

# On multiple infections by parasites with complex life cycles

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## 11 **Statement of Authorship**

12 Both authors developed the theory. P.L.N developed and implemented the computational  
13 model. Both authors wrote the manuscript.

## 14 **Data and Code Availability**

15 All data and simulation codes for generating figures are available on [https://anonymous.](https://anonymous.4open.science/r/multipleinfections)  
16 [4open.science/r/multipleinfections](https://anonymous.4open.science/r/multipleinfections)

17        Host manipulation is a common strategy of parasites with complex lifecycles. It  
18 directly affects predator-prey dynamics in trophically transmitted parasites. The-  
19 oretical studies suggest that predation-enhancing manipulation often decimates  
20 the prey population, making parasites prone to extinction. Host manipulation,  
21 however, can also suppress predation due to conflicting interests when multiple  
22 parasites infect a host, often neglected in theoretical studies. Misaligned interests  
23 of coinfecting parasites can occur due to limited carrying capacity or parasitoid  
24 developmental stage. Including this realistic complexity in a mathematical model,  
25 the results depart from previous studies substantially. We show that coinfect-  
26 ing multi-trophic parasites can preserve the predator-prey system and themselves  
27 through a combination of manipulation and reproduction parameters. Our study  
28 highlights the necessity and provides the means of incorporating the reality of  
29 multiple parasites and their multi-trophic life cycles in the theory of parasite  
30 ecology.

## Introduction

Parasites infect life on earth ubiquitously, and many of these parasites have complex life cycles (Zimmer, 2001). While a complex lifecycle can be defined as abrupt ontogenic changes in morphology and ecology (Benesh, 2016), a complex parasitic lifecycle typically involves numerous hosts that a parasite needs to traverse to complete its life cycle. This complex lifecycle results in the evolution of various strategies that enable the success of parasite transmission from one host to another. One famous strategy that inspires many science fiction movies and novels is host manipulation, where a parasite can alter the morphology and/or behaviour of its host to enhance its transmission to the next host (Hughes et al., 2012). Host manipulation has been shown in many host-parasite systems, from parasites with simple life-cycle to those with complex life-cycle that involves more than one host (Hughes et al., 2012; Molyneux and Jefferies, 1986). For instance, sand flies infected by *Leishmania* parasites bite more and take more time for a blood meal from mammals (the definitive host of *Leishmania*) compared to their uninfected counterparts (Rogers and Bates, 2007). Copepods infected by cestode parasites are more active and accessible to sticklebacks (the definitive hosts of the cestodes) compared to uninfected copepods (Wedekind and Milinski, 1996).

Theoretical studies have long attempted to understand the ecological and evolutionary consequences of host manipulation. Roosien et al. (2013) and Hosack et al. (2008) showed that manipulative parasites could increase the disease prevalence in an epidemic. Gandon (2018) studied the evolution of the manipulative ability of infectious disease parasites, showing different evolutionary outcomes depending on whether the pathogen can control its vector or host. Haderler and Freedman (1989); Fenton and Rands (2006) and Rogawa et al. (2018) showed that host manipulation could stabilise or destabilise the predator-prey dynamics depending on how manipulation affects the predation response function and the assumption on the fertility of the definitive infected host. Seppälä and Jokela (2008) showed that host manipulation could evolve even when it increases the risk of the intermediate host being eaten by a non-host predator, given that the initial predation risk is sufficiently low. These models, however, lack a crucial aspect of parasite dynamics, multiple infections (Kalbe et al., 2002).

Typical studies do not consider multiple infections, a phenomenon that is the norm rather than an exception in parasitism. Multiple infections result in the coinfection of more than one parasite inside a host, which may alter the manipulative outcomes (figure 1). An alignment of interest between coinfecting parasites may enhance manipulation, while a conflict of interest may reduce the manipulative effect. Indeed, Hafer and Milinski (2015) showed that copepods infected by two cestode parasites reduce the activity of copepods when both parasites are at the same noninfectious stage, i.e. both parasites are not ready to transmit. Thus the reduction in mobility is suggested to reduce the predation rate by the definitive hosts. When two infectious parasites infect the copepods, the copepods' activity increases, and so does the predation risk for the copepod. However, when the copepods are infected by one infectious and one noninfectious parasite, their interests clash, and one parasite wins over the other.

Theoretical work that considers multiple infections often focuses on the evolution of virulence (van Baalen and Sabelis, 1995; Alizon et al., 2013; Alizon and van Baalen, 2008; Choisy and de Roode, 2010; Alizon, 2012). They show multiple infections can increase vir-



**Figure 1: Who is in control?.** *Schistocephalus* eggs, which overwinter at the bottom of bodies of water, hatch into microscopically small swimming larvae. These larvae are eaten by copepods (also known as Cyclops due to its single eye), where they develop to the second larval stage. However, the copepod is only the first intermediate host. The larvae are then eaten by sticklebacks, where they reach the third larval stage and grow significantly in size and weight. For the parasite to successfully reach its final host, a warm-blooded animal like a bird, it manipulates its intermediate hosts. The timing is crucial as the chances of success are greatest if the larvae develop in the copepod for 13 to 15 days before entering the stickleback. The presence of multiple parasites in the same host can lead to competition and strategic decision pertaining to investment in manipulation and growth. And indeed a stickleback can be infected by numerous tapeworms as shown above by Martin Kalbe.

74 ulence (van Baalen and Sabelis, 1995; Choisy and de Roode, 2010). Evolutionary branching  
 75 of a less virulent and a hypervirulent parasite can occur when considering within-host dy-  
 76 namics (Alizon and van Baalen, 2008), and a reduction in virulence is possible if parasites  
 77 are co-transmitted (Alizon, 2012). These studies also involve host manipulation to a certain  
 78 extent, as it can affect transmission rates, even though they do not explicitly consider the  
 79 trait. Host manipulation in trophically transmitted parasites receives less attention. Although  
 80 manipulation correlates with the transmission rate in trophically transmitted parasites and  
 81 infectious diseases, there are differences. Host manipulation influences the predation rate in  
 82 trophically transmitted parasites, predominantly affecting predator-prey dynamics. Theoreti-  
 83 cal studies on host manipulation in trophically transmitted parasites with multiple infections  
 84 are rare (Parker et al., 2003; Vickery and Poulin, 2009). Moreover, they do not consider the  
 85 prey-predator dynamics, which will likely have important feedback on the evolution of host

manipulation. A few studies considering the prey-predator dynamics do not incorporate multiple infections (Rogawa et al., 2018; Iritani and Sato, 2018; Haderler and Freedman, 1989; Fenton and Rands, 2006). More importantly, they assume that transmission from definitive hosts to intermediate hosts is due to direct contact between the two types of hosts. This is often not the case, as parasites are released from the definitive hosts into the environment. Transmission happens only when intermediate hosts have contact with this free-living parasite pool.

Our study addresses the gap in the theoretical work on host manipulation in trophically transmitted parasites. We include multiple infections and consider the dynamics of the free-living parasite pool. Our compartment model helps illustrate a parasite's complex lifecycle with two hosts: an intermediate host preyed upon by a definitive host. Transmission from the intermediate host to the definitive host occurs when predation on infected intermediate hosts happens. Reproduction only happens in the definitive hosts. New parasites then enter the environment, where the cycle continues. We focus on the intermediate host manipulation, such that the parasite increases the uptake of the intermediate host by the definitive host to increase its transmission rate. We then analyse the effect of host manipulation on the ecological dynamics in the prey-predator-parasite system. In contrast to the abovementioned examples, our model consists of a single intermediate host as it already provides enough complexity to discuss between transmission and manipulation. We found that sabotage in host manipulation almost always pushes the dynamical system toward bistability, provided the reproduction in a single infection is sufficiently small. The bistable nature suggests that the predator-prey parasite system is finely balanced and susceptible to extinction via ecological disturbances. Initially surprising, we showed that cooperation in host manipulation and enhanced reproduction in co-infecting parasites is not always beneficial and might expose the parasite population to the risk of extinction.

## Model and Results

Our model concerns the complex lifecycle of a trophically transmitted parasite that requires two hosts: an intermediate host and a definitive host. Reproduction only happens inside the definitive hosts, releasing new parasitic progeny in the environment. An intermediate host can be infected if it encounters this free-living parasite pool. Finally, when a definitive host consumes an infected intermediate host, the definitive host gets infected, and the parasite completes its lifecycle.

For simplicity, we assume that hosts can be infected by one (single infection) or, at most, two parasites (double infections). Our model is, therefore, more relevant to the macroparasitic system. Given that infection occurs, the probability that two parasites from the parasite pool co-transmit to an intermediate host is denoted by  $p$ . Thus  $1 - p$  is the probability that a single parasite enters an intermediate host. When a definitive host consumes an intermediate host infected by two parasites, there is a probability  $q$  that the parasites co-transmit to the definitive host. With probability  $1 - q$ , only one parasite successfully transmits. This formulation assumes that infection always happens when hosts encounter parasites. The dynamics of a complex lifecycle parasite that requires two hosts is described by the following system

127 of equations, firstly for the intermediate host as,

$$\begin{aligned}
\frac{dI_s}{dt} &= R(I_s, I_w, I_{ww}) - dI_s - P_s(D_s, D_w, D_{ww})I_s - \eta I_s \\
\frac{dI_w}{dt} &= (1-p)\eta I_s - (d + \alpha_w)I_w - P_w(D_s, D_w, D_{ww}, \beta_w)I_w \\
\frac{dI_{ww}}{dt} &= p\eta I_s - (d + \alpha_{ww})I_{ww} - P_{ww}(D_s, D_w, D_{ww}, \beta_{ww})I_{ww}
\end{aligned} \tag{1}$$

128 where  $R(I_s, I_w, I_{ww})$  represents the birth rate of the intermediate hosts, a function of both  
129 infected and uninfected individuals.  $P_s$ ,  $P_w$ ,  $P_{ww}$  are the predation functions of definitive  
130 hosts on susceptible, singly infected and doubly infected intermediate hosts. The predation  
131 function depends on the density of the definitive hosts and the manipulative strategies of  
132 parasites in the intermediate hosts. In particular, if a single parasite infects an intermediate  
133 host, the manipulation strategy is  $\beta_w$ . However, if the intermediate host is co-infected, the  
134 manipulation strategy is  $\beta_{ww}$ . In the scope of this model, we assume no specific relationship  
135 between  $\beta_w$  and  $\beta_{ww}$  to explore all possible ecological outcomes of the system. The force  
136 of infection by parasites in the environment is denoted by  $\eta = \gamma W$ . Since parasites can ma-  
137 nipulate intermediate and definitive hosts, here, whenever we mention host manipulation, it  
138 specifically refers to the manipulation in intermediate hosts, which correlates to the predation  
139 rate.

140 For the definitive hosts we have,

$$\begin{aligned}
\frac{dD_s}{dt} &= B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww}) - \mu D_s - (\lambda_{ww} + \lambda_w)D_s \\
\frac{dD_w}{dt} &= (\lambda_w + (1-q)\lambda_{ww})D_s - (\mu + \sigma_w)D_w - ((1-q)\lambda_{ww} + \lambda_w)D_w \\
\frac{dD_{ww}}{dt} &= q\lambda_{ww}D_s + ((1-q)\lambda_{ww} + \lambda_w)D_w - (\mu + \sigma_{ww})D_{ww}
\end{aligned} \tag{2}$$

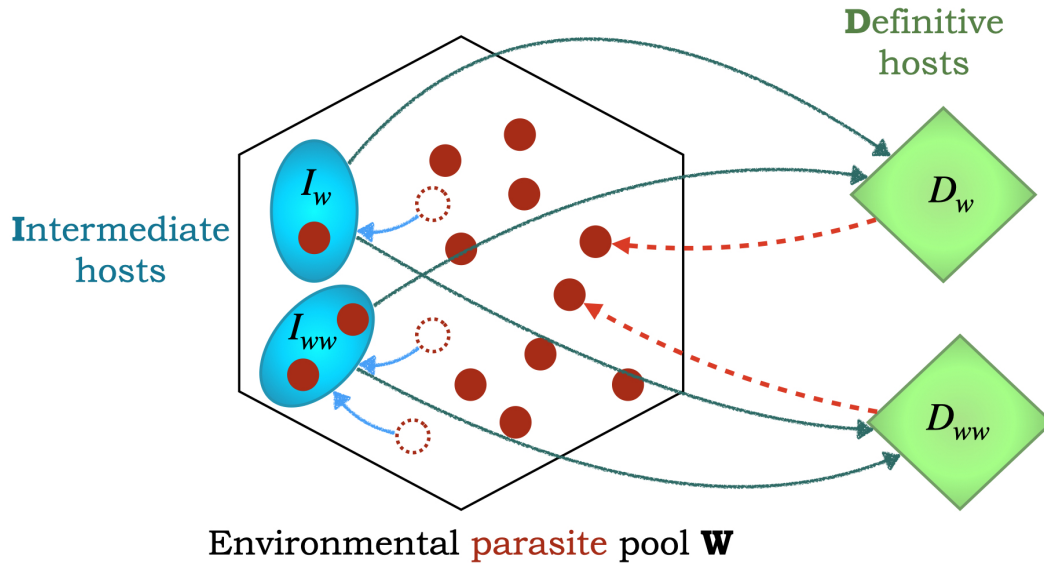
141 where  $B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww})$  represents the birth rate of definitive hosts. The birth  
142 rates depend on the density of both intermediate and definitive hosts, infected or uninfected.  
143 The force of infection that corresponds respectively to singly infected intermediate host ( $I_w$ )  
144 and doubly infected intermediate hosts ( $I_{ww}$ ) is denoted respectively by  $\lambda_w = h(\rho + \beta_w)I_w$   
145 and  $\lambda_{ww} = h(\rho + \beta_{ww})I_{ww}$ , where  $\rho$  is the baseline predation rate and  $h$  is the probability  
146 that the parasite successfully establishes inside the host. If there is no manipulation, that is,  
147  $\beta_w = \beta_{ww} = 0$ , the parasite is still transmitted via the based line predation. The dynamics  
148 of the free-living parasites in the environment are then given by,

$$\frac{dW}{dt} = f_w D_w + f_{ww} D_{ww} - \delta W - \eta I_s. \tag{3}$$

149 Definitions of different parameters can be found in Table SI.1.

150 Here, we focus on manipulation that enhances transmission from intermediate hosts to  
151 definitive hosts; we thus simplify the transmission from the parasite pool to intermediate  
152 hosts such that no sequential infection. This assumption is motivated given that the prey'

153 lifecycle is often shorter than that of the predator. A prey likely encounters the free-living  
 154 parasite pool once and then dies due to predation, making sequential transmission less likely  
 155 at this state. Sequential infection can happen when parasites transmit from intermediate  
 156 hosts to definitive hosts. Therefore, a singly infected definitive host can be further infected  
 157 by another parasite if it consumes infected intermediate hosts. Figure (2) illustrates the  
 system's dynamics.



**Figure 2:** Schematic of the model. Blue ovals represent intermediate host compartment, green diamonds represent definitive host compartment, and the transparent hexagon represents the parasite pool compartment with red circles illustrating individual parasites.

158

### 159 Basic reproduction ratio $R_0$ of the parasites

160 The basic reproduction ratio  $R_0$  (or basic reproduction number as often used in epidemiology)  
 161 indicates parasite fitness. It can be understood as the expected number of offspring a parasite  
 162 produces during its lifetime when introduced to a susceptible host population. We calculate  
 163 the basic reproduction ratio  $R_0$  using the next-generation method (Diekmann et al., 1990,

164 2009; Hurford et al., 2010) (See SI1 for details).

$$\begin{aligned}
 R_0 = & \overbrace{\gamma I_s^* \frac{pqh(\rho + \beta_{ww})}{\alpha_{ww} + d + P_{ww}} \frac{D_s^*}{\mu + \sigma_{ww}} \frac{f_{ww}}{\delta + \gamma I_s^*}}^{\text{Double infections}} + \\
 & \underbrace{\gamma I_s^* \left( \frac{(1-p)h(\rho + \beta_w)}{\alpha_w + d + P_w} + \frac{p(1-q)h(\rho + \beta_{ww})}{\alpha_{ww} + d + P_{ww}} \right) \frac{D_s^*}{\mu + \sigma_w} \frac{f_w}{\delta + \gamma I_s^*}}_{\text{Single infection}} \quad (4)
 \end{aligned}$$

165 where  $I_s^*$  and  $D_s^*$  are the densities of susceptible intermediate and definitive hosts at the  
 166 disease-free equilibrium. Here, the expression of  $R_0$  contains the possible reproduction routes  
 167 of a parasite, which can be via double or single infections. The first component corresponds  
 168 to the double infections route, in which the focal parasite co-transmits with another par-  
 169 asite into a susceptible intermediate host, then co-transmits into a susceptible definitive  
 170 host and reproduces. Here, parasites are so rare that only co-transmission matters and the  
 171 compartments with sequential infections are therefore neglected. The second component  
 172 corresponds to the single infection route, wherein the focal parasite infects a susceptible  
 173 intermediate host via single or double infections. The parasite then transmits alone into the  
 174 susceptible definitive host and eventually reproduces.

175 If  $R_0 > 1$ , a parasite spreads when introduced into the disease-free equilibrium of prey and  
 176 predator. Intuitively, the higher the density of susceptible intermediate and definitive hosts,  
 177 the larger the value of  $R_0$  as the infection reservoir is more extensive. In contrast, regardless  
 178 of the explicit form of the predation function, the higher the predation rate  $P_w$  and  $P_{ww}$ , the  
 179 lower the value of  $R_0$  given the smaller reservoir of intermediate hosts. The effect of host  
 180 manipulation on the value of  $R_0$  is not so straightforward; as host manipulation becomes  
 181 efficient, the transmission rate from the intermediate host to the definitive host increases,  
 182 but so does the predation rate. A higher predation rate results in a smaller intermediate  
 183 host reservoir available for the parasites to infect. To understand the effect of manipulation  
 184 on parasites' fitness and the system's ecological dynamics, we next specify the predation  
 185 functions. We consider linear functions for predation to begin with,

$$\begin{aligned}
 P_s(D_s, D_w, D_{ww}) &= \rho D_{total} \\
 P_w(D_s, D_w, D_{ww}, \beta_w) &= (\rho + \beta_w) D_{total} \\
 P_{ww}(D_s, D_w, D_{ww}, \beta_{ww}) &= (\rho + \beta_{ww}) D_{total}
 \end{aligned}$$

186 where  $D_{total} = D_s + D_w + D_{ww}$  is the total density of the definitive hosts, and  $\rho$  is the  
 187 baseline capture rate of the predator on the prey. If an intermediate host is infected, it is  
 188 captured by the definitive hosts with rate  $\rho + \beta_w$  if it is singly infected and with rate  $\rho + \beta_{ww}$   
 189 if it is doubly infected. Zero values for  $\beta_w$  and  $\beta_{ww}$  suggest no manipulation, and predation  
 190 is at the baseline value  $\rho$ .

191 For simplicity, we also consider a linear function of the birth of definitive hosts

$$B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww}) = \rho c D_{total} I_{total}$$



where  $c$  is the efficiency of converting prey into predator's offspring, and  $I_{total} = I_s + I_w + I_{ww}$  is the total density of the intermediate hosts. It is important to note that host manipulation affects the population dynamics via its influence on predation rate but not the physiological aspect of the definitive host, i.e. the predator. The birth rate of the predators thus depends on the capture rate, but it is not affected by host manipulation; as to our best knowledge, there is no supporting evidence to consider otherwise.

The explicit form of  $I_s^*$  and  $D_s^*$ , capturing the predator-prey dynamics, depends on the precise form of all birth and predation functions  $B, R, P_s, P_w$  and  $P_{ww}$ . But, it does not depend on the manipulation ability or any other parameter of the parasite. Given that the birth rate of the predator and the predation rate are linear functions in prey and predator density, the form of the birth rate  $R$  of the prey has a significant effect on the susceptible intermediate and definitive host dynamics.

## Birth function of intermediate hosts

The simplest form of the prey's birth rate is a linear function, in which case the disease free equilibrium is always unstable. In particular, it has a cyclic behaviour because, at this equilibrium, the jacobian matrix of the system (1, 2, 3) always has two pure imaginary eigenvalues (see SI2). This follows from the Lotka-Volterra system using linear functions for prey birth and predation (Lotka, 1920). Since the disease-free dynamics is cyclic, it is difficult to analyse the spread of a parasite using the basic reproduction ratio, which is evaluated when the disease-free state is stable. Here,  $R_0 > 1$  happens when  $\gamma$ , the transmission rate from the environment to intermediate hosts, and the reproduction rates  $f_w, f_{ww}$  are significantly large (the specific mathematical conditions can be found in SI3). However, even when this condition is satisfied, the parasite may not be able to spread and persist in cyclic susceptible host dynamics (Figure SI1). This result agrees with the conclusion in (Ripa and Dieckmann, 2013), which suggests that it is difficult for a mutant to invade a cyclic resident population. In our case, it is not the invasion of a mutant in a resident population but the invasion of a parasite in a cyclic disease-free host population; the argument, however, remains valid in both cases. This issue deserves a more thorough investigation, which is out of the scope of this article. Here, we choose a non-linear birth function of the intermediate hosts to obtain a stable disease circulation state and focus on the effect of host manipulation on the ecological dynamics.

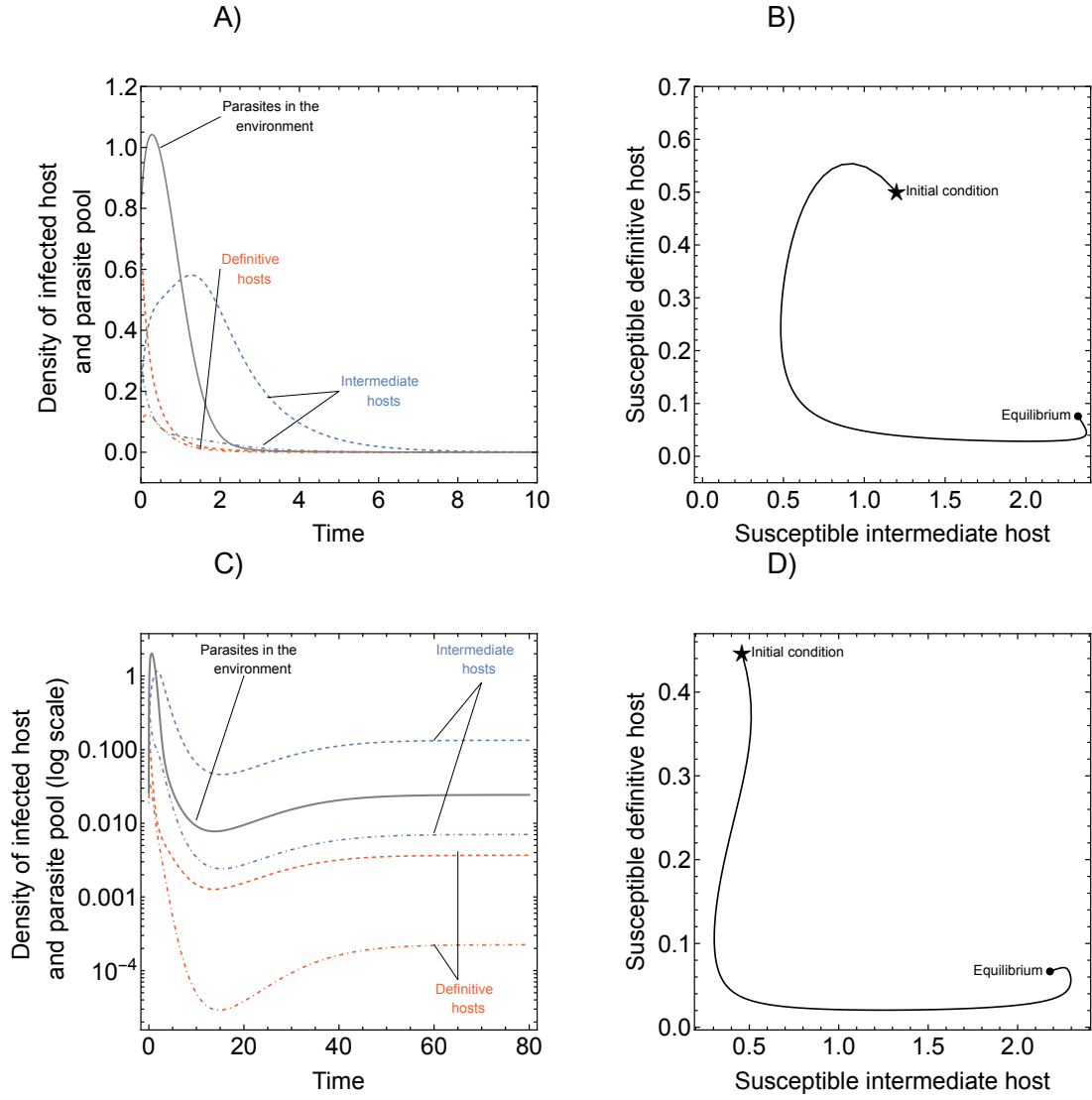
The logistic growth for the non-linear birth function follows by

$$R(I_w, I_s, I_{ww}) = rI_{total}(1 - kI_{total})$$

where  $k$  is the intraspecific competition coefficient. The disease-free equilibrium is as follows

$$I_s^* = \frac{\mu}{c\rho} ; D_s^* = \frac{c\rho(r - d) - k\mu r}{c\rho^2}$$

This equilibrium is positive and stable if components of the parasite, such as reproduction and transmission are sufficiently small, details of the condition can be found in SI4 (Figure 3B).



**Figure 3:** A, B) Disease free equilibrium where parasite cannot persist. C, D) Disease stable equilibrium. Solid gray line indicate the density of free-living parasites, blue lines indicate infected intermediate hosts while red lines indicate infected definitive hosts. Dashed lines indicate singly infected hosts while dot-dashed lines indicate doubly infected hosts. Parameters for disease free equilibrium  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = \alpha_{ww} = 0$ ,  $\beta_w = \beta_{ww} = 1.5$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = \sigma_{ww} = 0$ ,  $q = 0.05$ ,  $f_w = f_{ww} = 7.5$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $h_1 = h_2 = 0.6$ . Disease stable equilibrium have the same parameter values except for higher host manipulation  $\beta_w = \beta_{ww} = 4.5$  and parasite reproduction  $f_w = f_{ww} = 45$

228 When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio  
 229  $R_0 > 1$ . Since the expression is complicated, we could not obtain analytical solutions for

this inequality without assumptions. We assume the same parasite virulence,  $\alpha_w = \alpha_{ww}$ ,  $\sigma_w = \sigma_{ww}$ , and reproduction in double infection as a linear function concerning reproduction in single infections,  $f_{ww} = \epsilon f_w$ . When  $\epsilon > 1$ , reproduction in double infections is enhanced as compared to in single infections, whereas  $\epsilon \leq 1$ , reproduction in double infections is depressed or equal to reproduction in single infections. We found that the parasite can establish if its reproduction value in a single infection  $f_w$  is more significant than a threshold (Figure 4, see SI5).

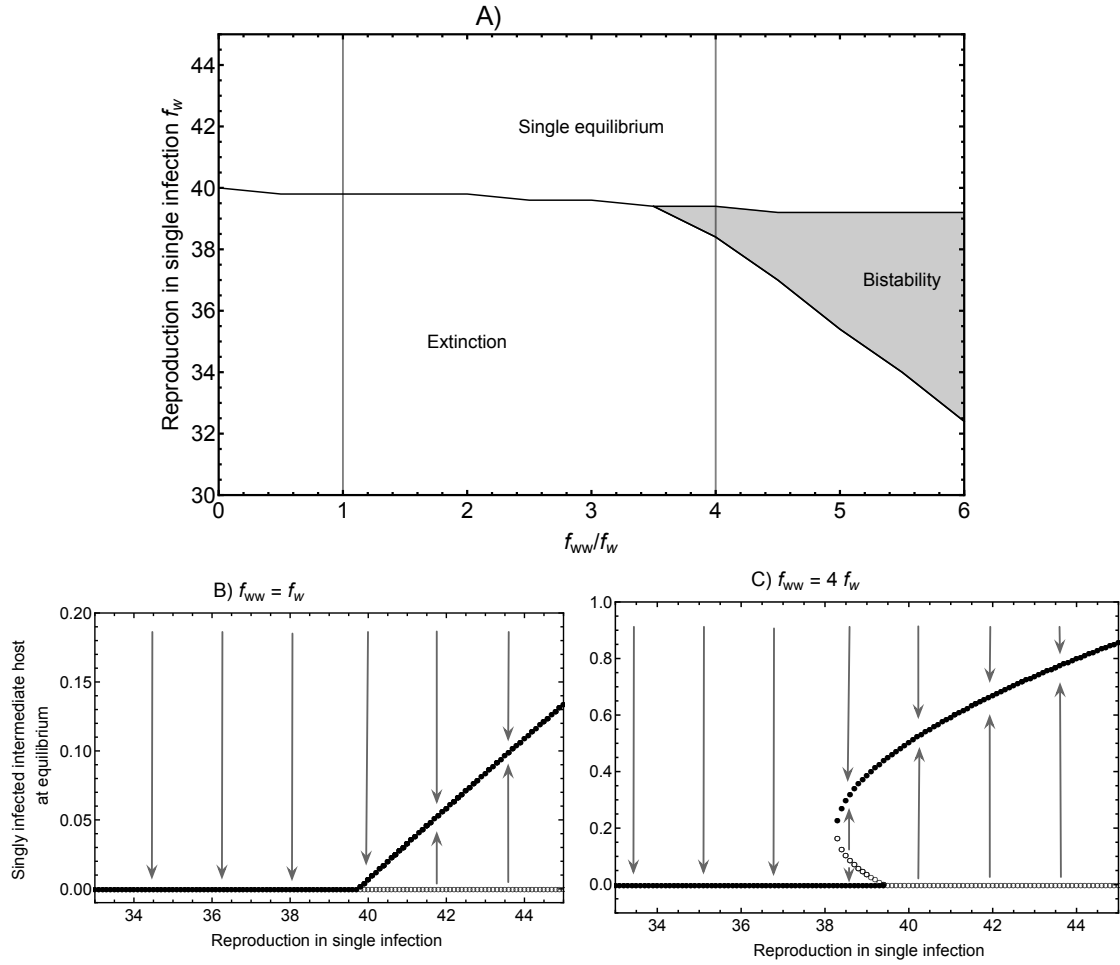
Our numerical results show that the parasite reproduction is substantial compared to other parameters (its value is nearly 40 times greater than other parameters). This observation suggests that trophically transmitted parasites must release many offspring into the environment to persist. Interestingly, bistability occurs if the reproduction rate of the parasite in double infections is enhanced (Figure 4A, B). In the bistable region, the parasite population can reach a stable equilibrium if the initial density is large enough. In contrast, with sufficient disturbance, the parasite population could go extinct.

## The effect of host manipulation on ecological dynamics

Host manipulation can be cooperative; two parasites increase the predation rate on intermediate hosts, or  $\beta_{ww} > \beta_w$ . However, it can also be uncooperative; the predation rate on doubly-infected intermediate hosts is lower than that on singly-infected ones or  $\beta_{ww} < \beta_w$ . Cooperation in parasite manipulation increases the parasite's basic reproduction ratio  $R_0$ , but the manipulation in a single infection substantially affects the value of  $R_0$  (Figure 5 Left). Intuitively, if the manipulation in a single infection is minor, there is not enough transmission, and the parasite goes extinct. However, suppose the ability to manipulate the host in a single infection is merely enough for the parasite population to escape extinction. In that case, cooperation in host manipulation leads to a bistable system state. Within the bistable region, the basic reproduction ratio can be less than one, suggesting that the parasite cannot spread when its manipulative values are within this area of weak manipulation when coinfecting.

Co-infecting parasites can influence each other in different life history traits besides manipulation. Parasites can have an enhanced reproduction rate in coinfections, i.e.  $f_{ww} > f_w$ . Likewise, they can compete for resources, so reproduction in double infection is depressed as compared to in single infection. Without any assumption on the relationship between manipulative ability and reproduction, we explore all possible combinations of cooperation-sabotage range in manipulation and depressed-enhanced range in reproduction. If parasites are uncooperative in manipulations and shows depressed reproduction, they cannot persist (Figure 5). In contrast, if they are highly cooperative in manipulation and show enhanced reproduction (i.e.  $\beta_{ww}/\beta_w \rightarrow \infty$  and  $f_{ww}/f_w \rightarrow \infty$ ), there is a guaranteed single equilibrium for parasite existence.

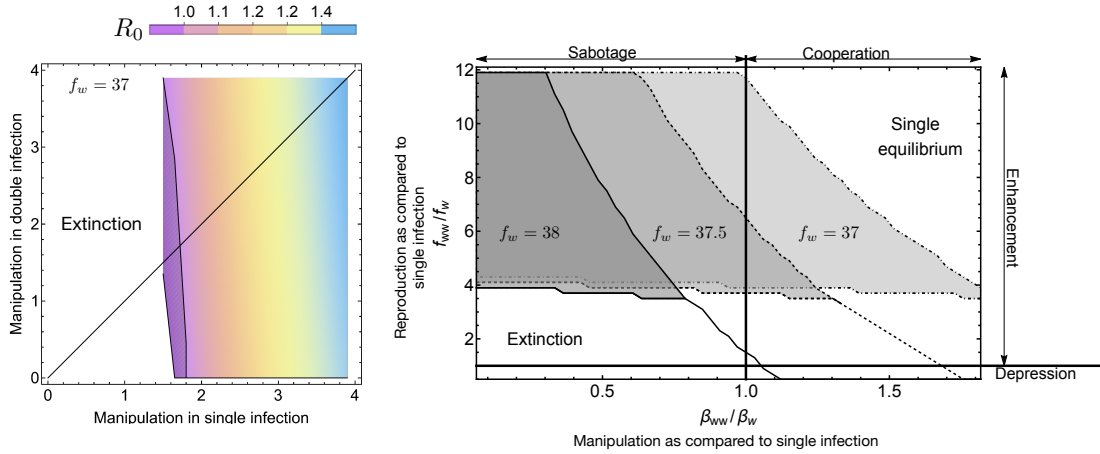
For intermediate levels of coordination in reproduction and manipulation, a bistable area could occur. However, the size of this area is sensitive to the value of reproduction and manipulation in a single infection. In particular, higher values of these two parameters reduce the bistability area, whereas larger values increase the bistability area (Figure 5, Figure SI1). If the parasites sabotage each other, the system is highly prone to bistability



**Figure 4:** Effect of parasite reproduction on the ecological dynamics. A) Enhanced reproduction in double infection leads to bistability, B, C) Density of singly infected host at equilibrium when reproduction of parasites are the same in singly and doubly infected hosts  $f_{ww} = f_w$ , and when reproduction of parasites in doubly infected hosts is enhanced four times than those in singly infected hosts  $f_{ww} = 4f_w$ . Filled circles indicate stable equilibrium and open circles indicate unstable equilibrium. Parameter  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $\beta_w = 1.5$ ,  $\beta_{ww} = 1.5$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $h_1 = h_2 = 0.6$

and only has a single equilibrium when reproduction is especially enhanced. Interestingly, sufficiently high reproduction enhancement leads to bistability (i.e.  $f_{ww}$  is at least four times  $f_w$ ), and depressed reproduction always leads to a single equilibrium of the system (Figure 5). While a single equilibrium guarantees the existence of a parasite population, bistability indicates that a disturbance of the system may likely lead to the extinction of the parasite population. This suggests that the benefits of coordination in reproduction and manipulation are context-dependent. Coordinating holds an advantage if there are no significant tradeoffs

279 and if reproduction or manipulation in single infections are large enough.

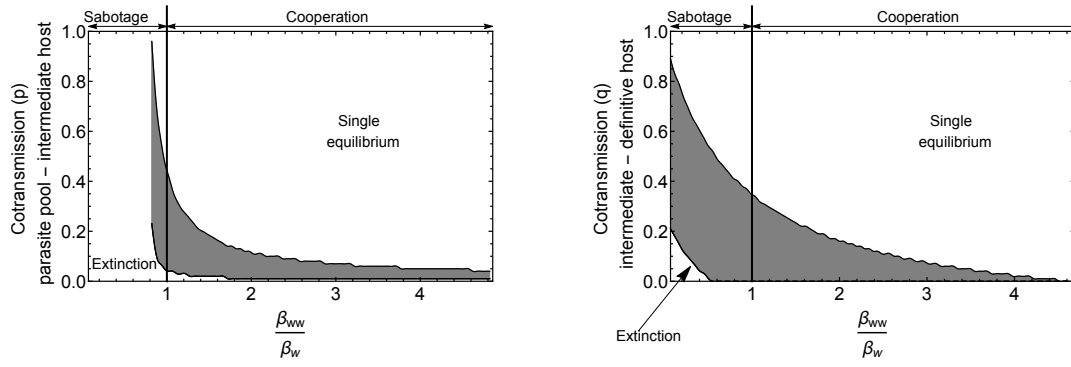


**Figure 5:** Left:  $R_0$  values increase with more efficient manipulation in both single and double infection. Hatched area indicates bistable region. As manipulation in single infection increases, the system only has one stable equilibrium. On the black line, manipulation is indifference between single infection and double infection ( $\beta_w = \beta_{ww}$ ). Right: Changes of the bistability area (shaded areas) with respect to different reproduction rates in single infection (different boundary styles). Manipulation and reproduction is indifference between single infection and double infection on the vertical and horizontal lines respectively. Common parameter:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\beta_w = 1.65$ ,  $h_1 = h_2 = 0.6$ .

280 Co-transmission probability from the parasite pool to intermediate hosts  $p$  has the opposite  
 281 effect on the bistable area compared to co-transmission probability  $q$  from intermediate hosts  
 282 to intermediate hosts (Figure 6). In particular, when the parasite sabotages the manipula-  
 283 tion, increasing  $p$  enlarges the bistable area, whereas increasing  $q$  reduces it. In contrast,  
 284 when parasites cooperate in manipulation, reducing  $p$  decreases the bistable area while re-  
 285 ducing  $q$  widens it. If cooperation in manipulation is exceptionally high, the population will  
 286 always exist with one stable equilibrium regardless of the co-transmission value. However,  
 287 as there are always limitations and trade-offs, high values may not be possible. Bistability  
 288 indicates vulnerability to disturbance, suggesting that cooperation in manipulation may be  
 289 beneficial when the co-transmission from the pool to the intermediate host increases. How-  
 290 ever, cooperation in manipulation may harm the population when the co-transmission from  
 291 the intermediate host to the definitive host increases.

## 292 Discussion & Conclusion

293 Host manipulation is a ubiquitous phenomenon suggested to affect the prey-predator dynam-  
 294 ics in trophically transmitted parasites. In particular, manipulation of infected intermediate



**Figure 6:** Left: Effect of cotransmission from parasite pool to intermediate host. Right: Effect of cotransmission from intermediate to definitive host. Common parameters:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\epsilon = 4.5$ ,  $\beta_w = 1.45$ ,  $f_w = 38$ ,  $h_1 = h_2 = 0.6$ .

hosts to increase the predation rate of definitive hosts may result in a heavy burden of predators on the intermediate host population. This pressure can make parasites more vulnerable to extinction (Hader and Freedman, 1989; Fenton and Rands, 2006).

Our model shows that parasites cannot spread quickly in a cyclic predator-prey system. This delay is an expected result since even though the parasite's basic reproduction ratio  $R_0$  is larger than one, it is estimated at the predator and prey's unstable equilibrium (or cyclic equilibrium). Thus, when the density of the prey and predator is at the minimum value of the cycle, the "effective"  $R_0$  of the parasite can be smaller than one. Another interesting result is that the reproduction value is much larger than other parameter values. This result is likely due to the introduction of a free-living parasitic pool. Our model shows that in making the system more realistic, we also obtain a more realistic quantitative value for parasitic reproduction.

In the study by Rogawa et al. (2018), a non-manipulative parasite can invade a susceptible prey-predator population and cause the system to cycle. The system stops cycling and approaches a fixed point when the parasite becomes manipulative, and this stability increases with increased manipulation. In our model, non-manipulative parasites cannot persist in the system, and the parasite never leads to cyclic dynamics. These results may contradict with Rogawa et al. (2018), where non-manipulative parasites can still exist via cyclic behaviour. We suggest that the different results may be due to our introduction of a parasite pool and multiple infections, unlike the model of Rogawa et al. (2018). In their system, transmission from the definitive host to the intermediate host was assumed to result from direct contact between the two hosts. Such immediate transmission could directly accelerate the feedback loop between prey and predator. Hence, faster predator-prey dynamics occur, which may lead to cyclic dynamics when parasites are introduced.

In our study, population dynamics exhibit bistability under certain circumstances. This is very likely due to the introduction of co-transmission, which has been shown to result in bistable population dynamics in plant virus Allen et al. (2019) and infectious diseases Gao

et al. (2016). In this bistability region, if the system is disturbed (e.g. migration of the intermediate or definitive hosts or predation of intermediate hosts by other predators), then the density of the infected hosts may crash, leading to parasite extinction. The bistability region widens as parasites show enhanced reproduction but sabotage manipulation. This extension is because the density of the doubly infected hosts is always much smaller than the singly infected hosts, limited by sequential transmission and a small probability of co-transmission. If manipulation in a single infection is not sufficient then the transmission of the parasites depends mainly on the doubly infected hosts, which is rare. So, extinction is possible if manipulation in double infections is low.

Iritani and Sato (2018) show that manipulative parasites persist if they can alternate manipulation between boosting and suppressing predation rate. In our model, the parasite cannot switch its manipulative strategy. Sabotaging manipulation reduces the basic reproduction ratio  $R_0$  and makes the system bistable, exposing the parasite to the risk of extinction. This result contrasts with Iritani and Sato (2018) because in our model, sabotage decreases transmission rate from intermediate to definitive host, and does not benefit the parasite.

Finally, our study focuses on the ecological dynamics of the trophically transmitted parasite. However, investigating the evolution of host manipulation is a natural extension beyond the scope of a single manuscript, given the complexities that arise in the ecological dynamics itself. Studying the evolution of host manipulation, considering the free-living parasite pool, calls for thorough analyses, which could be a standalone study. For example, we would need to include differences between the traits of the multiple parasites and hence the ecological model becomes more complex than presented in this study. The combinatorics and orderings of sequential infections will then become important. In addition, the occurrence of bistability in our model suggests that the evolution of host manipulation may drive the parasite to extinction simply because of the rarity of the mutant and the Allee effect as per Adaptive dynamics approaches. The coinfecting parasites can increase manipulation and enhance reproduction freely if there exist no tradeoffs. Nevertheless, our model shows that the benefits of this strategy are context-dependent, making it suboptimal in certain cases. Evolutionary dynamics would therefore depend on the tradeoff between host manipulation and other traits of the parasites, such as reproduction, virulence, and survivorship in the parasite pool, to list a few. This extension deserves thorough analysis, and we will treat it as a separate matter.

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## References

- Alizon, S., 2012. Parasite co-transmission and the evolutionary epidemiology of virulence. *Evolution* 67:921–933. URL <https://doi.org/10.1111/j.1558-5646.2012.01827.x>.
- Alizon, S. and M. van Baalen, 2008. Multiple infections, immune dynamics, and the evolution of virulence. *The American Naturalist* 172:E150–E168. URL <https://doi.org/10.1086/590958>.
- Alizon, S., J. C. de Roode, and Y. Michalakis, 2013. Multiple infections and the evolution of virulence. *Ecology Letters* 16:556–567. URL <https://doi.org/10.1111/ele.12076>.
- Allen, L. J. S., V. A. Bokil, N. J. Cuniffe, F. M. Hamelin, F. M. Hilker, and M. J. Jeger, 2019. Modelling Vector Transmission and Epidemiology of Co-Infecting Plant Viruses. *Viruses* 11:1153. URL <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6950130/>.
- van Baalen, M. and M. W. Sabelis, 1995. The dynamics of multiple infection and the evolution of virulence. *The American Naturalist* 146:881–910. URL <https://doi.org/10.1086/285830>.
- Benesh, D. P., 2016. Autonomy and integration in complex parasite life cycles. *Parasitology* 143:1824 – 1846.
- Choisy, M. and J. C. de Roode, 2010. Mixed infections and the evolution of virulence: Effects of resource competition, parasite plasticity, and impaired host immunity. *The American Naturalist* 175:E105–E118. URL <https://doi.org/10.1086/651587>.
- Diekmann, O., J. Heesterbeek, and J. Metz, 1990. On the definition and the computation of the basic reproduction ratio  $r_0$  in models for infectious diseases in heterogeneous populations. *Journal of Mathematical Biology* 28. URL <https://doi.org/10.1007/bf00178324>.
- Diekmann, O., J. A. P. Heesterbeek, and M. G. Roberts, 2009. The construction of next-generation matrices for compartmental epidemic models. *Journal of The Royal Society Interface* 7:873–885. URL <https://doi.org/10.1098/rsif.2009.0386>.
- Fenton, A. and S. A. Rands, 2006. The impact of parasite manipulation and predator foraging behavior on predator - prey communities. *Ecology* 87:2832–2841. URL [https://doi.org/10.1890/0012-9658\(2006\)87\[2832:tiopma\]2.0.co;2](https://doi.org/10.1890/0012-9658(2006)87[2832:tiopma]2.0.co;2).
- Gandon, S., 2018. Evolution and manipulation of vector host choice. *The American Naturalist* 192:23–34. URL <https://doi.org/10.1086/697575>.
- Gao, D., T. C. Porco, and S. Ruan, 2016. Coinfection dynamics of two diseases in a single host population. *Journal of Mathematical Analysis and Applications* 442:171–188. URL <https://www.sciencedirect.com/science/article/pii/S0022247X16300841>.



396 Haderler, K. P. and H. I. Freedman, 1989. Predator-prey populations with parasitic infec-  
 397 tion. *Journal of Mathematical Biology* 27:609–631. URL [https://doi.org/10.1007/](https://doi.org/10.1007/bf00276947)  
 398 [bf00276947](https://doi.org/10.1007/bf00276947).

399 Hafer, N. and M. Milinski, 2015. When parasites disagree: evidence for parasite-induced  
 400 sabotage of host manipulation. *Evolution* 69:611 – 620.

401 Hosack, G. R., P. A. Rossignol, and P. van den Driessche, 2008. The control of vector-borne  
 402 disease epidemics. *Journal of Theoretical Biology* 255:16–25. URL [https://doi.org/](https://doi.org/10.1016/j.jtbi.2008.07.033)  
 403 [10.1016/j.jtbi.2008.07.033](https://doi.org/10.1016/j.jtbi.2008.07.033).

404 Hughes, D. P., J. Brodeur, and F. Thomas, 2012. *Host Manipulation by Parasites*. Oxford  
 405 University Press, London, England.

406 Hurford, A., D. Cownden, and T. Day, 2010. Next-generation tools for evolutionary invasion  
 407 analyses. *Journal of The Royal Society Interface* 7:561–571.

408 Iritani, R. and T. Sato, 2018. Host-manipulation by trophically transmitted parasites: The  
 409 switcher-paradigm. *Trends in Parasitology* 34:934–944. URL [https://doi.org/10.](https://doi.org/10.1016/j.pt.2018.08.005)  
 410 [1016/j.pt.2018.08.005](https://doi.org/10.1016/j.pt.2018.08.005).

411 Kalbe, M., K. M. Wegner, and T. B. H. Reusch, 2002. Dispersion patterns of parasites in  
 412 0+ year three-spined sticklebacks: a cross population comparison. *Journal of Fish Biology*  
 413 60:1529–1542.

414 Lotka, A. J., 1920. Analytical note on certain rhythmic relations in organic systems. *Pro-*  
 415 *ceedings of the National Academy of Sciences* 6:410–415. URL [https://doi.org/10.](https://doi.org/10.1073/pnas.6.7.410)  
 416 [1073/pnas.6.7.410](https://doi.org/10.1073/pnas.6.7.410).

417 Molyneux, D. H. and D. Jefferies, 1986. Feeding behaviour of pathogen-infected vectors.  
 418 *Parasitology* 92:721–736.

419 Parker, G. A., J. C. Chubb, G. N. Roberts, M. Michaud, and M. Milinski, 2003. Optimal  
 420 growth strategies of larval helminths in their intermediate hosts. *Journal of Evolutionary*  
 421 *Biology* 16:47–54. URL <https://doi.org/10.1046/j.1420-9101.2003.00504.x>.

422 Ripa, J. and U. Dieckmann, 2013. Mutant invasions and adaptive dynamics in variable  
 423 environments. *Evolution* 67:1279–1290. URL [https://onlinelibrary.wiley.com/](https://onlinelibrary.wiley.com/doi/abs/10.1111/evo.12046)  
 424 [doi/abs/10.1111/evo.12046](https://onlinelibrary.wiley.com/doi/abs/10.1111/evo.12046).

425 Rogawa, A., S. Ogata, and A. Mougi, 2018. Parasite transmission between trophic levels  
 426 stabilizes predator–prey interaction. *Scientific Reports* 8. URL [https://doi.org/10.](https://doi.org/10.1038/s41598-018-30818-7)  
 427 [1038/s41598-018-30818-7](https://doi.org/10.1038/s41598-018-30818-7).

428 Rogers, M. E. and P. A. Bates, 2007. *Leishmania* manipulation of sand fly feeding behavior  
 429 results in enhanced transmission. *PLoS Pathogens* 3:e91. URL [https://doi.org/10.](https://doi.org/10.1371/journal.ppat.0030091)  
 430 [1371/journal.ppat.0030091](https://doi.org/10.1371/journal.ppat.0030091).

- 431 Roosien, B. K., R. Gomulkiewicz, L. L. Ingwell, N. A. Bosque-Pérez, D. Rajabaskar, and  
432 S. D. Eigenbrode, 2013. Conditional vector preference aids the spread of plant pathogens:  
433 Results from a model. *Environmental Entomology* 42:1299–1308. URL [https://doi.](https://doi.org/10.1603/en13062)  
434 [org/10.1603/en13062](https://doi.org/10.1603/en13062).
- 435 Seppälä, O. and J. Jokela, 2008. Host manipulation as a parasite transmission strategy  
436 when manipulation is exploited by non-host predators. *Biology Letters* 4:663–666. URL  
437 <https://doi.org/10.1098/rsbl.2008.0335>.
- 438 Vickery, W. L. and R. Poulin, 2009. The evolution of host manipulation by parasites: a  
439 game theory analysis. *Evolutionary Ecology* 24:773–788. URL [https://doi.org/10.](https://doi.org/10.1007/s10682-009-9334-0)  
440 [1007/s10682-009-9334-0](https://doi.org/10.1007/s10682-009-9334-0).
- 441 Wedekind, C. and M. Milinski, 1996. Do three-spined sticklebacks avoid consuming cope-  
442 pods, the first intermediate host of *Schistocephalus solidus*? - an experimental analysis  
443 of behavioural resistance. *Parasitology* 112:371–383. URL [https://doi.org/10.1017/](https://doi.org/10.1017/s0031182000066609)  
444 [s0031182000066609](https://doi.org/10.1017/s0031182000066609).
- 445 Zimmer, C., 2001. *Parasite Rex: Inside the Bizarre World of Nature's Most Dangerous*  
446 *Creatures*. Atria Books.