The life cycle and fitness domain of gregarine (Apicomplexa) parasites

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ABSTRACT

Theoretical demographic models with accompanying experimental programs provide an important framework to study the life history of organisms. In this paper we examine the fitness characteristics of gregarine parasites (Apicomplexa) and how these evolutionary long-lived parasites are shaped by their own life cycle stages inside and outside a definitive insect host. Although gregarines have been investigated in experimental works, their fitness and population characteristics have not been subject to modeling efforts to help understand their longevity or interactions with their host species. We develop a dynamic, mechanistic population model represented by a system of two differential equations for two of the parasite stages: the mature parasite, or trophont, inside a definitive insect host, and the infectious oocyst stage in the water environment of the host. In contrast to many classical macroparasite models, the force of infection between oocysts and hosts is of sigmoid type. Inside the host, production of the water borne infectious state is modeled by linear production rate in the trophont population with a density-independent trophont mortality. We examine stability of model's equilibria for different parameter values and different host populations. This leads to the definition of a fitness parameter that acts as a bifurcation parameter for the model. The model shows good cause for the establishment and long-time persistence of this common, widespread parasite. It is parameterized by extensive data gathered at Cedar Point Biological Station, and numerical calculations based on those parameters illustrate the dynamics. Possible applications include parasite control in aquacultures.

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1. Introduction

Parasitism is perhaps the most common way of life on Earth because every species of plant and animal that has been studied seriously has been shown to be parasitized by at least one, and often several, non-related species of protists, fungi, plants, or animals (Roberts and Janovy, 2009). Host–parasite systems are enormously varied with respect to modes of transmission, physiological relationships between species, and infection sites, and many are of serious economic importance. Thus it is not surprising that both experimental and theoretical research on host–parasite interactions is extensive. However, the parasite species examined in this paper have not been approached from a modeling viewpoint in spite of many experimental studies.

In this paper we focus on macroparasites and their insect hosts. In contrast to microparasites like viruses and bacteria, macroparasites have longer generations times, are larger, have at least one life cycle stage outside the definitive host, and do not multiply in the infective stage within the host. Good examples of macroparasites include helminths such as tapeworms and arthropods such

as fleas and ticks. Typically, macroparasite infections are aggregated, with most individual parasites occurring in a relative few host individuals and a large number of hosts being either lightly- or non-infected. Consequently, macroparasite populations often are described by negative binomial distributions in which variance-to-mean ratios are large, and quantitative models most often focus on parasite infrapopulations (number of parasites per host) instead of prevalence (fraction of hosts infected) (Nodtvedt et al., 2002; Pal and Lewis, 2004).

Host–parasite interactions in aquatic environments have not been modeled to the same extent as terrestrial ones of economic importance, but within the past few decades, with growing importance of aquaculture, efforts to control disease vectors, and impact of climate change, there has been increasing interest in such systems (Milner and Patton, 1999; Marcogliese, 2001; Esteva et al., 2006; Fenton et al., 2006; Revie et al., 2007). Parasites vary considerably in their dependence upon aquatic environments, ranging, for example, from trematodes that require molluscs as first intermediate hosts and live for a short time outside their hosts, to malarial parasites transmitted by vectors with aquatic life cycle stages. Aside from their economic importance, therefore, aquatic hosts offer many opportunities to explore avenues for, and constraints on, evolutionary change (Bolek and Janovy, 2007a,b). And among the most common and diverse of host–parasite systems

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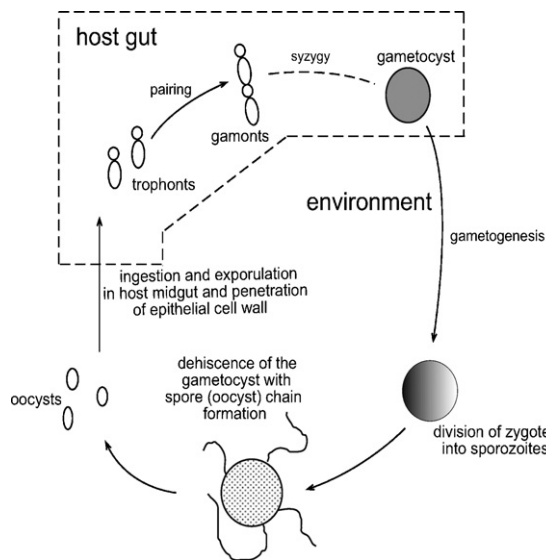E-mail address: dlogan@math.unl.edu (J.D. Logan).

Fig. 1. The direct life cycle of a gregarine parasite showing the transmission of the infection and the trophont production rate of infective agents (oocysts). The average life cycle is 8–11 days. For a more detailed diagram and discussion, see Roberts and Janovy (2009).

in aquatic environments are ones involving invertebrates and the so-called "gregarines" (Levine, 1988; Clopton, 2000).

Gregarine parasites (phylum Apicomplexa; class Conoidasida; order Eugregarinorida) are large, single-celled, eukaryotes that are transmitted by oocysts ingested by a host. All invertebrate phyla have been reported as hosts for gregarines, but they are most numerous in arthropods and annelids. Beetles alone (order Coleoptera) are infected with one to two species of gregarine per host species, leading to a reasonable estimate of approximately 500,000 gregarine species in coleopterans. Damselflies and dragonflies (order Odonata; "odonates") also have a rich gregarine fauna, with several species often occurring in single host species in a single collection site (Clopton et al., 1993; Percival et al., 1995). Differences in larval and adult odonate habitats, prey species, behaviors (including sex-specific mating behaviors), and potential for geographic distribution, as well as differences in parasite species' structure and development, all provide rich opportunities for using these insects as model systems for studying macroparasite population dynamics, especially in an evolutionary context.

A typical gregarine life cycle is direct, with numerous developmental stages both inside and outside the hosts (Fig. 1). Hosts ingest oocysts, containing infective sporozoites, which attach to or penetrate hosts' intestinal epithelium cells. Subsequent growth stages known as trophozoites, or trophonts, eventually detach from the epithelium and form associations with parasite cells of different mating types, thus becoming gamonts, which undergo syzygy and secrete a gametocyst wall. Gametocysts are shed into the environment and the contained gamonts undergo schizogony, forming gametes, which unite, forming zygotes inside. These zygotes then secrete an oocyst wall and go through zygotic meiosis to form haploid infective sporozoites, which are then released from the gametocyst by a variety of mechanisms known collectively as dehiscence. A gametocyst produces several thousand oocysts, each containing about eight sporozoites.

This paper reflects our efforts to establish insect–gregarine systems as models for host–parasite dynamics, and especially to inspire comparative work leading to evolutionary hypotheses. The aim is to understand factors affecting parasite fitness in nature, with special focus on the interplay among basic parameters of reproduction rates, infection rates, mortality, and host populations.

One key assumption is that the host population is constant. This assumption is not an uncommon one in infectious disease models (Anderson and May, 1978; Dobson, 1989; Dobson and Hilton, 1992; Mangel, 2006). We regard the constant host population as a parameter and we investigate the fitness properties of the parasites in terms of bifurcations associated with that parameter. In this context, we also assume that damselflies are not affected by gregarines, a position consistent both with the idea that well-adapted parasites are relatively harmless to their hosts (Nowak, 2006), and with studies that demonstrate little if any negative impact on insect hosts by Eugregarinorida (see Klingenberg et al., 1997; Hecker et al., 2002; Canales-Lazcano et al., 2005; Rodriguez and Omoto, 2007). This property is in stark contrast to some macroparasite models.

We track two parasite stages, the free-living infectious stage (oocysts), and trophonts inhabiting the definitive host (larval *Ischnura verticalis*, Odonata, Coenagrionidae). Our model represents a simplification of classical models such as those presented or reviewed by Anderson and May (1978), Dobson (1989), Dobson and Hilton (1992), Mangel (2006), and many others. One difference between our model and others is that although our parasite loads are strongly clumped, or negative binomially distributed, we do not use a traditional pairing probability to determine the prevalence of pairings; because gregarine mating behavior is not fully understood, we adopt a more mechanistic assumption of reproduction.

Our philosophy reflects that eloquently expressed by Maynard-Smith (1974, p. 35), namely, that the value of a theoretical population model is not in its precise description of the biological situation or in its ability, or inability, to predict population abundances exactly. What we do ask is what kind of change in the behavior of our model is made by particular changes in parameters; the assumption is that a comparable change will occur in the behavior of a real biological system. This brings up the issue of structural stability of the model with respect to changes in parameters. We want to know which parameters are critical and which are not. Therefore, a common approach is to select a fixed set of baseline parameters and perform a simulation of the dynamics; the magnitude of the change is measured from the baseline.

In summary, we want to understand the fitness characteristics and longevity of gregarine parasites and how they are shaped by their own life stages inside and outside a definitive insect host. The population model is a system of differential equations for the parasite stages (the trophonts in the definitive host and the free-living infectious oocysts), and we examine its possible equilibria under change of key life history parameters. A stability analysis leads to values, among them the basic reproductive number, that characterize population fitness and give good cause for the establishment and long time permanence of these common parasites. The model is parameterized by extensive data gathered at Cedar Point Biological Station in western Nebraska, and numerical calculations and bifurcation analysis based on those parameters illustrate the dynamics.

The plan of the paper is to introduce the model equations (Section 2) with reasonable justifications for our assumptions, and then examine the model's predictions regarding equilibria and stability (Section 3). In Section 4 we present simulations based on specific parameter values taken from our experimental work and the accompanying literature. The final section contains an analytical stability analysis and other mathematical features of the model.

2. Model formulation

In this section we develop a mechanistic population model of host–parasite interactions in a damselfly larva–gregarine system.
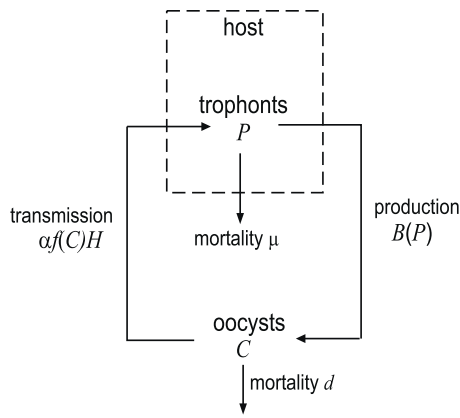
Fig. 2. Simplified life-cycle of the typical gregarine parasite showing the transmission of the infection and the production rate of infective agents. The force of infection $\alpha f(C)$ is assumed to by a sigmoid function of C and the production rate $B(P)$ a linear function of P. The state variables in the model are P and C, the per host population of trophonts and oocysts, respectively.

The simplified gregarine life cycle is shown in Fig. 2. In a water environment the larval hosts ingest infective oocysts, which then exporulate up to 8 sporozoites that attach to or penetrate hosts' intestinal epithelial cells. A gametocyst may produce several thousand oocysts.

In epidemiological language, the gregarine life cycle is characterized as direct. There are four items we require for a model: host population size, adult parasite (trophont) population size, infectious parasite oocyst population size, and in some cases, the distribution of the adult parasites in the host. The two distinct parasite populations, oocysts and adults, occupy separate habitats (aquatic and inside the host); both are controlled by immigration and mortality. An emigration process also affects the oocysts. Technically, there is no true "birth" process because birth of an oocyst does not directly affect the adult population; an oocyst can be regarded as a immigrant into the pool of the free-living oocyst community. From a modeling viewpoint, confronting these issues with data is difficult; simply, it is often impossible to obtain accurate data from experiments, in particular infection rates.

The model we develop tracks trophonts inside the definitive host and oocysts in the environment. We denote populations of these stages by $P(t)$ and $C(t)$, respectively, where t is time given in days. Both P and C are measured in units of 'per host'. Therefore, we think of $P(t)$ as the average parasite load. We let H denote the constant number of host. Therefore, there is no mortality of trophonts caused by host deaths. Further, we ignore time delays and any abiotic effects.

The same complex life cycle issues apply to most all macroparasite models: the infection, or transmission, rate of the infective stages, the production rate of infective stages by adults inside the host, and the mortality of both the mature parasites and the external infective stages. All require constitutive assumptions about the mechanisms involved. As in many parasite models, gregarines appear to have a low infection probability, but it is balanced by a high productivity to maintain a suitable level of fitness.

2.1. Infection transmission

The contact rate between infective agents (oocysts) in the water environment and insect hosts can be modeled in many ways. Often this is the most difficult part of the model because of measurements of oocyst populations and contact rates are difficult or impossible. In the next few paragraphs we summarize key approaches and justify our choices.

Deterministic models are mechanistic, requiring the specification of the effective contact rate between oocysts and hosts, and the fraction of those contacts that result in a mature, host-inhabiting adult. If c is the *total* number of oocysts in the environment, then the simplest model for the rate of effective contacts would be βcH, where β is the *contact rate*. Then, up to eight infective sporozoites exporulate from each ingested oocyst, and a fraction σ of those survive and establish mature trophonts. The transmission coefficient a is by $a \approx 8\sigma\beta$, which leads to the mass-action transmission rate acH, where a is the effective transmission coefficient. The quantity $f(c) = ac$ is the force of infection. Unfortunately, this simple mass-action type of infection rate does not yield appropriate dynamics in a model because it couples with a linear production rate (strongly supported by our field data), leading only to an extinct, stable persistent state. Alternately, one can argue that the force of infection should be a saturating function, for example, is a type II (Holling) or type III (sigmoid) functional response. Another phenomenological form of the force of infection could be chosen from the zeroth term of a negative binomial distribution. [Escapement models of this sort occur often in host–parasitoid equations as a stabilizing mechanism, where parasitoids are assumed to search in a heterogenous environment; e.g., see Britton (2003), pp. 48–53, for a simple discussion.]

From a stochastic viewpoint, the encounter between hosts and oocysts is a random event. Models of encounter rates are often defined by Poisson or Poisson-like processes, where the host ingests a single oocyst, or several oocysts, at random intervals of time. Although a Poisson process can be shown to lead to a negative binomial distribution of parasites in hosts when the rate parameter is gamma-distributed (e.g., see Mangel, 2006), it does not model our data. Our collected field data show that the average parasite load does not increase over time in damselfly larvae, suggesting perhaps that parasites are contacted in 'clumps' rather than one at a time. This would lead to a *compound* Poisson process, where the number of parasites ingested in the interval $[0, t]$ is

$$X(t) = \sum_{j=0}^{N(t)} Y_j,$$

where $N(t)$ is a Poisson process and the Y_j are nonnegative i.i.d. random variables representing the number of infective oocysts taken in one encounter. (See, for example, Tallis and Leyton, 1969, who couple this randomness with the probability of the infectious stage becoming established and mature in the host.) This strategy, as applied to our data, is a topic of an ongoing study.

In the present paper we model the infection transmission process deterministically by a sigmoid function

$$Ha\frac{mc^2}{\overline{\gamma}^2 + c^2},$$

where $\overline{\gamma}$ is the half-saturation, m is the saturation, and a is effective transmission coefficient. The sigmoid response is justified by measurements of feeding rates of certain insect stages; this is discussed in Section 4 in the context of parameter identification. Assuming a density-independent mortality of oocysts and a linear production rate we obtain the basic model equation

$$\frac{dc}{dt} = -dc - \alpha H\frac{c^2}{\gamma^2 + c^2} + bp, \tag{1}$$

where p is the total number of trophonts, b is the production rate, and $\alpha = am$, which we call the transmission. This linear production rate, as we observe later, has substantial support from our from our field data, as well as from the data of others.

The rate of change of trophont numbers is the rate they are established from oocyst infection rate minus the mortality rate.

Quantity	Description and units
$P(t)$	Parasite load (per host)
$C(t)$	Oocysts (per host)
H	Host population (constant)
α	Transmission constant (per host per day)
μ	Mortality of trophonts (per day)
d	Mortality of oocysts (per day)
b	Production rate (oocysts per trophont per day)
γ	Half saturation (oocysts per host)

Assuming density-independent mortality leads to the model equation

$$\frac{dp}{dt} = -\mu p + \alpha H \frac{c^2}{\gamma^2 + c^2}. \tag{2}$$

We point out, in contrast to most models, in this insect/gregarine system there is no evidence of parasite mortality induced by host mortality or effect of a host immune system response.

Introducing *per host* variables $P = p/H$ and $C = c/H$ and substituting into equations (1) and (2) gives our working model

$$\frac{dC}{dt} = bP - dC - \alpha \frac{C^2}{\gamma^2 + C^2},$$
$$\frac{dP}{dt} = -\mu P + \alpha \frac{C^2}{\gamma^2 + C^2},$$

where $\gamma = \overline{\gamma}/H$. For reference, Table 1 defines the basic quantities in the transmission process and their dimensions. Note that the host population has been scaled out of the final equations.

2.2. Production rate

In the next section we analyze the properties of the model, and in the following section we discuss in detail the confrontation of the model with data. First, however, we comment on the assumed linear production rate, in contrast to some standard models in the literature.

The infective oocysts are increased by the mating of trophonts through the gametogenesis process, and they are decreased by natural mortality in the environment and ingestion by the hosts. The production rate of oocysts per host is generally given by $B(P)$, which depends on the trophont population. It has been well documented, especially in the case of schistosomiasis, that an important issue is how mature males and females pair. It seems clear that not all of the trophonts will pair; some of the mature parasites will die, some will be washed out of the host's gut, and some will not be able find a mate. Details of this pairing model, based on the probability distribution of parasites per host, are discussed thoroughly in May (1977), Bradley and May (1978), and Anderson and May (1978). As in many other macroparasite infections, our dissections show a strong negative binomial distribution of trophonts in their definitive damselfly hosts. In the case of gregarines, trophonts are monogamous, forming a single pair, but it is difficult, if not impossible, to identify their sexual types prior to mating. If we assume, however, that the sexual types of mature trophonts are negative binomially distributed together (rather than separately) with parameters P and k (P is the average trophont load and k is the clumping parameter of Bradley and May (1978), or the negative binomial exponent), the probability argument of Bradley and May (1978) may be applied. The probability that a given host has i parasites of mating type 1 and j females of mating type 2 is

$$Pr(i, j | P, k) = (1 - a)^k \frac{\Gamma(i + j + k)(a/2)^{i+j}}{\Gamma(k) i! j!}, \quad a = \frac{P}{P + k}.$$

The probability of pairing is then given by

$$\varphi(P, k) = 1 - \frac{(1 - a)^{1+k}}{2\pi} \int_0^{2\pi} \frac{(1 - \cos 2\theta) d\theta}{(1 + a \cos \theta)^{1+k}}. \tag{3}$$

This integral expression must be calculated numerically for most parameter values. The average number of pairs formed is therefore

$$\frac{1}{2} P \varphi(P, k).$$

What is significant in our model regarding (3) is the shape of the plots of φ vs. P for different values of the clumping parameter k. In the limit as $k \to \infty$, the probability φ approaches a Poisson distribution, which plots as a type II (Holling) response saturating at 1. For $k \le 1$, which is the case of clumped distributions (where the variance is much larger than the mean), the probability φ again plots as a type II response. This gives reason to assume, mechanistically, that the probability of pairing can be modeled by a function of the type

$$\varphi(P, k) = \frac{P}{\gamma + P},$$

where γ is the half-saturation. Therefore, the rate of production of oocysts would be, in this case,

$$B(P) = \frac{1}{2} b' P \frac{P}{\gamma + P},$$

where b' number of oocysts produced per gametocyst. This conclusion, however, does not conform with our measurements (Bunker et al., submitted for publication) or those of Schwank (2004), both of whom measure the oocyst production $B(P)$ as a linear function of P.

We summarize the key assumptions of the model, which are based on our, and other's, experimental data.

1. There is no evidence in gregarines that hosts are affected by their presence or that there is a host immune system response.
2. The mortalities of trophonts and oocysts are density-independent.
3. The infection rate between oocysts and hosts is given by sigmoid function of the oocyst population. The host population is constant.
4. The production rate of infectious oocysts is a linear function of the mature trophont population.
5. Abiotic effects are not included.

3. Equilibrium analysis

We now examine the equilibria in our model and the conclusions concerning its bifurcation properties. So as not to interrupt the flow of modeling discussion, many of the details of the mathematical analysis may be found in the final section of the paper.

From the previous section, the model equations are

$$\frac{dC}{dt} = bP - dC - \alpha \frac{C^2}{\gamma^2 + C^2}, \tag{4}$$

$$\frac{dP}{dt} = -\mu P + \alpha \frac{C^2}{\gamma^2 + C^2}. \tag{5}$$

The isoclines, or the set of points C, P in the CP-phase plane where $C' = 0$ and $P' = 0$, are given by, respectively,

$$P = \frac{1}{b} \left(dC + \alpha \frac{C^2}{\gamma^2 + C^2} \right), \quad (C \text{ isocline}) \tag{6}$$
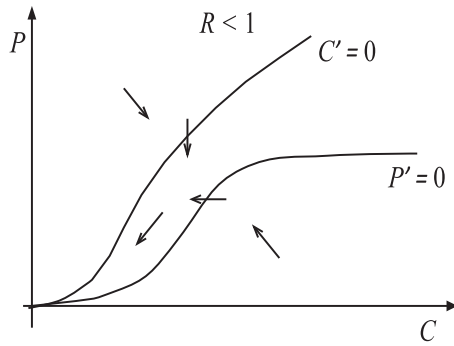
Fig. 3. Phase diagram showing the isoclines and direction field in the case $R < 1$. The only equilibrium is the origin (extinction), which is globally asymptotically stable.

$$P = \frac{\alpha}{\mu}\frac{C^2}{\gamma^2 + C^2}. \quad (Pisocline) \qquad (7)$$

Equilibria are the intersections of these two isoclines. Both have a sigmoid shape with the C isocline gradually increasing, linearly, as $C \to \infty$. Clearly, $P = C = 0$, which represents extinction, is one equilibrium. Then, equating the two isoclines and canceling C (corresponding to the extinction state) gives

$$\frac{1}{b}\left(d + \alpha\frac{C}{\gamma^2 + C^2}\right) = \frac{\alpha}{\mu}\frac{C}{\gamma^2 + C^2}.$$

Multiplying by $\gamma^2 + C^2$ and rearranging yields a quadratic equation for C:

$$C^2 + \frac{\alpha}{d}\left(1 - \frac{b}{\mu}\right)C + \gamma^2 = 0. \qquad (8)$$

Introducing the parameter

$$R \stackrel{\text{def}}{=} \frac{\alpha}{2\gamma d}\left(\frac{b}{\mu} - 1\right), \qquad (9)$$

we can write the roots, or equilibrium values of C, as

$$C^* = \gamma\left(R \pm \sqrt{R^2 - 1}\right). \qquad (10)$$

Correspondingly, the equilibrium values of P are

$$P^* = \frac{\alpha}{\mu}\frac{C^{*2}}{\gamma^2 + C^{*2}}. \qquad (11)$$

Note that $R > 0$ because the reproduction rate is much larger than the mortality rate μ. There are three cases: if $R < 1$ there is only a single equilibrium (extinction); if $R = 1$ there are two intersections, extinction and a positive equilibrium where the isoclines are tangent; if $R > 1$ there are three intersections, extinction, and two positive equilibria where the isoclines cross. Therefore, the parameter R is a bifurcation parameter for the problem. As it increases from small values to larger ones there is a bifurcation from one, to two, to three equilibria.

The parameter R, which we call a *fitness* parameter, plays a role similar to the basic reproduction number in epidemic models. Observe that b/μ is the rate that trophonts produce oocysts times the average lifespan $1/\mu$ of trophonts; similarly, we can relate $\alpha/2\gamma$ to the average number of mature trophonts produced by oocysts and $1/d$ as the average lifespan of an oocyst.

Figs. 3–5 show how the intersections of the isoclines change as R increases. It is clear by the structure of the isoclines and direction fields what the stability of each equilibrium is. A detailed stability analysis is outlined in Section 5.

From Figs. 6 and 7 we infer a bifurcation diagram showing equilibria changes as a function of the parameter R.

We can infer additional, immediate results regarding the orders of magnitude of some of the parameters. Because of the longevity
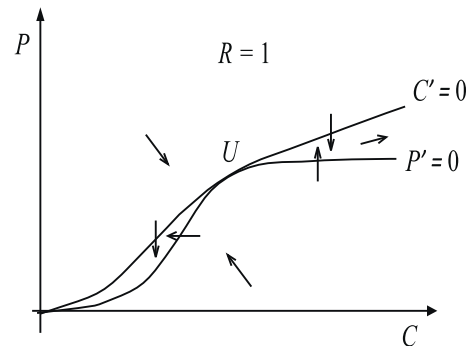

Fig. 4. Phase diagram showing the isoclines and direction field in the case $R = 1$. The isoclines are tangent at the point U, which is a locally unstable equilibrium. The origin (extinction) is stable.
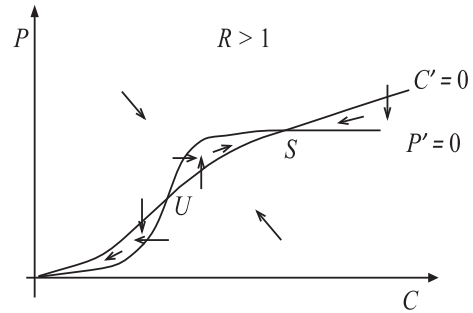

Fig. 5. Phase diagram showing the isoclines and direction field in the case $R > 1$. There are three distinct equilibria: the stable extinction state $(0, 0)$, the intersection U, which is unstable, and the larger intersection S, which is stable.
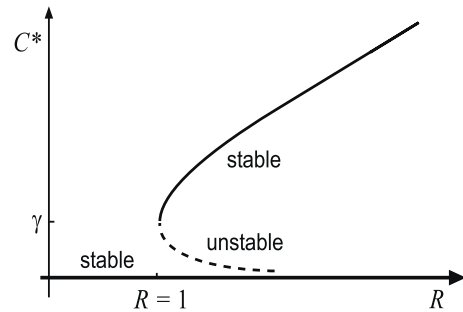

Fig. 6. Bifurcation diagram showing the equilibrium state C^* as a function of the fitness R. The extinction state $C^* = 0$ is stable for all R. At $R = 1$ the extinction state bifurcates into two additional equilibria; the upper branch is stable, and the lower branch is unstable.
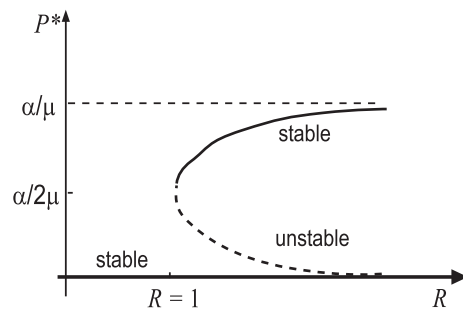

Fig. 7. Bifurcation diagram showing the equilibrium state P^* as a function of the fitness R. In contrast to C^*, P^* remains bounded as the fitness R increases. Thus the trophont population remains limited even though the infectious oocyst population increases.

of gregarine parasites, we certainly expect the fitness parameter R given by (9) to be larger, or even much larger, than unity. If b is large, i.e., a large oocyst production rate, we expect $R > 1$ unless the infection rate is extremely small. Our field observations confirm b is order 10^4. We can also meet this fitness condition when the mortality rate of oocysts d is small. Observations of many gregarine systems imply that some oocysts survive a long time (on the order of years). We note that this fact is in stark contrast to free-living stages of some parasite taxa.

4. Results and simulation

Confronting a mathematical model with data is never an easy task; in fact, it is nearly always the hardest task. The model was parameterized in part from results of a two-year program undertaken at Cedar Point Biological Station (41°12′33″N, 101°38′54″W). Studies included the census and collection of larval and adult damselflies over two summer periods and the dissection of the insects' intestines and counting of mature trophonts. The program with data and data analysis is detailed in Bunker et al. (submitted for publication). We used some of the data from this work to choose key aspects of our model: a density independent mortality of trophonts, a negative binomial distribution of trophonts per host, which gives an equilibrium value of the trophont population, and a linear production rate of oocysts. The counts and measurements reveal the age makeup of the parasite population and ongoing infection events, of 23,755 identified parasites from 1198 larval, 183 teneral, and 446 adult hosts (five sites, 9 collections each), over a three month period in 2010. Gametocyst production rates were estimated from the work of Schwank (2004); oocyst longevity is based on infection experiments of Clopton (1993); oocyst production is based on haemocytometer counts from sporulated gametocysts made during the summer of 2011. The host population estimate (10,000) is based on capture rates and pond areas of the various collection sites.

Field data show convincingly that adult *Ishnura verticalis* have a different parasite fauna than larvae. Because adult damselflies are terrestrial feeders, and gametocysts must be under water to sporulate and produce oocysts, and transmission to adults must be by way of a transport host or carrier, as in the case of some other gregarines (Wise et al., 1999). When this prey item is in very low numbers, damselfly predation must be minimal, but when prey is present in abundance, predation rate will be limited by a variety of factors, including predator satiation and digestion rate (Richardson and Baker, 1997). Thus encounters between *I. verticalis* adults and oocyst-bearing prey should become saturated at some point, implying a type 3, or sigmoid, functional response describing the force of infection. Finally, the mortality rate μ of trophonts is based on data by Watwood et al. (1997) and Schwank (2004), who measured the total number of gametocysts shed per trophont over a time period equivalent to three complete parasite developmental periods (8–10 days per period) in experimental infections. Their experiments gave an estimate of the lifespan of trophonts to be 10–12 days, giving our selected value of $\mu = 0.089$ as the trophont mortality rate. We elaborate that obtaining data on adult parasites in a host is a difficult process (e.g., see Anderson and May, 1978)—it requires a one-time, destructive dissection, and so we only a single data point can be found per host. Dissecting many hosts and 'averaging' in some manner is confounded by the fact that feeding rates (ingestion of parasites) are variable among the hosts. This is especially true for gregarines, in which the infective stages are so small that they cannot be manipulated singly. The best data available have been obtained in laboratory settings (referenced above). Several insect hosts were fed oocysts in separate environments and the number of gametocysts shed into the water environment

Table 2
Parameters α and γd versus the fitness R.

R	1.0	1.05	1.10	1.25	1.50
α	1.07	0.82	0.75	0.67	0.61
γd	7740	5650	4960	3870	1700

were counted. After several days the dissections were done. This gave the number in minus the number out and an estimate of the mortality rate. Some of these experiments were performed with *Tribolium* and *Tenebrio* hosts rather than damselfly hosts, whose biology essentially prevents similar studies (e.g., see Richardson and Janovy, 1990; Detwiler, 2004; Detwiler and Janovy, 2008).

A set of baseline parameters for simulations can be developed as follows. There are 5 parameters in the model: b, α, μ, d, γ. The fitness parameter R depends on these five, and the equilibria values of P^* and C^* provide two additional relations. For easy reference we rewrite these relations in terms of the fitness R:

$$C^* = \gamma \left(R + \sqrt{R^2 - 1} \right), \tag{12}$$

$$P^* = \frac{\alpha}{\mu} \frac{\left(R + \sqrt{R^2 - 1} \right)^2}{1 + \left(R + \sqrt{R^2 - 1} \right)^2}, \tag{13}$$

$$R = \frac{\alpha}{2\gamma d} \left(\frac{b}{\mu} - 1 \right) \approx \frac{\alpha b}{2\gamma d\mu}. \tag{14}$$

Our experimental results give estimates for the three quantities:

$$P^* = 6, \qquad b = 1290, \qquad \mu = 0.089. \tag{15}$$

These values confirm our approximation b/μ is much greater than 1 in (14). To repeat, the average trophont load P^* was estimated to lie in the range between 5 and 6 by dissection of a large number of intestines of larvae from different locations. We measured the average number of viable gametocysts shed per trophont, 0.043; then, estimating 30,000 oocysts produced per gametocyst gives $b = 1290$. The latter is consistent with the data of Schwank (2004) and Bunker et al. (submitted for publication).

The values of α, γ, and d, all properties of the force of infection side of the life cycle (see Fig. 2), are the most difficult to obtain and were not able to be measured directly. This is common in many parasite studies. However, equations (13) and (14), along with the values in (15), provide estimates of α and product γd, respectively, in terms of the fitness R and the other measured parameters. In particular,

$$\alpha = \mu P^* \frac{1 + \left(R + \sqrt{R^2 - 1} \right)^2}{\left(R + \sqrt{R^2 - 1} \right)^2}$$

and

$$\gamma d = \frac{\alpha b}{2\mu R} = \frac{bP^*}{2R} \frac{1 + \left(R + \sqrt{R^2 - 1} \right)^2}{\left(R + \sqrt{R^2 - 1} \right)^2}.$$

Therefore, one strategy is to ask how the left sides of these two equations change as the fitness R changes through a reasonable range of values, say, from $R = 1$ to $R = 1.5$ (from 0% growth to 50% growth). For selected fitness values the associated values of α and γd are shown in Table 2.

The table shows order of magnitude changes in a narrow range of values. We conclude that the stable equilibrium is robust in changes of these parameters.

Table 3
Baseline parameter values.

Quantity	Value
b	1290
μ	0.089
α	0.82
d	0.09
γ	$4.16(10)^6$

A reasonable set of baseline parameters for simulations is $R = 1.05$ and $\alpha = 0.82$. To separate γ and d we note, from (12), that $C^* = 1.37\gamma$; so γ and C^* are of the same order. Taking $b = 1290$ and $P^* = 6$, we observe that $b = 1290 P^* = 7740$ oocysts produced. So, C^* is on the order of 10^4. Moreover, the mortality of oocysts, as we have noted, is low. After multiple simulations we chose $d = 0.09$ and $\gamma = 6.28(10)^4$. These parameter values, which are used for our sample simulations, are collected in Table 3. These parameters lead to the equilibrium value $C^* = 8.8(10)^4$, $P^* = 6.19$ with fitness $R = 1.06$ for the stable equilibrium. [The unstable equilibrium is $C^* = 4.4(10)^4$, $P^* = 3.10$.]

Numerical calculations were performed in MATLAB. Fig. 8 shows time series plots of $P(t)$ and $C(t)$ in the case of a stable equilibrium.

Figs. 9 and 10 show time series plots and the phase diagram for small initial conditions. In this case the populations become extinct because of the small initial population at one life cycle stage or another.

4.1. Summarizing remarks

Gregarines form one of the oldest and most abundant parasite groups in the world, and odonates have a long fossil record going back well into the Mesozoic (Bechly, 2010). Yet no mathematical models have been developed for gregarine life cycle dynamics associated with their damselfly hosts in order to attempt to understand the fitness characteristics of these organisms. The reason for this, and for the comparatively few experimental programs on gregarines, is certainly caused by the fact that their insect hosts have been of less interest than other parasites, like schistosomes, which cause fatal infections in the human species (Anderson and May, 1978). But this interest level is changing due the recent appearance of aqua-cultures for farming seafood and fish. Infected insect hosts form part of the diet of fish and other aquatic species.

In this paper we have formulated a direct life-cycle model based on a type III force of infection rate between the hosts and the water-born infectious stage; the production rate of oocysts from trophonts is linear. We showed the existence of a stable equilibrium when the fitness coefficient exceeds one, and the result is robust in that the fitness narrows the values of parameter ranges to ones found in our, and other's, field studies. Thus, the model supports conclusions regarding gregarine longevity and their high level of fitness, thereby addressing a key issue in evolutionary biology. As is the case for other parasite species, the large production rate of infective aquatic agents overcomes the small contact rate with hosts to maintain fitness. Moreover, gregarines are different from many other parasite species with a direct life cycle because of the long life span of the infective stage (oocysts) in the aquatic domain. Again, that aids in the fitness. One of our key results is that our model and field data do not conform with the classic model of parasite pair-mating in the host, in spite of the common negative binomial distribution of adult parasites per host.

5. Model analysis

In this final section we briefly supply some of the technical details concerning our statements about the model in Section 2. In particular, we carry out a standard linearized stability analysis (e.g., Logan and Wolesensky, 2009, p. 165ff.) to confirm analytically the stability properties shown in Figs. 3–5. Moreover, we show that there can be no periodic orbits, or limit cycles, and that there is a compact basin of attraction that is therefore an invariant set for the flow. Put together, these results will show that there are no other bifurcations possible in our system, other than those stated. See
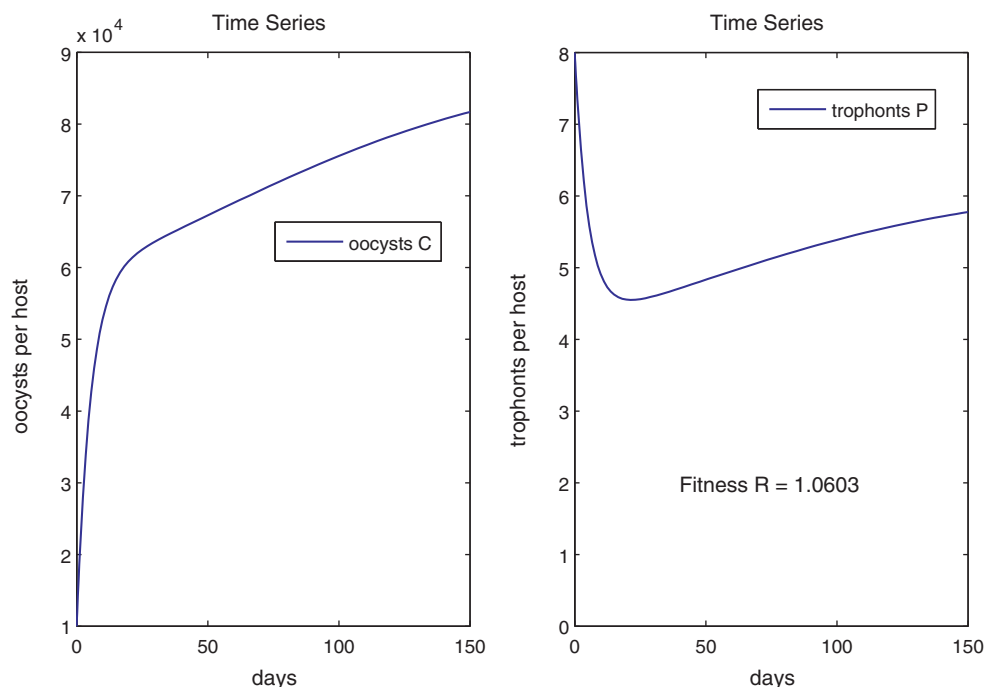

Fig. 8. Time series plots of the oocyst and trophont populations over a 150-day period with initial conditions $C(0) = 10^4$ and $P(0) = 8$. The remaining parameters are given in Table 3. The curves approach the stable equilibrium $C^* = 8.8(10)^4$, $P^* = 6.19$.
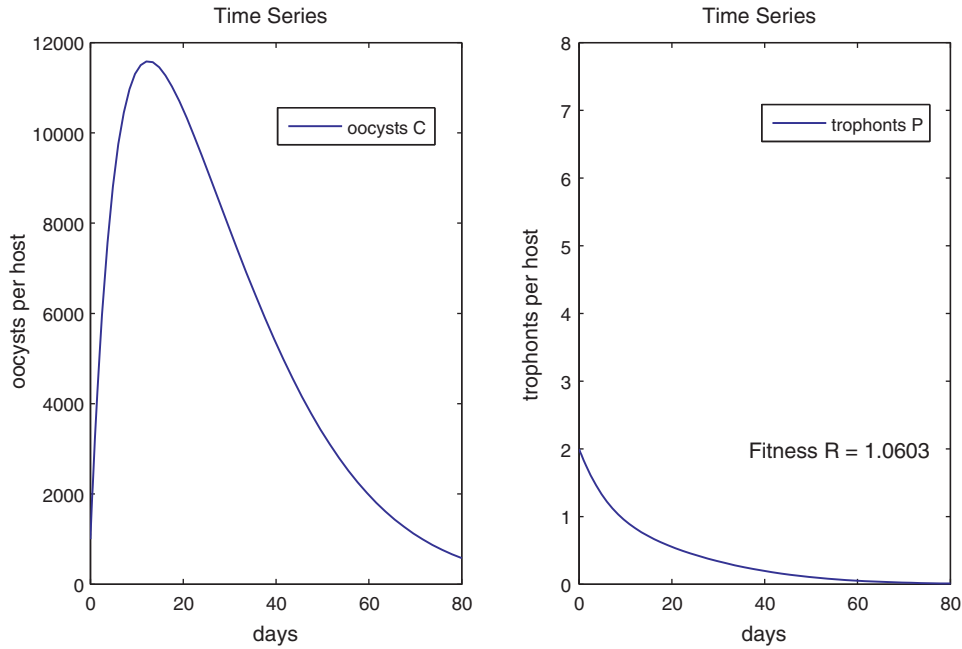
Fig. 9. Time series plots of the oocyst and trophont populations over a 80-day period with initial conditions $C(0) = 10^3$ and $P(0) = 2$. The remaining parameters are given in Table 3. The curves approach zero, which is the extinction state.

Nedorezov (1997) for a discussion of several other possibilities for related systems occurring in population dynamics.

The model system consists of Eqs. (4) and (5); we denote their right hand sides by $F(C, P)$ and $G(C, P)$, respectively. The Jacobian (or, community) matrix for the systems is

$$J(C, P) = \begin{pmatrix} \dfrac{\partial F}{\partial C} & \dfrac{\partial F}{\partial P} \\ \dfrac{\partial G}{\partial C} & \dfrac{\partial G}{\partial C} \end{pmatrix} = \begin{pmatrix} -d - \dfrac{\alpha H(2\gamma^2 C)}{(\gamma^2 + C^2)^2} & b \\ \dfrac{\alpha H(2\gamma^2 C)}{(\gamma^2 + C^2)^2} & -\mu \end{pmatrix}, \quad (16)$$

Fig. 10. Phase plane plot of oocyst and trophont populations over a 80-day period with initial conditions $C(0) = 10^3$ and $P(0) = 2$. The remaining parameters are given in Table 3. The curves approach extinction.

where we have used

$$\frac{\partial}{\partial C}\left(\frac{C^2}{\gamma^2 + C^2}\right) = \frac{2\gamma^2 C}{(\gamma^2 + C^2)^2},$$

and where C and P denote equilibrium values. The test for asymptotic stability of an equilibrium is that trace$(J) < 0$ and det$J > 0$. Clearly, from (16), the diagonal entries are negative and so the first condition is satisfied for all equilibria. When $C = 0$ (extinction) we have det$J > 0$, and therefore the extinction state is always asymptotically stable for any set of parameters. It is easy to check that $(0, 0)$ is a nodal point.

It remains to find the determinant for positive values of the equilibrium C. By definition,

$$\det J = \mu d + \mu \frac{2\alpha H\gamma^2 C}{(\gamma^2 + C^2)^2} - b\frac{2\alpha H\gamma^2 C}{(\gamma^2 + C^2)^2}$$
$$= \mu d + \frac{2\alpha H\gamma^2 C}{(\gamma^2 + C^2)^2}[\mu - b].$$

Next we use the quadratic relation (8) and definition of R to write

$$\gamma^2 + C^2 = \frac{\alpha H}{2\gamma d}\left(\frac{b}{\mu} - 1\right)C = 2\gamma RC.$$

Then the determinant becomes, upon simplification,

$$\det J = \mu d + \frac{\alpha H}{2R^2 C}(\mu - b) = \mu d - \frac{\mu\alpha H}{2R^2 C}\left(\frac{b}{\mu} - 1\right).$$

Hence, det$J > 0$ if, and only if,

$$C > \frac{\alpha H((b/\mu) - 1)\gamma}{2d\gamma R^2} = \frac{\gamma}{R}.$$

For the larger equilibrium this statement is valid because $C/\gamma = R + \sqrt{R^2 - 1} > R$, since $R > 1$. Therefore the larger equilibrium is asymptotically stable. Again, it is a node.

For the smaller positive equilibrium $C/\gamma = R - \sqrt{R^2 - 1} < R$, so det$J < 0$ and therefore the smaller nonzero equilibrium is unstable. Again, it straightforward to check that, at this equilibrium, there are two independent eigenvectors corresponding to the positive and
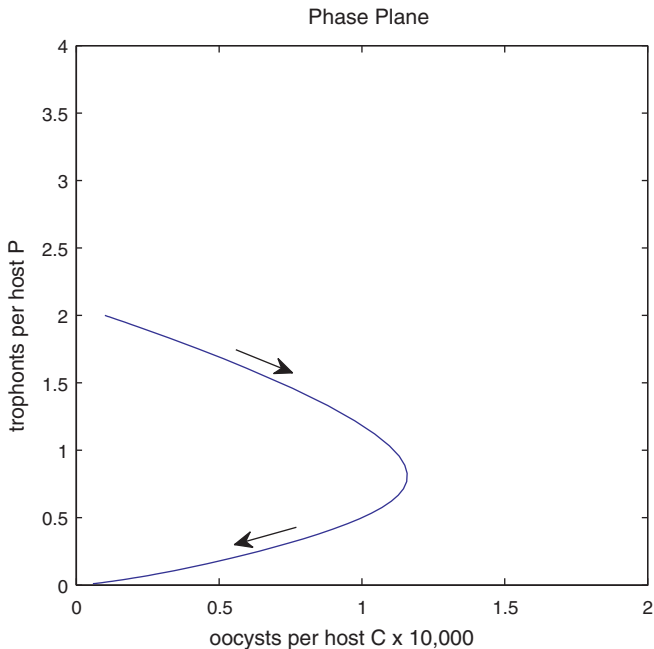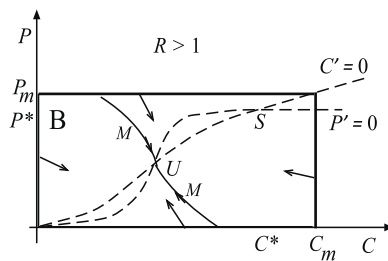
Fig. 11. Basin of attraction B, $R > 1$. The equilibria are the origin (extinction), the saddle point U, and the asymptotically stable node $S = (C^*, P^*)$. The two stable manifolds associated with the saddle point U, labeled M, separate the plane into two regions; to the right, all solutions approach S, and to the left all solutions approach the origin. The size of the basin of attraction, determined by C_m and P_m can be arbitrarily large. Thus, the behavior is global.

negative eigenvalues. These eigenvectors determine the directions of the separatrices, or the unstable manifolds exiting the equilibrium and the stable manifolds entering the equilibrium. Thus, we have a saddle structure at the unstable node. It is important to note that the unstable manifolds, which cross the $C4$ and P axes with negative slope, divide the phase plane into two regions: to the left all orbits approach extinction, and on the right the orbits approach the stable equilibrium ($R > 1$), or approach infinity (only for $R = 1$). Thus, at $R = 1$ a saddle–node bifurcation occurs.

An easy application of the Bendixson–Dulac theorem (e.g., see Logan and Wolesensky, 2009, pp. 168–169) shows there are no periodic orbits in the positive PC-plane. We compute the divergence of the vector field ($F(C, P), G(C, P)$) for $C > 0$, $P > 0$:

$$\frac{\partial F}{\partial C} + \frac{\partial G}{\partial P} = -d - \frac{2\gamma^2 C}{(\gamma^2 + C^2)^2} - mu < 0.$$

By the referenced theorem there can be no periodic solutions, or limit cycles, in the region $C > 0$, $P > 0$ for any values of the parameters.

Finally, for $R > 1$ we show there is a compact (closed, bounded) basin of attraction that traps the solution curves. Refer to Fig. 11. Let $C_m > C^*$ be any fixed value, where $C^* = \gamma \left(R + \sqrt{R^2 - 1} \right)$ is the the stable equilibrium, and let P_m be the unique value of P where the intersection of $C = C_m$ with the C–isocline occurs. Clearly, $P_m > P^*$, the P-equilibrium. Let B be the closed rectangle with vertices (0, 0), (C_m, 0), (C_m, P_m), (0, P_m). It follows easily from the definition of the vector field that the boundary of B consists of ingress points; the vector field points inward. Thus, B is a basin of attraction that traps the solution curves. The Poincare–Bendixson theorem limits the behavior of the the orbits entering B. Because they cannot escape and there are no periodic solution inside B, orbits must be an equilibrium (point) or approach an equilibrium. This constrains the possibilities to those stated in Section 2.

Acknowledgements

References

Anderson, R.M., May, R.M., 1978. Infectious Diseases of Humans. Oxford University Press, Oxford, UK.
Bechly, G., 2010. Additions to the fossil dragonfly fauna from the Lower Cretaceous Crato Formation of Brazil (Insecta: Odonata). Palaeodiversity 3 (Suppl. S), 11–77.
Bolek, M.G., Janovy Jr., J., 2007a. Small frogs get their worms first: the role of non-odonate arthropods in the recruitment of *Haematoloechus coloradensis* and *Haematoloechus complexus* in newly metamorphosed northern leopard frogs, *Rana pipiens*, and Woodhouse's toads, *Bufo woodhousii*. Journal of Parasitology 93, 300–312.
Bolek, M.G., Janovy Jr., J., 2007b. Evolutionary avenues for, and constraints on, the transmission of frog lung flukes (*Haematoloechus* spp.) in dragonfly second intermediate hosts. Journal of Parasitology 93, 593–607.
Bradley, D.J., May, R.M., 1978. Consequence of helminth aggregation for the dynamics of schistosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 72, 262–273.
Britton, N., 2003. Essential Mathematical Biology. Springer-Verlag, NY.
Bunker, B.E., Tracey, E., Barnes, A., Duba, A., Shuman, M., Janovy Jr., J., Logan, J.D. Macroparasite transmission among geographical locations and host life cycle stage, submitted for publication.
Canales-Lazcano, J., Contreras-Garduno, J., Cordoba-Aguilar, A., 2005. Fitness-related attributes and gregarine burden in a non-territorial damselfly *Enallagma praevarumhagen* (Zygoptera: Coenagrionidae). Odonatolgica 34, 123–130.
Clopton, R.E., 1993. Specificity in the Gregarine Assemblage Parsitizing *Tenebrio molitor*. Ph.D. Dissertation. University of Nebraska Lincoln, Lincoln, NE.
Clopton, R.E., Percival, T.J., Janovy Jr., J., 1993. *Nubenocephalus nebraskensis* n. gen., n. sp. (Apicomplexa: Actinocephalidae) from adults of *Argia bipunctulata* (Odonata: Zygoptera). Journal of Parasitology 79, 533–537.
Clopton, R.E., 2000. Order Eugregarinorida. In: Lee, J.J., Leedale, G.F., Bradbury, P. (Eds.), An Illustrated Guide to the Protozoa. , 2nd ed, pp. 205–288.
Detwiler, J.T., 2004. Host Specificity Amongst Gregarine Species and their Tenebrionid Hosts From an Experimental and Phylogenetic Perspective. M.S. Thesis. University of Nebraska Lincoln, Lincoln, NE.
Detwiler, J.T., Janovy Jr., J., 2008. The role of phylogeny and ecology in experimental host specificity: insights from a eugregarine-host system. Journal of Parasitology 94, 7–12.
Dobson, A.P., 1989. The population biology of Parasitic helminths in animal populations. In: Levin, S.A., Hallam, T.G., Gross, L.J. (Eds.), Applied Mathematical Ecology. Springer-Verlag, Berlin, pp. 144–175.
Dobson, A.P., Hilton, P.J., 1992. Regulation and stability of a free-living host–parasite system: *Trichostrongylus* in red grouse. II. Population models. Journal of Animal Ecology 61, 487–498.
Esteva, L., Rivas, G., Yang, H.M., 2006. Modelling parasitism and predation of mosquitoes by water mites. Journal of Mathematical Biology 53, 540–555.
Fenton, A., Hakalahti, T., Bandilla, M., Valtonen, E.T., 2006. The impact of variable hatching rates on parasite control: a model of an aquatic ectoparasite in a Finnish fish farm. Journal of Applied Ecology 43, 660–668.
Hecker, K.R., Forbes, M.R., Leonard, N.J., 2002. Parasitism of damselflies (*Enallagma boreale*) by gregarines: sex biases and relations to adult survivorship. Canadian Journal of Zoology 80, 162–168.
Klingenberg, C.P., Barrington, L., Rosilind, H., Keddie, B.A., Spence, J.R., 1997. Influence of gut parasites on growth performance in the water strider (Hemiptera: Gerridae). Ecography 20, 29–36.
Levine, N.D., 1988. The Protozoan Phylum Apicomplexa, vol. 1. CRC Press, Boca Raton, FL, 203 pp.
Logan, J.D., Wolesensky, W.R., 2009. Mathematical Methods in Biology. John Wiley and Sons, New York.
Mangel, M., 2006. The Theoretical Biologist's Toolbox. Cambridge University Press, Cambridge, UK.
Marcogliese, D., 2001. Implications of climate change for parasitism of animals in the aquatic environment. Canadian Journal of Zoology 79, 1331–1352.
Milner, F.A., Patton, C.A., 1999. A new approach to mathematical modeling of host–parasite systems. Computers and Mathematics with Applications 37, 93–110.
May, R.M., 1977. Togetherness among schistosomes: Its effects on the dynamics of infection. Mathematical Biosciences 35, 301–343.
Nedorezov, L.V., 1997. Escape effect and population outbreaks. Ecological Modelling 94, 95–110.
Nodtvedt, A., Dohoo, I., Sanchez, J., Conboy, G., DesCoteaux, L., Keefe, G., Leslie, K., Campbell, J., 2002. The use of negative binomial modelling in a longitudinal study of gastrointestinal parasite burdens in Canadian dairy cows. Canadian Journal of Veterinary Research 66, 249–257.
Nowak, M.A., 2006. Evolutionary Dynamics. Harvard University Press, Cambridge, MA.
Pal, P., Lewis, J.W., 2004. Parasite aggregations in host populations using a reformulated negative binomial. Journal of Helminth 78, 57–61.
Percival, T.J., Clopton, R.E., Janovy Jr., J., 1995. Two new menosporine gregarines, *Hoplorhynchus acanthatholius* N. Sp. and *Steganorhynchus dunwoodyi* N. G., Sp, (Aplicomplexa: Eugregarinorida: Actinocephalidae) from coenagrionid damselflies (Odonata: Zygoptera). The Journal of Eukaryotic Microbiology 42 (4), 406–410.
Revie, C.W., Hollinger, E., Gettinby, G., Lees, F., Heuch, P.A., 2007. Clustering of parasites within cages on Scottish and Norwegian salmon farms: alternative sampling strategies illustrated using simulation. Preventive Veterinary Medicine 81, 135–147.

Richardson, J.M.L., Baker, R.L., 1997. Effect of body size and feeding on fecundity in the damselfly *Ischnura verticalis* (Odonata: Coenagrionidae). Oikos 79, 477–483.

Richardson, S., Janovy, J., 1990. *Actinocephalus carrilynnae* N. Sp. (Apicomplexa: Eugregarinorida) from the blue damselfly, *Enallagama civile* (Hagen). Journal of Protozoology 37, 567–570.

Roberts, L.S., Janovy Jr., J., 2009. Foundations of Parasitology, 8th ed. McGraw-Hill, Dubuque, IA, 701 pp.

Rodriguez, Y., Omoto, C.K., 2007. Individual and population effects of eugregarine, *Gregarina niphandrodes*, on *Tenebrio molitor* (Coleoptera: Tenebrionidae). Environmental Entomology 36, 689–693.

Schwank, S., 2004. Effects of Infection Intensity on Gametocyst Shedding in Gregarines. M.S. Thesis. University of Nebraska Lincoln, Lincoln, NE.

Smith, J.M., 1974. Models in Ecology. Cambridge University Press, Cambridge, UK.

Tallis, G.M., Leyton, M.K., 1969. Stochastic models of populations of helminthic parasites in the definitive host, I. Mathematical Biosciences 4, 39–48.

Watwood, S., Janovy Jr., J., Peterson, E., Addison, M.A., 1997. *Gregarina triboliorum* (Eugregarinida: Gregarinidae) n. sp. from *Tribolium confusum*, and resolution of the confused taxonomic history of Gregarina minuta Ishii 1914. Journal of Parasitology 83, 502–507.

Wise, M.R., Janovy Jr., J., Wise, J.C., 1999. Host specificity in *Metamera sillasenorum*, n. sp. a gregarine parasite of the leech *Helobdella triserialis* with notes on transmission dynamics. Journal of Parasitology 86, 602–606.