sex differences in intensity of gregarine infection, but only for two samples where prevalence of gregarines was high and for which relatively young males and females were compared (in truth, only one of these comparisons was significant, the other approached significance). I did not necessarily expect males to differ from females for samples collected later in the season in the first year. At that time, ages of hosts were not controlled and variance of intensity of infection may be so high at this time as to mask any relations that do exist (R. Clopton, pers comm.). The other early sample was plagued by lower overall prevalence, but if the effect of foraging ecology on exposure to gregarines was strong, I should have detected a sex difference in intensity (and I did find weak support for a sex bias). Thus, a sex difference in intensity of gregarines (if real for E. boreale) is difficult to detect, using these methods. Perhaps more importantly, the difference is in the likelihood of becoming infected (prevalence), though there may be less difference in the numbers of gregarines once infected (as my data on intensity of infections indicate).

The final part of my study concerned what effect gregarines might have on their hosts. Parasites that exert sublethal effects, or weaken their host only under certain detrimental conditions, may have a subtle and largely unrecognized influence on natural selection on a population (Levin and Pimental 1981). A common way of testing effects of parasites on hosts is to subject the hosts to stressful conditions, and measure longevity relative to their degree of infection (Zuk 1987). In my study, I found a significant positive relationship between number of gregarines and survivorship for both sexes. Condition of an individual damselfly is influenced by the quantity of food eaten. Gregarines, as parasites, harm the host to some extent. However, few species of damselfly show obvious effects of parasitism by gregarines (Åbro 1987) unless intensity of infection is very high (Åbro 1971, 1974). If the amount of food eaten by a damselfly is roughly proportional to the number of gregarines ingested (as I assume it is), and the intensity of infection by gregarines is relatively low, then as the individual host eats more food it ingests more gregarines. However, the host's condition will improve more from the nutrients and fat storage than it would decline from the effects of the gregarines (this point has been raised recently by Lafferty 1992). Importantly, the slope of the relationship between number of gregarines and survivorship was not significantly different for males and females, suggesting that the sexes were not affected differentially by gregarines. Yet, females, on average, lived longer than males, potentially due to greater fat reserves. Experiments with a related species (Enallagma ebrium) showed that males survived less well than females under semi-natural conditions (Forbes and Leung 1995).

That males survived less well than females is important for two reasons. It is

entirely consistent with the idea that males take risks with energy acquisition, and it also sheds light on an important issue in testing for sex differences in parasitism. That is, if males survive bouts of cool rainy weather less well than females, the populations sampled following these bouts will contain proportionately more older females than older males and this may give the impression of a sex bias in parasitism by gregarines. This result may explain the nearly significant sex bias found at UD on the second sampling date in 1997.

Natural infections of gregarines in E. boreale were not lethal, or even obvious. This contrasts with many authors who found gregarines to be detrimental to their hosts. Siegel et al (1992) showed a correlation between gregarine infection and increased mortality in the mosquito Aedes triseriatus. Gregarines also contributed to reduced average longevity in some damselflies (Åbro, 1971). Åbro (1974) found that Coenagrion hastulatum (Charp.), Enallagma cyathigerum (Charp.), and Pyrhosoma nymphula (Sulz.) were heavily infected, and feeble, discoloured specimens exhibited extensive damage to the midgut, which likely progressed to bacterial septicemia (also, Åbro 1990). However, in the same study, Sympetrum danae was not affected by gregarines. Åbro (1987) also claims that there is gregarine-induced susceptibility to predation in some species of damselfly, as infected individuals are more feeble fliers. The effects of gregarines appear to be widely variable, as well (Åbro 1974; Taylor and Merriam 1996). My results seem to be specific to my study species. If gregarines do harm E. boreale, the effects are subtle, and likely masked by other factors, such as age of host, and seasonal or environmental factors.

CHAPTER 3

Parasitism of Nehalennia irene (Hagen) damselflies by gregarines: testing for sex and morph biases, and relations to adult survival.

3.1 Introduction

3.1.1 Background for study

In Chapter 2, I investigated whether sexes of Enallagma boreale predictably differ in either prevalence or intensity of parasitism, or in the fitness costs associated with parasitism by gregarines. I did not find differential fitness costs, but found some support for a sex bias in parasitism that may be explained, in part, by differences in exposure to infective stages of gregarines. I further noted that seasonal changes in parasite prevalence and changes in age structure of the male and female subpopulations could both obscure sex-related biases in parasitism and, in some cases, make them apparent.

In this Chapter, I test the same ideas concerning sex biases in parasitism by gregarines using another Coenagrionid damselfly, Nehalennia irene. I also extend the questions to include female morphs of this species. In this introduction, I first describe similarities and differences between the ecology of these two host species and their bearing on predictions concerning sex biases in parasitism for N. irene. I then outline our general understanding of factors favoring polymorphism in populations before illustrating specifically what is known about costs and benefits to female morphs of N. irene.

Finally, I highlight why this species was chosen to examine potential morph differences in parasitism, or fitness costs associated with parasitism.

N. irene, like E. boreale, has a mating system characterized by "scramble mate competition", where males are indiscriminate in their sexual activity (as in Fincke 1994b). Unlike E. boreale, sex ratios at ponds are not heavily biased toward males (see Forbes et al. 1995). N. irene males also show contact mate guarding, which means that single males are unlikely to dislodge males already in tandem with females. However, single females are often the objects of unwanted male attention, and non-receptive females show a suite of male rejection behaviours (Forbes et al. 1997). These male rejection behaviours appear to be expressed more readily by one of the female morphs (the andromorph; coloured and patterned like the male). This morph shows a greater overlap in habitat use with males than does the other female morph (the heteromorph; coloured and patterned cryptically), although compared to E. boreale, both female morphs of N. irene utilize habitats close to the pond (where the density hosts, and therefore of infective cysts, should be highest).

As mentioned in Chapter 2, females eat copious quantities of prey items to increase their fecundity, while males eat as little as possible to increase their mate-searching success. Andromorphs spend the majority of their time at the very edge of the pond, where the density of infective cysts should be highest, while heteromorphs spend their time in an area with lower densities of infective cysts. I predict that females will have higher rates of parasitism than same or similar-aged males, and this will be more pronounced for the andromorph. The heteromorph will still have greater rates of parasitism than the males, because the high foraging rate contributes more to rate of

infection than does the proximity to infective gregarine cysts.

As indicated, I am also interested in whether the female morphs differ in measures of parasitism by gregarines, or in the fitness costs associated with parasitism (another reason for the survivorship experiments). Before detailing specific predictions with respect to female morphs, I first describe the rationale for investigating differences between morphs in parasitism.

Polymorphism is the existence of two or more discrete, genetically determined forms within a population that differ in specific proteins or enzymes, colours, and shapes. These forms are not due to variations in sex or maturity, and are more frequent than can be explained by recurrent mutation (arbitrarily set at $> 5\%$ frequency in the population) (Pough et al. 1996). Researchers have proposed that frequency-dependent selection imposed by parasites may partly or wholly explain polymorphism in host populations (Hamilton and Zuk 1982); although other explanations exist (e.g., strains of the larch bud moth (*Zeiraphera diniana*) exhibit colour polymorphism associated with important life history traits, such as egg development and survival rates (Baltensweiler 1993)).

Damselfly (Zygoptera) populations often exhibit coexistence of two or more female colour morphs (Walker 1953). Generally, the “andromorph” (or androchromatype) is coloured and/or patterned like the male, while the “heteromorph” (or heterochromatype) is unlike the male in pattern or colour, often cryptically coloured (Hilton 1987, Cordero 1990). Studies of colour polymorphism in female damselflies show that it is a heritable trait (e.g., diallelic single locus with andromorphy recessive: Johnson 1964, Johnson 1966; but see Cordero 1990). However, the origin of this polymorphism is unknown, as is the mechanism that maintains it (although the

mechanism is debated, see Forbes et al, 1995). Most researchers attempting to explain female-limited polymorphism in Odonates and Lepidopterans (the only other insect order where the phenomenon occurs) have focused on costs or benefits imposed by mate-searching males on one morph, versus supposed higher predation rates on either that same morph (butterflies: Vane-Wright and Ackery 1984), or the other morph (damselflies: Johnson 1975).

Whether differential treatment by males results in different costs to the female morphs has been debated (Thompson 1989, Cordero 1992, Forbes 1994, Fincke 1994a 1994b, Cordero 1998). Forbes et al. (1997) conclude that in N. irene, differential harassment of females can occur, and that differential use of habitats or sites by female morphs is expected if male harassment to females is costly and falls disproportionately to one morph. However, the andromorphs seem to gain all of the benefits at high population densities, while the heteromorph is continually at a disadvantage (see also Table 1 in Cordero 1992). Yet in N. irene, the andromorph remains the minority morph at both low- and high-density sites.

Interestingly, some researchers have found that at high population densities, prevalence of parasites with direct transmission increases (Anderson and May 1981). This leads to the interesting possibility that one morph increases at high population densities because of advantages in social interactions, but is thwarted from further increases by differential exposure or sensitivity to parasites.

3.1.2 Specific predictions

I predicted that females would show higher rates of gregarine parasitism than same or similar aged males. It is clear from Chapter 2 that this prediction is difficult to test with natural populations of zygopterans, where spatial or temporal variation in parasite prevalence and structure of age cohorts exist. It would also be illuminating to see if sexes differed in their survivorship relative to one another under conditions of food limitation as found for E. boreale. I expected that males would be shorter-lived, as in E. boreale. This may account for occasional sex biases in age cohort representation and degree of parasitism.

Circumstantial evidence from previous studies show that heteromorphs are harassed by males more than andromorphs (Johnson 1975, Robertson 1985, Hinnekint 1987), and that andromorphs avoid unnecessarily long matings (Robertson 1985). Andromorphs have not been proven to have a greater risk of predation than heteromorphs (Thompson 1989). All benefits thus far appear to be for the andromorph, which should lead to natural selection for andromorphs. However, even at sites with high male density, which had high proportions of andromorphs, the percentage of andromorphic females in the population never rose above 28.1% (Forbes et al., 1995), which hints at some mitigating factor that is specifically detrimental to the andromorphs.

Importantly, Åbro (1976) showed that gregarines were excreted by the damselflies, underwent a period of maturation, then were picked up by another host individual. Because the cysts are excreted, areas with high damselfly density should have high density of infective cysts. Edges of the pond or marsh are where mate-searching

males are in highest density would, therefore, have the highest density of infective cysts. Forbes et al. (1995) found that proportionately more andromorphs than heteromorphs were found at the edges of ponds, where male density was high. Both of these factors should contribute to rates of parasitism. If both andromorphs and heteromorphs are maximizing their food intake, the andromorphs may experience substantially higher levels of parasitism. Amount of food ingested likely has more effect on parasite loads than density of infective cysts in foraging area. This factor could create balancing selection on the andromorph, causing the 30% threshold that andromorphs never seem to pass.

Sites with high population density should have the highest densities of infective cysts. Many parasites affect the hosts most severely when the host is stressed (Zuk 1987), and high density population is generally regarded as a stressful situation (Wallace et al. 1991). Taking these three ideas into consideration, I expected that differences in parasitism between males, heteromorphs, and andromorphs would be most easily detected at high density sites. My prediction was that all damselflies would have the greatest infections at high density sites, ranked within site from andromorph (greatest) to heteromorph, to males (least). I sampled sites with a gradient of damselfly densities, to determine whether host population density had an effect on measures of parasitism. I also examined the data for existence of seasonal trends in parasitism (for reasons outlined in Chapter 2), expecting similar patterns as found in E. boreale.

When indirectly measuring the costs of parasites on the overall fitness of N. irene, using a survivorship experiment, I predicted that males and andromorphs would be more severely affected than heteromorphs. Males, as mentioned above, may take greater risks

when acquiring energy, in order to maximize the time spent mate-searching. This may lead to shorter lifespans for males than females, which could cause occasional sex biases in parasitism, simply because more old, heavily parasitised females are in the population. If gregarines are more costly to andromorphs, that could help to balance the overall fitness of the two female morphs.

3.2 Materials and Methods

3.2.1. Study Species

Nehalennia irene is a small damselfly (body length 25-28mm; Cannings and Stuart 1977, in Forbes et al. 1995) that is ubiquitous in most of Canada (Walker 1953). *En mass* emergence generally begins in the first week of June. The teneral stage (when the adult exoskeleton is soft and brownish, the soft wings have a rainbow sheen, and the adult is reproductively immature) is generally very short in N. irene (< 24 hours) (Walker 1953). As indicated, female Nehalennia irene occur as two distinct colour morphs, which are not age-related (Forbes et al., 1995). The morph that is male-like in colour and pattern is termed the “andromorph”. Nehalennia irene andromorphs have blue markings on the abdomen and thorax, in contrast to the “heteromorphs”, which are more cryptic. The ventral side of the abdomen and thorax is pale yellow in heteromorphs. Forbes et al. (1995) found that mean head widths do not differ between andromorphs and heteromorphs in Nehalennia irene. This lack of difference in structural size corresponds with findings in female morphs of other zygopteran species such as Enallagma boreale

(wing length, head width; Forbes 1994), and Ischnura graellsii (body length; Cordero 1992). Therefore, the genes that control colour and pattern polymorphism do not appear to have pleiotropic effects on body size, nor are they linked to the genes that affect body size. Andromorphs of Nehalennia irene are less frequent in the population than heteromorph (2.1 - 28.1%, Forbes et al. 1995).

3.2.2 Gregarines

The parasites in this study are readily identifiable as Gregarines (Apicomplexa: Eugregarinida) (Sleigh 1989). Although more specific keys to the taxonomy of Eugregarine parasites of damselflies are under construction (R. Clopton pers. comm., R. Clopton in press), all have similar life cycles and characteristics (Grell 1973, Sleigh 1989). The gut parasites in N. irene are likely Hoplorhynchus spp, and one as yet unidentified genus (R. Clopton pers.comm). They have three distinct body parts and are thus septate gregarines in the Class Eugregarinidae. Gregarines are intestinal parasites of most of the Arthropods (Sleigh 1989), including cockroaches and crickets (Orthoptera), beetles (Coleoptera), moths (Lepidoptera), and dragonflies (Odonata). The gregarine life cycle is univoltine, with no free-living stage.

Gregarines in their infective stage (oocytes) are ingested, along with insect prey, by foraging damselflies. Mid-sized chironomid midges and small Lepidopterans have been found with gregarine oocysts stuck between the setae on their legs (Åbro 1976); such insects can be completely macerated by damselflies before they are ingested as a

bolus, along with the entire oocytes. The method by which this parasite's infective stages become attached to the tarsal setae of damselfly prey is not known (Åbro 1976). Once in the midgut, the oocytes split to produce numerous sporozoites (eight sporozoites per oocyte, Åbro 1976). The sporozoites then attach to the gut wall as trophozoites, and absorb digesta. After growing in size for some time, trophozoites detach from the gut epithelium, and develop into gamonts that move about freely in the gut. Gamonts proceed to find a "mate", and undergo syzygy, after which the two attached individuals are enclosed in a protective covering, and become a gametocyst. Approximately two weeks (the actual amount of time is temperature dependent; Åbro 1974) after ingestion of the sporozoites, the gametocysts are excreted by the host. Each gametocyst undergoes development and eventually releases many oocysts (the infective stage); the proper development of oocysts may include an obligatory period of cool temperatures (Åbro, 1976).

Previous studies have determined the impact and effect of gregarines on many species of Odonate (Åbro 1974, 1976, 1987, 1990, 1996; Taylor and Merriam, 1996). Some damselfly-gregarine interactions are visibly detrimental to the damselfly (Åbro 1971, 1974), yet others seem to cause the host little distress (Åbro 1987).

3.2.3 Study sites

Five sites were chosen for the comparison of parasitism between males and female morphs, in order to have a gradient of andromorph : heteromorph : male proportions. These five flooded beaver ponds were within a 10km radius of the Queen's University

Biological Station, near Chaffey's Lock, Ontario, Canada (44° 34' N; 79° 15' W). Lindsey Lake Marsh (LLM) is >1 ha. in size at the southern edge of Lindsey Lake, beside a small grassy area. Barb's Marsh (BM) is approximately 1 ha. in size, and adjoins a large hay field. Elgin Pond (EP) is <1 ha. and has a marshy circumference, with mature oak forest and grassy understory surrounding it. Upper Dowsley Lake (UD) is < 1 ha and approximately rounded in shape, with a grassy margin (including sedges) bordered by mixed deciduous forests. Indian Lake Bight (ILB) is a reedy lake with shallow water, and a small grassy verge along the eastern side. The emergent vegetation at all sites is mainly Typha spp. and Carex spp., and dead trees protrude from the surface of the water, providing emergence sites for eclosing Nehalennia irene.

3.2.4 Infection Rates

Reproductively mature adults were collected from 16 June - 27 July, 1997 (Table 3.1). Nehalennia irene were sweep-netted and sexed, and further classified as andromorph or heteromorph (as described above). After decapitation, the entire body was weighed. The gut was removed by gently pulling on the posterior abdominal segment with forceps until the entire digestive tract emerged. The gut was then preserved in sugared ethanol, an inexpensive, osmotically balanced preservative (1L H₂O : 1L 95% EtoH : 40g sucrose), and stored in an eppendorf microcentrifuge tube. Using a dissecting microscope, the gut was located and then dissected with 00 insect pins. The pins were used to separate gregarines from the gut wall and digesta. During dissection, a drop of 2.5% pharmaceutical iodine was placed on the specimen to increase contrast between

gregarines and tissue. The gregarine trophozoites, gamonts, and gametocysts were enumerated. Data from individual sites were analyzed with two-way ANOVA, with main effects of sex/morph and site, and dependent variable of $\ln(\text{number of gregarines})$. Then, the data were analyzed using ANCOVA, with sex/morph, and sampling site as the main effects, $\ln(\text{number of gregarines})$ as the dependent variable, and date as covariate. The transformed data were normally distributed.

3.2.5 Density

Samples to determine the density of N. irene were taken in the 1997 field season. Simple density samples were taken at four locations: ILB, BM, EP, and LLMS. UD was not sampled. Each site had between three and five 3.3 m x 3.3 m quadrats at similar distances from the water's edge. Each quadrat was sampled by sweep-netting as many individuals within the quadrat as possible in a five-minute period. This method was used to standardize both the area sampled, and the time sampled, between sites. These density estimates were compared among sites using a one-way ANOVA.

3.2.6 Survivorship

Adult males and females were collected to determine the effects of parasites on the sexes. The individuals were placed singly in plastic cups containing 2 ml of water beneath window screen mesh, and a wooden dowel for a perch. Each cup was covered with perforated aluminum foil. The cups were placed in a dark, cool room (14.0°C - 19.5°C)

until individuals died. These conditions simulated a natural period of food deprivation resulting from prolonged cool, cloudy weather (Forbes and Baker 1991). To ascertain the time of death, cups were checked every 12 hours for dead or moribund individuals. Any dead individuals were recorded, weighed, and subsequently dissected to count gregarines. This experiment was repeated three times and the effects of various factors on survivorship were tested in an ANCOVA.

3.3 Results

3.3.1 Infection Rates

Body size (measured by wet mass of the damselfly) was not correlated with gregarine load for males ($F_{(1, 527)} = 0.22$, $p = 0.64$, $r^2 = 0.0004$), but was correlated with gregarine load for females, ($F_{(1, 579)} = 5.44$, $p = 0.02$, $r^2 = 0.009$). While andromorphs showed no correlation between gregarine load and mass ($F_{(1, 91)} = 1.02$, $p = 0.32$, $r^2 = 0.01$), heteromorphs did show a correlation ($F_{(1, 484)} = 4.14$, $p = 0.043$, $r^2 = 0.008$). However, in both significant correlations, the r^2 was so low as to be biologically meaningless.

Andromorphs, overall, had greater prevalence of infection than heteromorphs (andromorph = $59.6 \pm 10\%$, heteromorph = $43.0 \pm 4\%$, confidence intervals do not overlap: these are the sum of all site x date samples). When each site-by-date sample was tested independently, however, only one sample showed significant difference between the morphs (June 23, LLM; andromorph = $64 \pm 28\%$, heteromorph = $20 \pm 12\%$, confidence intervals do not overlap; Table 3.1), and there was no trend among other

sampling dates. Other samples of males and females were statistically indistinguishable from each other (confidence intervals overlapped for all). Overall, males and females had similar prevalences of infection (male = $47.45 \pm 4.3\%$, females = $45.52 \pm 4.1\%$, confidence intervals overlap). This finding is representative of the general trend for sampling days (all confidence intervals overlapped; Table 3.1).

Two-way ANOVAs were performed for each site, with main effects of date and sex/morph. For one site, LLM, sex/morph accounted for significant variation in intensity of infection ($F_{(1, 114)} = 4.84$, $p = 0.0096$), and post-hoc Tukey's honest significant difference (HSD) test for uneven N showed that andromorphs had significantly greater intensity of infection than heteromorphs ($p = 0.0056$) and males ($p = 0.014$). Males and heteromorphs did not have significantly different intensity of infection ($p = 0.79$). The rest of the sites did not support the hypothesis of differences in intensity of infection among males, andromorphs, and heteromorphs, although the results were close to significant (BM, $F_{(2, 106)} = 3.02$, $p = 0.053$; EP, $F_{(2, 125)} = 2.83$, $p = 0.063$; ILB, $F_{(2, 100)} = 2.67$, $p = 0.074$; UD, $F_{(2, 37)} = 1.71$, $p = 0.19$). At all sites, date was not a significant factor (BM, $F_{(2, 106)} = 2.94$, $p = 0.057$; LLM, $F_{(2, 114)} = 0.83$, $p = 0.44$; EP, $F_{(2, 125)} = 2.95$, $p = 0.056$; ILB, $F_{(2, 100)} = 0.0064$; UD had only one sampling date), and neither was the date by sex/morph interaction (BM, unable to calculate; LLM, $F_{(4, 114)} = 1.23$, $p = 0.31$; EP, $F_{(4, 125)} = 0.22$, $p = 0.93$; ILB, $F_{(4, 100)} = 1.09$, $p = 0.34$); UD had only one sampling date). ANCOVA was then performed using data from the week when density measurements were taken (3 July 1997 - 12 July 1997), with main effects of sex/morph and site, the dependent variable of $\ln(\text{intensity of infection})$, and the covariate of date. Over all sites,

Table 3.1. Measures of parasitism for Nehalennia irene over thirteen date-by-site samples. Confidence intervals were calculated following Snedecor and Cochran (1980). ANDRO = andromorphs, HETERO = heteromorphs.

DATE	SITE	Prevalence			+/- 95% CI			Intensity			+/- S. E.		
		MALE	N	ANDRO	N	HETERO	N	MALE	N	ANDRO	N	HETERO	N
17 Jun, 1997	BM	19±9%	64	0	1	27±11%	60	9.8±3.6	12	N/A	0	9.0±2.3	16
22 Jun, 1997	UD	48±14%	46	30±28%	10	34±2%	44	7.9±1.6	22	2.0±1.0	3	7.7±1.7	15
23 Jun, 1997	LLM	28±20%	47	64±28%	11	20±12%	44	6.2±1.3	13	9.1±2.8	7	6.1±3.5	9
26 Jun, 1997	EP	45±16%	38	60±43%	5	42±14%	45	7.8±1.6	17	9.3±3.1	3	14.7±3.5	19
28 Jun, 1997	ILB	54±15%	41	55±10%	11	41±19%	27	13.1±3.2	22	5.5±1.8	6	8.4±2.1	11
3 Jul, 1997	BM	60±15%	44	74±18%	23	76±18%	21	5.8±1.2	26	15.0±5.1	17	12.8±2.7	16
10 Jul, 1997	LLM	69±14%	45	33±53%	3	47±16%	38	9.1±2.9	31	26±N/A	1	4.2±3.5	18
11 Jul, 1997	EP	70±14%	40	67±53%	3	67±14%	43	7.6±2.7	28	3.0±1.4	2	12.1±5.4	29
12 Jul, 1997	ILB	70±13%	44	72±21%	18	61±15%	38	10.3±2.7	31	16.2±7.8	13	19.0±5.4	23
20 Jul, 1997	BM	30±16%	33	0	1	38±15%	42	5.1±1.7	10	N/A	0	34.3±28.0	16
21 Jul, 1997	LLM	49±13%	55	50±49%	4	38±15%	40	3.9±0.1	27	34.0±32.0	2	8.6±5.0	15
24 Jul, 1997	EP	38±17%	32	50±49%	4	50±15%	44	5.3±1.9	12	1.5±0.5	2	15.8±6.7	22

sex/morph was a significant factor ($F_{(3, 223)} = 4.65$, $p = 0.01$) explaining the variation in these samples. Post-hoc Tukey's HSD test for unequal N showed that males had lower intensity of infection than heteromorphs ($p = 0.033$) or andromorphs ($p = 0.020$) for the week when density was sampled. Site did not account for significant variation within intensities ($F_{(3, 223)} = 1.77$, $p = 0.15$). The interaction between sex/morph and site was not significant ($F_{(6, 223)} = 1.74$, $p = 0.11$). Prevalence of infection (subsampled; one sample from each site from 3 July 1997 - 12 July 1997) did not differ between sites (See Table 3.1).

3.3.2 Seasonal Biases

Season biases for rates of infection were different from those in E. boreale (Chapter 2). The beginning of the season was characterized by low prevalence, which increased non-significantly though the season, decreasing near the end (see Table 3.1). Unlike the trend for E. boreale (see Chapter 2), intensity of infection simply increased slightly (though not significantly) throughout the season (see Table 3.1).

3.3.3 Density

A one-way ANOVA with main effect of site and dependent variable of density of N. irene showed that the site accounted for significant variation in density of damselflies ($F = 4.69$, $p < 0.05$; samples taken within one week for all four sites, 3 July, 1997 - 12 July, 1997). Post-hoc Tukey's honest significant difference test for samples with uneven N

showed that ILB had significantly higher density than BM ($p = 0.039$) and EP ($p = 0.05$), however LLM was not significantly different from either ILB or BM, and EP was not significantly different from BM (Figure 3.1).

The highest density of males was at LLM (5.0 ± 1.7 individuals/sweep), and lowest density of males was at EP (1.7 ± 0.43 individuals/sweep). Density of andromorphs was highest at ILB (3.6 ± 0.87 individuals/sweep), and lowest at LLM (0.1 ± 0.11 individuals/sweep). Heteromorphs were in greatest numbers at ILB (4.2 ± 1.22 individuals/sweep), and at lowest concentrations at EP (1.4 ± 0.67 individuals per sweep).

3.3.4 Survivorship

A one-way ANOVA was performed with a main effect of sex/morph, and a dependent variable of hours survived. Sex/morph had a significant effect on longevity ($F_{(2, 180)} = 6.03$, $p = 0.0029$; Table 3.2). Post-hoc Tukey's HSD tests for unequal N showed that males did not live as long as andromorphs ($p = 0.019$) or heteromorphs ($p = 0.031$), while andromorphs and heteromorphs did not differ significantly from each other in longevity ($p = 0.58$).

I compared the relationship between number of gregarines and hours survived for males, andromorphs, and heteromorphs. The regression was not significant for males, andromorphs, or heteromorphs in any trial. Neither was that "relationship" different among the males and two female morphs; ANCOVA, with sex/morph as the main effect, hours survived as the dependent variable, and $\ln(\text{number of gregarines})$ as the covariate, showed no difference (test for parallelism, $F(3, 177) = 0.94$, $p = 0.39$).

3.4 Discussion

3.4.1 Infection rates

Forbes et al. (1995) found that andromorphs and heteromorphs showed differences in foraging ecology; andromorphs were more likely to be at the edge of the pond. Also, Anholt (1992) found that for some Coenagrionid damselflies, males differ in foraging ecology from females; males are time minimizers, and females are energy maximizers. I assumed that foraging ecology played a role in proportional transmission of ingested parasites (in this case, gregarines) (following Åbro 1976, Zuk 1987, Taylor and Merriam 1996). My data show that although male and female Nehalennia irene are equally likely to become infected, the females, once infected, accumulate more parasites than the males. At several sites, there is a tendency for females to have more gregarines than males (the data are close to significant; $p = 0.053$ to $p = 0.19$). When replicated over a number of sites this tendency produces significant overall results, even though significance might not have been discovered in most site-by-date comparisons. This supports my prediction based on different foraging habits for males and females, namely that females, as “energy maximizers” eat more prey, and incidentally infective gregarine cysts, than same or similar-aged males, which are “time minimisers” as described in the introduction.

However, contrary to my hypothesis, andromorphs are equally likely to be infected as heteromorphs, and the two female morphs also have similar average numbers of gregarines, once infected. This was unexpected, particularly at ILB, the site with highest damselfly density. However, this paradox can be explained if my assumptions

Figure 3.1. Total density of Nehalennia irene damselflies at four sampling sites. Density is measured as number of individuals sweep-netted in 5 minutes in a 3m x 3m quadrat.

Table 3.2. Differences in survivorship times of males and female morphs of Nehalennia irene - three different trials. Mean = mean number of 12-hour periods survived. ANDRO = andromorphs, HETERO = heteromorphs.

TRIAL	Mean $\pm$ SE					
	MALE	N	ANDRO	N	HETERO	N
1	11.5 $\pm$ 0.7	22	13.0 $\pm$ 0.9	9	13.4 $\pm$ 1.0	21
2	5.6 $\pm$ 0.6	19	10.3 $\pm$ 1.3	7	7.5 $\pm$ 0.4	28
3	6.3 $\pm$ 0.3	33	8.7 $\pm$ 0.5	17	8.0 $\pm$ 0.6	28
Combined	7.7 $\pm$ 0.4	74	10.2 $\pm$ 0.6	33	9.3 $\pm$ 0.5	77

about transmission of the parasites were incorrect. Åbro (1976) showed that infective gregarine cysts were likely ingested along with insect prey such as chironomids. Along with that explanation, I assumed that areas with high density of infective cysts would produce a higher proportion of prey items that had gregarine cysts in their tarsal setae. If the small chironomids actually move between the areas where male and female damselflies spend their time, this assumption would be invalid. Males and females would still show differential parasitism, but the female morphs would be indistinguishable in rates of parasitism.

3.4.2 Seasonal biases

N. irene showed seasonal variation in prevalence of infection, a confounding variable which needs to be taken into consideration. This is a typical pattern in seasonal parasites (Zuk, 1987, Arnqvist and Mäki 1990). Damselflies emerge *en mass* at certain times throughout the species' flight season, so at the start of the season the cohorts may be predominately young individuals, and at the end of the season the majority of cohorts may be old, but as the season progresses, either old or young cohorts may predominate depending on mortality of specific age classes. This may cause misleading trends in the data from point counts of parasitism, namely that if one sex or morph has a shorter average lifespan, any differences in prevalence may be due to the skewed age ratio in the population, not foraging ecology. I was not able to control for age in N. irene, as conventional aging methods were either difficult and inconsistent (cuticular sectioning, Neville 1967, Zuk 1988), or unavailable for this species (ectoparasitic mite engorgement,

see Chapter 2). Although the prevalence of infection shows seasonal trend, the intensity of infection does not. This may indicate that ages are similar between groups in the population, as the gregarines are, theoretically, accumulated over time. Åbro (1990) found that in all Zygoptera, the load of gregarines increased with advancing adult age, owing to recurrent ingestion of infective gregarine oocysts.

3.4.3 Survivorship

If a parasite is indirectly costly to a host, the individual host may lose resources that would generally be allocated to its survival in trying environmental conditions (Anderson and May 1981). Based on this idea, I collected adult damselflies and subjected them to conditions simulating a natural period of food deprivation resulting from prolonged cool, cloudy weather (Forbes and Baker 1991). An incidental observation from these survivorship trials was that females lived longer, on average, than males. This is potentially due to differences in fat reserves, as females store more fat than males; I found similar results in Chapter 2, and experiments with other Coenagrionid species also showed that females survived longer than males (*Enallagma ebrium*, Forbes and Leung 1995, *Lestes disjunctus*, Anholt 1997). Although females lived longer than males, I found that the number of parasites did not influence longevity for males, females, andromorphs, or heteromorphs. There was also no evidence that gregarines are more costly to one sex or female morph than to the other in *Nehalennia irene*. Using this approach, gregarines seem to have no detectable effect on the damselflies, either beneficially or detrimentally. This begs the question whether gregarines are in fact

parasites of these damselflies, as one of the conditions for classification of an organism as a parasite is that the organism must “harm” its host in some way (Anderson and May 1981).

Anderson and May (1981) state that prevalence of infection is inversely related to pathogenicity of the parasite. Parasites of low pathogenicity are likely to be very prevalent but to have little effect of the population dynamics of their host (Anderson and May 1981). Nehalennia irene, in this study, showed overall prevalence of infection around 45%, which is a moderate level of infection. My survivorship experiment showed that gregarines had little effect on the longevity of stressed individuals. Perhaps the reason that I found no difference between males and females, and female morphs is that the effect size for the impact of gregarine parasites is so small as to be biologically irrelevant. This conclusion is contradicted by studies showing gregarines affecting Odonates detrimentally (Taylor and Merriam 1996), even causing death (Åbro 1971, 1974, 1987, 1990). Åbro (1990) even found that male and female Coenagrion puella had different intensities of parasitism, however, this study showed no statistical results, and details given were too few to determine whether this relationship was significant.

3.4.4 Influence of host population density

Forbes et al. (1995) found that the proportion of andromorphs in the population was correlated with the density of males at that study site. I, also, found that the site with the highest overall population density had the highest proportion of andromorphs (ILB, 33% of the population; this is one of the eight sites studied in Forbes et al. 1995).

Theoretically, parasites do well when the host population is under stress or at high density (Anderson and May 1981). If there was an effect of foraging ecology on parasitism, and the foraging ecologies were different, I would likely have seen my significant results at the study sites with the highest density. These sites were ILB (highest density) and LLM (next highest density). I found no differences in the data for either site.

However, I found that the site with highest host density (ILB) did have the highest intensity of infection for males and females, although there was no correlation between intensity of infection and density of hosts overall. Prevalence of infection showed no relationship to host population density.

3.4.5 General conclusions

In some systems, polymorphism eventually disappears, because one morph has lower fitness than another, and is excluded from the population by natural selection. For Nehalennia irene, however, the least frequent morph shows no signs of exclusion. While andromorphs may be the predominant morph in some zygopteran populations (Enallagma boreale, 57 - 78%; Forbes, 1994), Forbes et al (1995) showed that andromorphs of Nehalennia irene are in the minority (2.1 - 28.1%). This proportion of andromorphs appears to fluctuate from year to year ($\leq 8\%$; Forbes et al., 1995) but neither morph is ever out-competed to exclusion. This continued presence of both female morphs has been tentatively explained by many arguments such as: differential mortality of morphs from visual predators (Johnson 1975, Robertson 1985), differential harassment of morphs by males (Hinneking 1987, Forbes 1995, Cordero 1998) and neutral selection (Fincke,

1994). I hypothesized that andromorphs might be parasitized more heavily than heteromorphs, and the effects of the gregarines might be more detrimental to andromorphs. My results show, however, that there is no difference between andromorphs and heteromorphs, either in susceptibility to or in cost of gregarine parasites.

Density of the host population appears to have some effect on rates of parasitism, specifically that intensity of infection is highest for males and females in the site with highest density of damselflies. However, when looking at the patterns of intensity of infection, the interaction between sampling site and female morph is difficult to explain. Prevalence of infection, however, does not seem to vary with host population density. This could be explained through individual preference of damselflies or individual immunity. Some damselflies prefer to capture and eat small insects that are carriers of the infective gregarine cysts, and other damselflies prefer to capture and eat small insects that do not carry the infective cysts. If this occurs, the individuals that eat carriers will be infected multiply, while the individuals that do not eat carriers will not be infected at all. On the other hand, some damselflies may be able to expel the infective gregarine cysts rather than allowing them to germinate.

There are three ways through which differential parasitism can occur: first, through differential susceptibility of the two groups; second, through differential responses to the parasite, and; third, through differential costs of the parasite. I have tested and found negative results related to the first point for female morphs. Males and females, however, show differential intensity of infection. The second point, which I did not address, is difficult to elucidate for insects, as immune and physiological responses

are either unknown or difficult to measure. I tested for indirect evidence of the third point, differential costs associated with parasitism by gregarines, with a survivorship experiment. This showed that although females live longer than males, the number of parasites does not influence the individual host's longevity when stressed. Males and females, as well as female morphs, show no differential costs of parasitism.

CHAPTER 4

General Discussion

The two studies I conducted for this thesis are very similar, and yet they have a few important differences. In Chapter 2, I surveyed Enallagma boreale larvae to deduce whether any patterns in gregarine distribution in adults was due to larval ecology. Interestingly, the larvae were highly infected, contrary to previous studies (Åbro 1974, Åbro 1976, Åbro 1987, Åbro 1990, Taylor and Merriam 1996, but see Geus, 1969, Foerster 1938), though there was no sex bias in parasitism. I could not test this assumption in Nehalennia irene (Chapter 2), as no larvae were sampled. However, I began my study of N. irene assuming that the newly emerged adults were relatively parasite-free, as found in E. boreale. In the study of E. boreale, I attempted to control for host age, so that all adults were close to the same age. This would prevent accumulation of gregarines over time from influencing the outcome, if there were proportionally more old females than males in the sample. Support for the idea of controlling for age came from the seasonal pattern of prevalence and intensity of infection. Controlling for age of hosts in the samples of N. irene, however, was not possible. Fortunately, the seasonal patterns of prevalence and especially intensity do not fluctuate as greatly as in E. boreale, and this may not have skewed the N. irene data. Finally, I did not compare female

morphs in the study of E. boreale. Enallagma species do show female polymorphism, and in E. boreale, the proportion of andromorphs in the population is generally higher than that of heteromorphs (57-78%, Forbes 1994). However, as I had no reason to believe that female morphs of E. boreale differed in their foraging ecology, I did not investigate that aspect of the parasite-host interaction in this particular species. The two studies are comparable in many ways, but the differences must be taken into account when comparing results.

The measures of parasitism used in this thesis were intensity and prevalence of infection. Although I do not know whether the species of gregarine were the same in both E. boreale and N. irene, I can compare rates of parasitism between the two host species. E. boreale adults showed prevalence of infection ranging from $13.9 \pm 10.5\%$ early in the season, to $92.0 \pm 7.52\%$ late in the season, while N. irene ranged from $18.75 \pm 9.6\%$ early in the season, to $76.19 \pm 18\%$ in mid-season (excluding prevalence of 0 with sample size < 3 in N. irene). Mean intensity of infection ranged from 5.2 to 10 in E. boreale, and ranged from 1.5 to 34.25 in N. irene. Prevalence of infection was similar between the two species, while intensity of infection was potentially greater in N. irene. These rates of infection are similar to some values in the literature, and different from others. Taylor and Merriam (1996) found mean intensities from approximately 5 to 115 in Calopteryx maculatum; Åbro (1974) found intensities ranging from 1 to > 900 gregarines per individual in Pyrrhosoma nymphula and Enallagma cyathigerum, 1 to 500 in Coenagrion hastulatum, 1 to 200 in Lestes sponsa, and 1 to 20 in Sympetrum danae. While most of these mean intensities are greater than those in this study, the mean intensity values for E. boreale and N. irene are not unreasonable when compared with other Odonate - Gregarine

relationships. Prevalence of gregarine infection in damselflies is reported in three studies. The values given for Calopteryx maculata in Taylor and Merriam (1996) have a narrower range: 60%-85%. However, the prevalence of gregarine infection in other studies (Åbro 1987, 1974) have a wider range : 28% - 98% and 0% - 100%. These values are also comparable to values for prevalence in E. boreale and N. irene.

Seasonal patterns in prevalence were similar between the two study species, agreeing with the literature (Zuk 1987, Scott 1988). Intensity of infection increased, then decreased through the season in E. boreale, however, N. irene did not show any pattern in variation of intensity throughout the season. One explanation for this difference may be that the lifespan of individual N. irene (average for mature adult in survivorship experiment = 4.42 ± 0.14 days, teneral stage is approximately 1 day) is shorter than the lifespan of E. boreale (average in survivorship experiment = 6.92 ± 0.29 days, teneral stage is at least 7 days). N. irene, therefore, would not have as many old individuals which have accumulated gregarines, which would decrease the overall intensity of infection in the population.

The crux of these two studies is the conflicting result when comparing parasitism of males and females. I found some support for the idea that females should have more gregarines than males when sampling E. boreale. One of two sampling dates at the beginning of the flight season (when all adult hosts were approximately the same age) showed that females had significantly higher prevalence of infection than males. Other samples showed a similar, non-significant trend. Although Nehalennia irene showed no differences in prevalence of parasitism between males and females, there was a significant difference between male and female intensity of parasitism. However, N.

irene did not show a difference in parasitism between andromorphs and heteromorphs, which I expected.

While not the focus of my thesis, it is interesting to compare the two species with respect to factors that may influence parasitism. First, transmission of parasites may be slightly different between the two species. Enallagma boreale is larger than N. irene, and may eat a wider range of prey items, as well as larger prey items. Åbro (1976) found the infective cysts of gregarines in among the tarsal setae of medium-sized Chironomids and Ceratopogonids. Gregarine cysts were not found on small or large chironomids, nor were they found on large or small Lepidopterans or other potential prey items. Although this study was not exhaustive, and only sampled one small area near Bergen, Norway, the results may be applicable. Perhaps some of the insects which are prey for E. boreale do not carry the infective gregarine cysts, while all of the smaller insects which are food for N. irene carry the infective cysts. This idea also explains why Åbro (1996) found that in Calopteryx virgo, a damselfly larger than Enallagma boreale, there is a trend for females to have more gregarines than males (a purely descriptive study).

There may also be another supplemental source of infection for both species. Åbro (1976) also hypothesized that damselflies may ingest the oocytes along with water. The fecal pellets of damselflies fall to the ground and disintegrate, and the gametocyst bursts to produce viable oocysts, which are then dispersed on the woodland floor, or in the litter on the ground. At least some oocytes are easily suspended on the surface film of a drop of water or dew (Åbro 1976). It is at this stage that the chironomids would pick up the gregarine cysts. Damselflies may also pick up the cysts at this point. Åbro (1976) observed damselflies resting on the ground to pick up dew drops, and potentially sipping

water from the surface of the pond. These two methods of ingestion of infective gregarine cysts (ingestion with prey and ingestion with water) may work together. Damselfly species may encounter the cysts at different rates, or by different means. This explanation may also encompass the data from Xanthocnemis zealandica (Appendix A), a damselfly which is approximately the same size as Enallagma species, and shows no sex bias or morph bias in parasitism by gregarines. However, the insect prey of New Zealand damselflies is unknown, and the insect carriers of gregarine cysts may not be prey items of X. zealandica.

In Chapters 2 and 3, I show that the effects of gregarine parasitism are not severe in the species studied. There was no effect of female polymorphism on rates of parasitism. This nullifies the idea that differential parasitism, namely greater parasitism of the andromorphs at high density sites, might explain why andromorphs do not seem to increase in representation at high density sites despite having advantages over heteromorphs in costly social interactions with males. Therefore, this study adds little to the argument over balanced selection versus neutral selection of female morphs in damselflies (as in Johnson 1975, Robertson 1985, Fincke 1988, Thompson 1989, DeMarchi 1990, Forbes 1991b, Fincke 1994a 1994b, Forbes et al. 1995).

Finally, it is unclear whether sex biases in parasitism observed in this study would translate into population effects. This is in contrast with some studies of parasitism in invertebrates which showed behavioural differences between the sexes, causing differential parasitism which may affect hosts at the population level (Poulin 1996, Wedekind and Jakobsen 1998). The effects of the gregarines in this thesis are not severe. In fact, for E. boreale, the relationship between longevity and number of parasites is

positive! This contradicts previous studies of damselflies and gregarines, which showed reduced host longevity (Åbro 1971), and ulceration and ruptures of the damselfly's gut wall (Åbro 1974, Åbro 1990) due to the parasite. Gregarines also cause reduced longevity and resilience, increased weight loss, and increased development time in field crickets (Zuk, 1987). In the systems studied in this thesis, gregarines may be reduced in virulence, moving towards a state of commensalism, rather than parasitism.

If gregarines are becoming commensals with damselflies in the species and sites in this study, their effect on the population of damselflies would be negligible. They would no longer have the potential to influence natural selection against specific groups in the population. The costs and benefit balance that determines fitness of an individual would be slightly different for damselflies in this study system as compared to other study systems.

PROSPECTUS

A scientific study of any kind should produce more questions to be answered. This thesis has done so. Some questions highlight inadequacies of the study, and others are based on the findings of this thesis.

Two questions would have contributed greatly to comprehension of my results, if answered. The first is: How are gregarines transmitted in Odonates? The specific factors that influence their transmission could help to explain why I see different patterns of parasitism in E. boreale and N. irene males and females, as well as in Coenagrion puella (Åbro 1996). Although Åbro found that gregarines are directly transmitted, and they sometimes become stuck between tarsal setae on Chironomids, little else is known about them. I assumed that ingestion of infective gregarines cysts was proportional to food intake, but if the damselflies are ingesting the cysts from another source, my predictions may have been different. The second question is: What effect do these parasites have on my study species, either physiologically, or immunologically? The evidence from this study indicates that gregarines do not damage their host measurably. Nor do I know if an insect can mount an immune response to a parasite in the lumen of the gut. Gregarines may be at the threshold between parasite and commensal in my host study species.

One interesting idea arose from the high levels of parasitism in larvae compared to the very low levels of infection in newly-emerged teneral. As the individual damselfly ecloses, the entire exoskeleton is shed - including the lining of the gut.

Together, these facts beg the question: is metamorphosis a mechanism for shedding parasites? And if so, is it incidental, or one of the main functions of eclosion? An interesting aside is that although I found high levels of gregarine parasitism in larvae, most published studies did not find any larvae with gregarines. No one has yet looked at the effects of gregarines on larval damselflies.

Another idea that would be interesting to follow up is the evolution of polymorphism. Assuming that the female polymorphism is a phylogenetic trait, ie. it was shared by a common ancestor to all taxa which have the trait, why did it disappear in some populations? Or, if it was not a phylogenetic trait, why was there convergent evolution to produce female polymorphism in so many closely related species? Investigation of this idea should begin by comparison of life histories of Coenagrionid species with and without female polymorphism, and those species with dominating heteromorphs versus species with dominating andromorphs.

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APPENDIX

Measures of parasitism in Xanthocnemis zealandica, New Zealand damselflies

Table A1. Measures of parasitism for Xanthocnemis zealandica over seven sampling dates. 95% confidence intervals were calculated following Snedecor and Cochran (1980). ANDRO = andromorphs, HETERO = heteromorphs.

DATE	Prevalence		+/- 95%		CI		Intensity		+/- S. E.			
	MALE	N	ANDRO	N	HETERO	N	MALE	N	ANDRO	N	HETERO	N
17 Dec, 1997	54±20%	24	67±53%	3	50±40%	6	27.2±8.5	13	13.0±12.0	2	13.0±7.5	3
19 Dec, 1997	43±37%	7	50±35%	8	50±28%	12	21.0±13.5	3	21.3±10.1	4	8.6±3.9	6
26 Dec, 1997	55±22%	20	36±28%	11	55±23%	18	17.5±6.4	11	15.3±7.0	4	33.9±19	10
2 Jan, 1998	60±25%	15	46±25%	15	60±25%	15	30.6±15.1	9	29.6±14.5	7	11.1±4.5	9
13 Jan, 1998	68±19%	22	36±25%	14	53±25%	17	25.5±7.6	15	38.8±14.3	5	27.6±10.9	9
19 Jan, 1998	85±13%	27	73±20%	11	67±22%	18	34.1±5.4	23	21.3±6.2	8	105.5±81.6	12
22 Feb, 1998	18±14%	28	9±17%	11	22±19%	18	5.4±2.5	5	0	1	4.75±0.75	4