

# **On multiple infections by parasites with complex life cycles**

Phuong L. Nguyen <sup>†</sup> and Chaitanya S. Gokhale <sup>‡,\*</sup>

<sup>†</sup> Department of Biology, University of Fribourg,

Chemin du musée 15, Switzerland

<sup>‡</sup>Max Planck Institute for Evolutionary Biology, Department of Theoretical Biology

August-Thienemann-Straße 2, 24306 Plön, Germany

\*Center for Computational and Theoretical Biology, University of Würzburg,

Klara-Oppenheimer Weg, 32, 97074, Würzburg, Germany

<sup>†</sup>linh.phuong.nguyen@evobio.eu

<sup>‡</sup>chaitanya.gokhale@uni-wuerzburg.de

## **Statement of Authorship**

Both authors developed the theory.

P.L.N developed and implemented the computational model.

Both authors wrote the manuscript.

1           **Abstract:** Host manipulation is a common strategy of parasites with complex  
2       life cycle. It directly affects predator-prey dynamics in trophically transmitted  
3       parasites. Theoretical studies suggest that predation-enhancing manipulation of-  
4       ten decimates the prey population, making parasites prone to extinction. Host  
5       manipulation, however, can also reduce predation due to conflicting interests  
6       when multiple parasites infect a host, which is often neglected in theoretical stud-  
7       ies. Misaligned interests of coinfecting parasites can occur due to limited carrying  
8       capacity or parasitoid developmental stage. Including this realistic complexity in  
9       a mathematical model, the results depart from previous studies substantially.  
10      We show that coinfecting multi-trophic parasites can preserve the predator-prey  
11     system and themselves through manipulation and reproduction parameters. Our  
12     study highlights the necessity of, and provides the means for, incorporating the  
13     reality of multiple parasites and their multi-trophic life cycles into the theory of  
14     parasite ecology.

15 **Introduction**

16 Parasites infect life on earth ubiquitously, and many of these parasites have complex life cycles  
17 (?). While a complex life cycle can be defined as abrupt ontogenetic changes in morphology  
18 and ecology (?), it typically involves numerous host species that a parasite needs to traverse  
19 to complete its life cycle. This complex life cycle results in the evolution of various strategies  
20 that enable successful parasite transmission from one host species to another. One famous  
21 strategy that inspires many science fiction movies and novels is host manipulation, where  
22 a parasite can alter its host's morphology and/or behaviour to enhance its transmission to  
23 the next host (?). Host manipulation has been shown in many host-parasite systems, from  
24 parasites with simple life cycles to those with a complex life cycle that involves more than  
25 one host species (??). For instance, sand flies infected by *Leishmania* parasites bite more  
26 and take more time for a blood meal from mammals (the definitive host of *Leishmania*)  
27 compared to their uninfected counterparts (?). Copepods infected by cestode parasites are  
28 more active and accessible to sticklebacks (the cestodes' definitive hosts) than uninfected  
29 copepods (?).

30 Theoretical studies have long attempted to understand the ecological and evolutionary  
31 consequences of host manipulation. ? and ? showed that manipulative parasites could  
32 increase the disease prevalence in an epidemic. ? studied the evolution of the manipulative  
33 ability of infectious disease parasites, showing different evolutionary outcomes depending  
34 on whether the pathogen can control its vector or host. ?? and ? showed that host  
35 manipulation could stabilise or destabilise the predator-prey dynamics depending on how  
36 manipulation affects the predation response function and the reproduction of the infected  
37 definitive host. ? showed that host manipulation could evolve even when it increases the risk  
38 of the intermediate host being eaten by a non-host predator, given that the initial predation  
39 risk is sufficiently low.

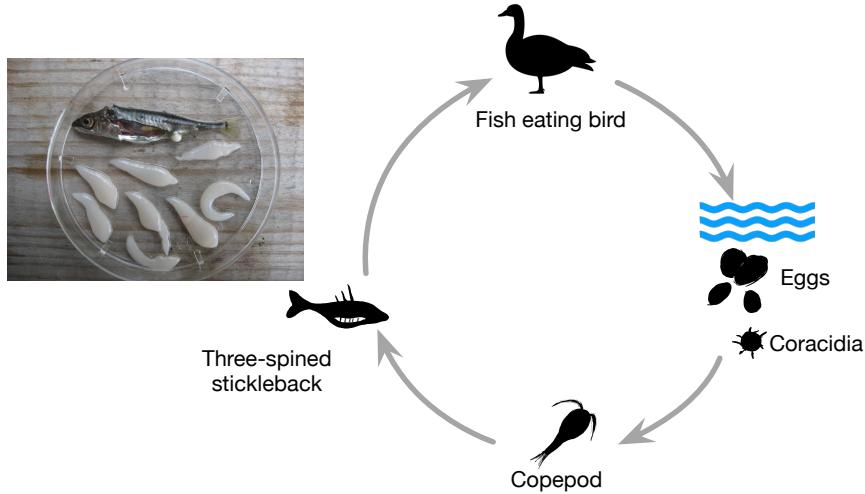
40 Most studies mentioned above have not explicitly considered a crucial aspect of parasite  
41 dynamics – multiple infections (?) i.e. the presence of multiple individual parasites within a

42 single host. Multiple infections are a norm rather than an exception in parasitism. They result  
43 in the coinfection of more than one parasite inside a host, which may alter the manipulative  
44 outcomes (figure 1). An alignment of interest between coinfecting parasites may enhance  
45 manipulation, while a conflict of interest may reduce the manipulative effect. Indeed, ?  
46 showed that copepods infected by two cestode parasites reduce the activity of copepods  
47 when both parasites are at the same noninfectious stage, i.e. both parasites are not ready  
48 to transmit. When two infectious parasites infect the copepods, the copepods' activity  
49 increases, and so does the predation risk for the copepod. However, when the copepods  
50 are infected by one infectious and one noninfectious parasite, their interests clash, and the  
51 infectious parasite wins.

52 Theoretical work that considers multiple infections often focuses on the evolution of vir-  
53 ulence (?????), while host manipulation in trophically transmitted parasites receives less  
54 attention. Even though host manipulation and virulence correlate with parasite transmis-  
55 sion, there are subtle differences, such that virulence implies an addition to the natural  
56 mortality rate of the infected host, whereas manipulation links to the immediate death of the  
57 intermediate host due to predation. Host manipulation in trophically transmitted parasites,  
58 therefore, strongly affects the entire predator-prey dynamics. Theoretical studies regarding  
59 host manipulation rarely consider multiple infections. Studies incorporating this feature ne-  
60 glect the predator-prey dynamics, which will likely have important feedback on the evolution  
61 of host manipulation (??). Moreover, these models assume that transmission from definitive  
62 hosts to intermediate hosts is due to direct contact between the two types of hosts (???).  
63 This is often not the case in nature, as parasites are released from the definitive hosts into the  
64 environment. Transmission thus happens only when intermediate hosts have contact with  
65 this free-living parasite pool. The inclusion of this free-living stage could have a profound  
66 effect on the dynamics of the whole predator-prey-parasite system.

67 Our study addresses the gap in the theoretical work on host manipulation in trophically  
68 transmitted parasites. We include multiple infections of the same parasite species and con-

Specific example of a parasite with a complex lifecycle



**Figure 1: Who is in control?** Schistocephalus eggs hatch into microscopically tiny swimming larvae. These larvae are eaten by copepods, where they develop to the second larval stage. However, the copepod is only the first intermediate host. The larvae are then eaten by sticklebacks, reaching the third larval stage and growing prominently 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 presence of multiple parasites in the same host can lead to competition and strategic decisions pertaining to investment in manipulation and growth. Indeed, a stickleback can be infected by numerous parasites, all vying for control, as shown and photographed by Martin Kalbe (?). While this is a specific example of a parasite with a complex lifecycle, our model abstracts the concept to generic multi-host lifecycles with an environmental component.

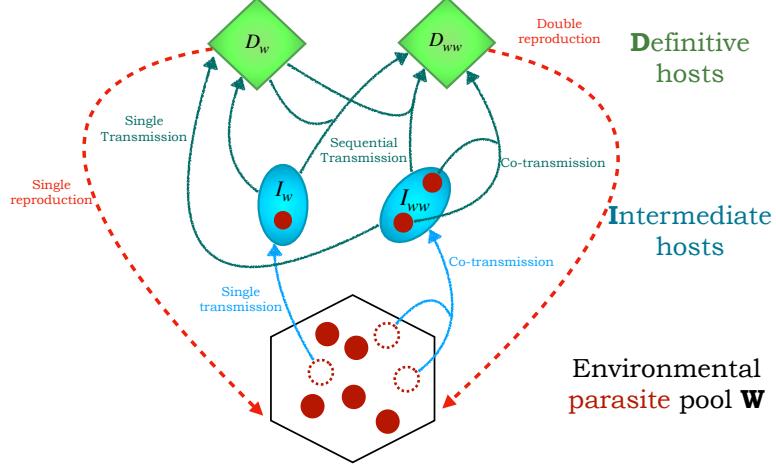
69 consider the dynamics of the free-living parasite pool. Our compartment model helps illustrate  
 70 a parasite's complex life cycle with two host species: an intermediate host preyed upon  
 71 by a definitive host. Transmission from the intermediate host to the definitive host occurs  
 72 when predation on infected intermediate hosts happens. Reproduction only happens in the  
 73 definitive hosts. New parasites then enter the environment, where the cycle continues. We  
 74 focus on the intermediate host manipulation, such that the parasite increases the uptake of  
 75 the intermediate host by the definitive host to increase its transmission rate. We then anal-  
 76 yse the effect of host manipulation on the ecological dynamics in the predator-prey-parasite  
 77 system. We found that sabotage in host manipulation almost always pushes the dynamical

78 system toward bistability, provided the reproduction in a single infection is sufficiently small.  
79 The bistable nature suggests that the predator-prey parasite system is finely balanced and  
80 susceptible to extinction via ecological disturbances. Initially surprising, we showed that co-  
81 operation in host manipulation and enhanced reproduction in co-infecting parasites is not  
82 always beneficial and might expose the parasite population to the risk of extinction.

83 **Model**

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

90 For simplicity, we assume that hosts can be infected by one (single infection) or, at  
91 most, two parasites of the same species (double infections). Thus, while  $I_s$  and  $D_s$  are the  
92 susceptible intermediate and definitive hosts, their singly and doubly infected counterparts  
93 are denoted by  $I_w$  and  $D_w$  and  $I_{ww}$  and  $D_{ww}$  respectively. Our model is, therefore, more  
94 relevant to the macroparasitic system. Figure (2) illustrates the transmission dynamics, and  
95 details of the model's variables and parameters are shown in Table 1. Note that multiple  
96 infections in nature often involve more than two parasites. Typically, the number of parasites  
97 in multiple infections follows a negative binomial distribution, i.e. most hosts are infected  
98 with a few parasites while very few hosts are infected with many parasites (?). However, since  
99 we use a compartmental model, enabling binomial distribution would mean infinitely many  
100 differential equations, making it impossible to formulate and analyze the model. Instead,  
101 we focus on another aspect of multiple infections, that is, co-transmission, which has been  
102 shown to affect the evolutionary trajectories of parasites in infectious disease (?). Given  
103 an infection, the probability that two parasites from the parasite pool co-transmit to an



**Figure 2: Schematics of the transmission routes.** Blue ovals represent the intermediate hosts, while green diamonds represent the definitive hosts. The hexagon represents the parasite pool compartment, with the red circles illustrating the free-living individual parasites. The parasites infect the intermediate hosts singly ( $I_w$ ) or doubly ( $I_{ww}$ ) upon encounter between the intermediate hosts and the parasite pool (blue arrows). These intermediate hosts are then predated upon by the definitive hosts (green arrows), thus moving the parasites to the final host (either as  $D_w$  or  $D_{ww}$ ) where they can reproduce and reenter the free-living stage in the environmental pool  $W$  (red dashed arrows).

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 intermediate hosts encounter free-living parasites and when definitive hosts consume infected intermediate hosts (Figure. 2). The dynamics of a complex life cycle parasite that requires two host species is described by the following system of equations, firstly for the intermediate host as,

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

112 where  $R(I_{total})$  represents the birth rate of the intermediate hosts, a function of both infected  
 113 and uninfected individuals  $I_{total} = I_s + I_w + I_{ww}$ . Intermediate hosts die at a natural rate  
 114  $d$ , and parasites cause additional mortality rate  $\alpha_w$  in single infection and  $\alpha_{ww}$  in double  
 115 infection.  $P_s$ ,  $P_w$ ,  $P_{ww}$  are the predation functions of definitive hosts on susceptible, singly  
 116 infected and doubly infected intermediate hosts. The predation function depends on the  
 117 density of all definitive hosts  $D_{total} = D_s + D_w + D_{ww}$  and the manipulative strategies of  
 118 parasites in the intermediate hosts. In particular, if a single parasite infects an intermediate  
 119 host, the manipulation strategy is  $\beta_w$ . However, if the intermediate host is co-infected, the  
 120 manipulation strategy is  $\beta_{ww}$ . We assume no specific relationship between  $\beta_w$  and  $\beta_{ww}$  to  
 121 explore all possible ecological outcomes of the system. The force of infection by parasites in  
 122 the environment is denoted by  $\eta = \gamma W$ , where  $\gamma$  represents the infection rate of free-living  
 123 parasites. The force of infection is a term often used in epidemiology, which represents  
 124 the rate at which a host gets infected by the parasites. Since parasites can manipulate  
 125 intermediate and definitive hosts, whenever we mention host manipulation, it specifically  
 126 refers to the manipulation in intermediate hosts, which correlates to the predation rate.

127 For the definitive hosts, we have,

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

128 where  $B(D_{total}, I_{total})$  represents the birth rate of definitive hosts. The birth rates depend  
 129 on the density of both intermediate and definitive hosts, infected or uninfected. The natural  
 130 mortality rate of definitive hosts is represented by  $\mu$ , and parasites induce additional mortality  
 131 rates  $\sigma_w$  and  $\sigma_{ww}$  in single and double infection, respectively. The force of infection that  
 132 corresponds respectively to singly infected intermediate host ( $I_w$ ) and doubly infected inter-  
 133 mediate hosts ( $I_{ww}$ ) is denoted respectively by  $\lambda_w = h(\rho + \beta_w)I_w$  and  $\lambda_{ww} = h(\rho + \beta_{ww})I_{ww}$ ,

134 where  $\rho$  is the baseline predation rate, i.e. the basic constitutive level of predation, and  $h$   
135 is the probability that the parasite successfully establishes inside the host. Without manipu-  
136 lation, that is,  $\beta_w = \beta_{ww} = 0$ , the parasite is still transmitted via the baseline predation  $\rho$ .  
137 The dynamics of the free-living parasites in the environment are then given by,

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

138 where  $f_w$  and  $f_{ww}$  are the reproduction rates of parasites in single and double infection,  
139 respectively, and parasites die naturally at a rate  $\delta$ .

140 Here, we focus on manipulation that enhances transmission from intermediate hosts to  
141 definitive hosts; we thus simplify the transmission from the parasite pool to intermediate  
142 hosts so that no sequential infection occurs. This assumption is motivated because the prey  
143 life cycle is often shorter than the predator's. A prey likely encounters the free-living parasite  
144 pool once and then dies due to predation, making sequential transmission less likely at this  
145 state. Sequential infection can happen when parasites transmit from intermediate hosts to  
146 definitive hosts. Therefore, a singly infected definitive host can be further infected by another  
147 parasite if it consumes infected intermediate hosts.

## 148 **Basic reproduction ratio $R_0$ of the parasites**

149 The basic reproduction ratio  $R_0$  (or basic reproduction number as often used in epidemiology)  
150 indicates parasite fitness. It can be understood as the expected number of offspring a parasite  
151 produces during its lifetime when introduced to a susceptible host population. We calculate

**Table 1:** Description of variables and parameters

Parameters and Variables	Description	Dimensionality
$I_i$	Density of intermediate hosts that are susceptible $i = s$ , singly infected $i = w$ , or doubly infected $i = ww$	$[I_i]t^{-1}$
$D_i$	Density of definitive hosts that are susceptible $i = s$ , singly infected $i = w$ , or doubly infected $i = ww$	$[D_i]t^{-1}$
$W$	Density of parasites released from definitive hosts into the environment	$[W]t^{-1}$
$r$	Reproduction rate of intermediate host	$t^{-1}$
$k$	Competition coefficient	$[I_i]^{-1}$
$d$	Natural death rate of intermediate hosts	$t^{-1}$
$\alpha_i$	Additional death rate of intermediate hosts due to infection by a single parasite ( $i = w$ ) or two parasites ( $i = ww$ )	$t^{-1}$
$p$	Probability that two parasites cotransmit from the environment to an intermediate host	dimensionless
$\gamma$	Transmission rate of parasites in the environment to intermediate hosts	$[W]^{-1}t^{-1}$
$\rho$	Baseline capture rate	$[I_i]^{-1}t^{-1}$
$c$	Coefficient of energy conversion into new definitive host	dimensionless
$\mu$	Natural death rate of definitive hosts	$t^{-1}$
$\sigma_i$	Additional death rate of definitive hosts due to infection by a single parasite ( $i = w$ ) or two parasites ( $i = ww$ )	$t^{-1}$
$\sigma_i$	Additional death rate of the hosts due to being infected by a singly parasite ( $i = w$ ) or two parasites ( $i = ww$ )	$t^{-1}$
$q$	Probability that two parasites cotransmit from intermediate hosts to definitive hosts	dimensionless
$\beta_i$	Transmission rate of parasites from intermediate hosts to definitive hosts	$[I_i]^{-1}t^{-1}$
$f_i$	Reproduction rate of parasites in singly infected definitive hosts ( $i = w$ ) or doubly infected hosts ( $i = ww$ )	$t^{-1}$
$\delta$	Natural death rate of parasites in the environment	$t^{-1}$
$h$	Probability that the parasites successfully established inside the definitive host	dimensionless

\*  $[I_i]$ ,  $[D_i]$ , and  $[W]$  have the same unit (*individual area<sup>-1</sup>*)

152 the basic reproduction ratio  $R_0$  using the next-generation method (???) (See SI1 for details).

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

153 where  $I_s^*$  and  $D_s^*$  are the densities of susceptible intermediate and definitive hosts at the  
 154 disease-free equilibrium. Here, the expression of  $R_0$  contains the possible reproduction routes  
 155 of a parasite, which can be via double or single infections. The first component corresponds  
 156 to the double infections route, in which the focal parasite co-transmits with another parasite  
 157 into a susceptible intermediate host, then co-transmits into a susceptible definitive host and  
 158 reproduces. Here, parasites are so rare that only co-transmission matters and the compart-  
 159 ments with sequential infections are neglected. The second component corresponds to the  
 160 single infection route, wherein the focal parasite infects a susceptible intermediate host via  
 161 single or double infections. The parasite then transmits alone into the susceptible definitive  
 162 host and eventually reproduces.

163 If  $R_0 > 1$ , a parasite spreads when introduced into the disease-free equilibrium of prey and  
 164 predator. Intuitively, the higher the density of susceptible intermediate and definitive hosts,  
 165 the larger the value of  $R_0$  as the infection reservoir is more extensive. In contrast, regardless  
 166 of the explicit form of the predation function, the higher the predation rate  $P_w$  and  $P_{ww}$ , the  
 167 lower the value of  $R_0$  given the smaller reservoir of intermediate hosts. The effect of host  
 168 manipulation on the value of  $R_0$  is more complex; as host manipulation becomes efficient,  
 169 the transmission rate from the intermediate host to the definitive host increases, but so does  
 170 the predation rate. A higher predation rate results in a smaller intermediate host reservoir  
 171 for the parasites to infect. To understand the effect of manipulation on parasites' fitness  
 172 and the system's ecological dynamics, we next specify the predation functions. We consider  
 173 linear functions for predation to begin with,

$$P_s(D_{total}) = \rho D_{total}$$

$$P_w(D_{total}, \beta_w) = (\rho + \beta_w)D_{total}$$

$$P_{ww}(D_{total}, \beta_{ww}) = (\rho + \beta_{ww})D_{total}$$

174 where  $\rho$  is the baseline capture rate of the predator on the prey. If an intermediate host is

175 infected, it is captured by the definitive hosts with rate  $\rho + \beta_w$  if it is singly infected and with  
176 rate  $\rho + \beta_{ww}$  if it is doubly infected. Zero values for  $\beta_w$  and  $\beta_{ww}$  suggest no manipulation,  
177 and predation is at the baseline value  $\rho$ .

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

$$B(D_{total}, I_{total}) = \rho c D_{total} I_{total}$$

179 where  $c$  is the efficiency of converting prey into predator's offspring. It is important to note  
180 that host manipulation affects population dynamics via its influence on the predation rate,  
181 not the physiological aspect of the definitive host, i.e., the predator. The birth rate of the  
182 predators thus depends on the capture rate, but it is not affected by host manipulation; to  
183 our best knowledge, there is no supporting evidence to consider otherwise.

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

## 190 Birth function of intermediate hosts

191 The simplest form of the prey's birth rate is a linear function, in which case the disease-free  
192 equilibrium is always in a cyclic regime (see SI 2). This follows from the Lotka-Volterra system  
193 using linear functions for prey birth and predation (?). Since the disease-free dynamics is  
194 cyclic, it is difficult to analyse the spread of a parasite using the basic reproduction ratio,  
195 which is evaluated when the disease-free state is stable. Here,  $R_0 > 1$  happens when  $\gamma$ , the  
196 transmission rate from the environment to intermediate hosts, and the reproduction rates  
197  $f_w, f_{ww}$  are quite large (as compared to the theoretical threshold shown by the mathematical

198 conditions in SI 3). However, even when this condition is satisfied, the parasite may not be  
199 able to spread and persist in cyclic susceptible host dynamics (Figure SI.1). This result agrees  
200 with the conclusion in (?), which suggests that it is difficult for a mutant to invade a cyclic  
201 resident population. In our case, it is not the invasion of a mutant in a resident population  
202 but the invasion of a parasite in a cyclic disease-free host population; the argument, however,  
203 remains valid in both cases. This issue deserves a more thorough investigation, which is out of  
204 the scope of this article. Therefore, we choose a non-linear birth function of the intermediate  
205 hosts to obtain a stable disease-free state and focus on the effect of host manipulation on  
206 the ecological dynamics (Figure 3).

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

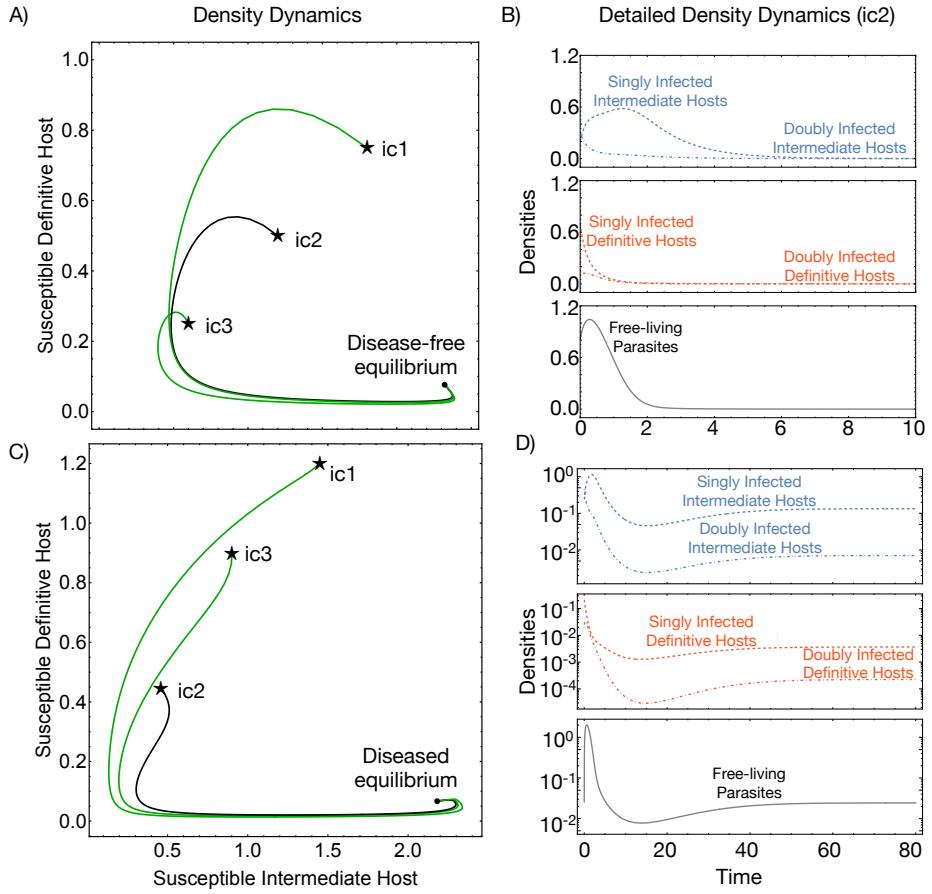
$$R(I_{total}) = rI_{total}(1 - kI_{total})$$

208 where  $r$  is the intrinsic growth rate of the intermediate hosts, and  $k$  is the intraspecific  
209 competition coefficient. The disease-free equilibrium is as follows,

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

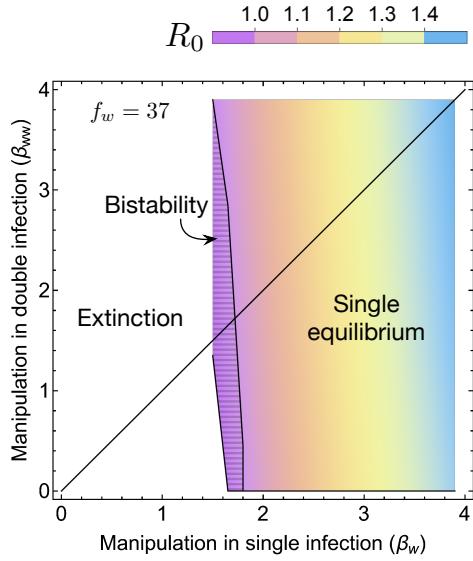
210 This equilibrium is positive and stable if components of the parasite, such as reproduction  
211 and transmission, are sufficiently small; details of the condition can be found in section SI  
212 4. Here, as reproduction and transmission value of the parasite are not sufficient, it goes  
213 extinct (Figure 3A, B), leaving the predator-prey dynamics attaining equilibrium (Figure 3C,  
214 D)

215 When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio  
216  $R_0 > 1$  (Figure 4). Since the expression is complicated, we could only obtain analytical  
217 solutions for this inequality with assumptions. We assume the same parasite virulence,  
218  $\alpha_w = \alpha_{ww}$ ,  $\sigma_w = \sigma_{ww}$ , and reproduction in double infection as a linear function concerning  
219 reproduction in single infections,  $f_{ww} = \epsilon f_w$ . When  $\epsilon > 1$ , reproduction in double infections



**Figure 3: Ecological dynamics of the predator-prey-parasite system.** On the left, we show the density dynamics of the susceptible intermediate and definitive hosts at different initial conditions (ic1, ic2, and ic3). The detailed dynamics of infected compartments are further shown for specific initial conditions (ic2), including the free-living parasite dynamics. A-B) A case of a disease-free equilibrium being reached from different initial conditions (ic). C-D) A case where the parasite survives. Parameters for disease free equilibrium  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = \alpha_{ww} = 0$ ,  $\beta_w = \beta_{ww} = 1.5$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = \sigma_{ww} = 0$ ,  $q = 0.05$ ,  $f_w = f_{ww} = 7.5$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $h = 0.6$ . Disease stable equilibrium has the same parameter values except for higher host manipulation  $\beta_w = \beta_{ww} = 4.5$  and parasite reproduction  $f_w = f_{ww} = 45$

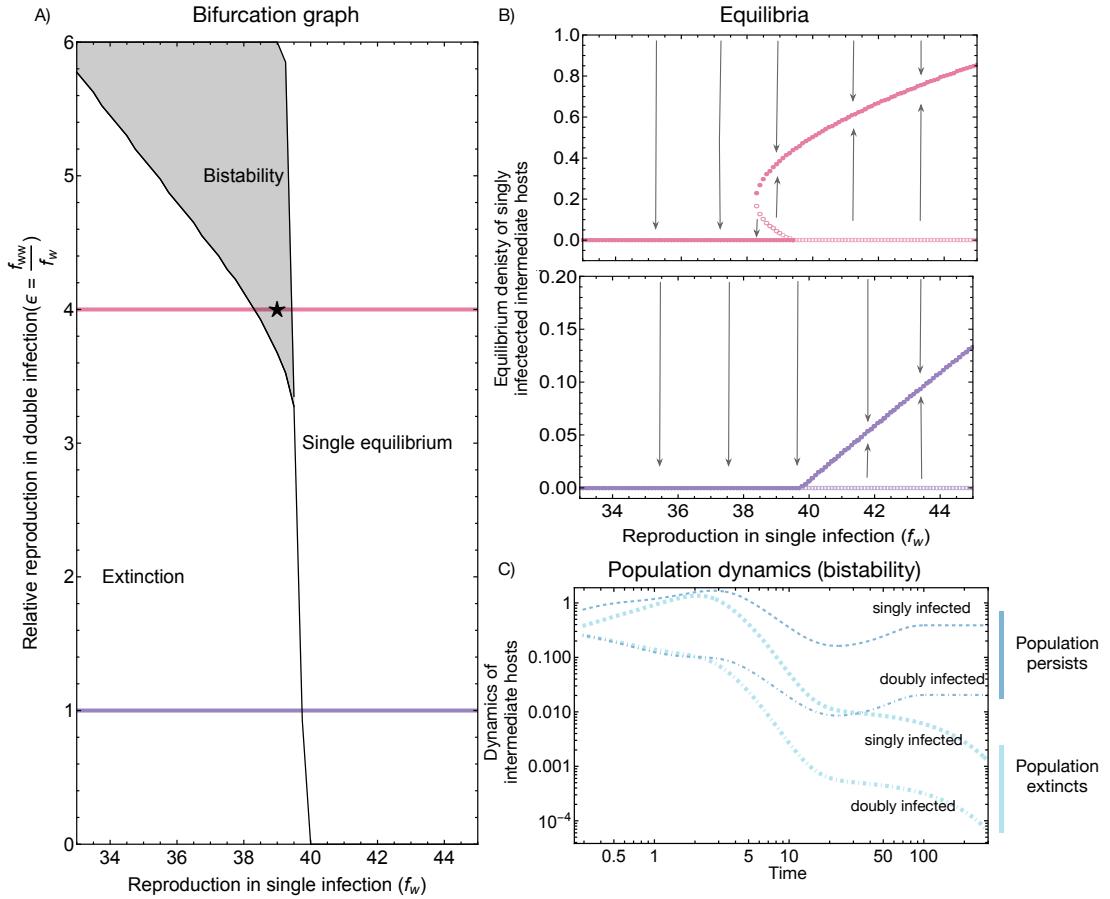
is enhanced compared to in single infections, whereas for  $\epsilon \leq 1$ , it is suppressed or equal to reproduction in single infections. We found that the parasite can establish if its reproduction value in a single infection  $f_w$  is more significant than a threshold (Figure 5, see section SI 5 and Eq. (SI.19)).



**Figure 4: Effect of manipulation in single and double infections on the reproduction ratio  $R_0$ .**  $R_0$  values increase with more efficient manipulation in single and double infection. The hatched area indicates the bistable region. As manipulation in single infection increases, the system only has one stable equilibrium. On the black line, the manipulation level is equal between single and double infection ( $\beta_w = \beta_{ww}$ ). In the upper triangular area, parasites cooperate, and in the lower triangular area, parasites sabotage. Common parameter:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $f_w = 37$ ,  $\epsilon = 4.5$ ,  $h = 0.6$ .

Our numerical results show that the parasite reproduction is substantial compared to other parameters (Figure 5A). For instance, in the parameter set used to generate Figure 5, to spread in the predator-prey system, the value of parasite reproduction ( $f_w$ ) has to be at least 20 times the value of intermediate host reproduction  $r = 2.5$ , given that both these parameters represent the *per capita* growth rate of the parasite and the intermediate host population. This observation suggests that trophically transmitted parasites should release many offspring into the environment to persist. Interestingly, bistability occurs if the reproduction rate of the parasite in double infections is enhanced. Bistability suggests that the parasite population is vulnerable to extinction. Specifically, if sufficient parasites are introduced into the disease-free predator-prey populations, the parasite population persists and reaches a stable equilibrium. In contrast, if only a few parasites are introduced into the

235 disease-free populations, or if sufficient disturbance occurs when the parasite population is  
 236 already established, the parasite population could go extinct (Figure 5C).



**Figure 5: Effect of parasite reproduction on the ecological dynamics.** A) A bifurcation graph for different reproduction values in single and double infections. B) Equilibrium density of intermediate host when  $\epsilon = 4$  when bistability occurs at high values of  $f_w$  (in pink), and  $\epsilon = 4$  when only one stable equilibrium exists at high values of  $f_w$  (in purple). C) Details of the parasite population dynamics in the case of bistability shown through the infected intermediate hosts. When the parasites start at high density, the parasite population persists, whereas when they start at lower density, they perish. Filled circles indicate stable equilibrium, and open circles indicate unstable equilibrium. Parameter  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $\beta_w = 1.5$ ,  $\beta_{ww} = 1.5$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $h = 0.6$ .

237 **The effect of host manipulation on ecological dynamics**

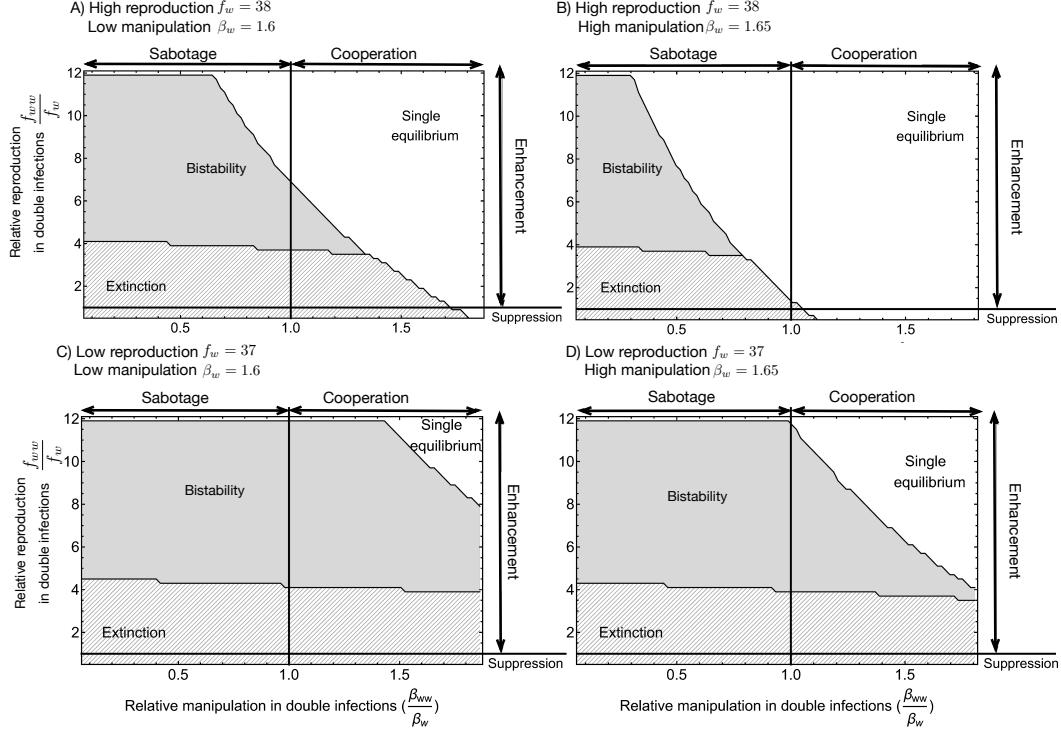
238 Host manipulation can be cooperative; two parasites increase the predation rate on interme-  
239 diate hosts, or  $\beta_{ww} > \beta_w$  (?). However, it can also be uncooperative; the predation rate on  
240 doubly-infected intermediate hosts is lower than that on singly-infected ones or  $\beta_{ww} < \beta_w$   
241 (?). Cooperation in parasite manipulation increases the parasite's basic reproduction ratio  
242  $R_0$ , but the manipulation in a single infection substantially affects the value of  $R_0$  (Figure  
243 4, see section SI 6 for analytical results). Intuitively, if the manipulation in a single infection  
244 is minor, there is not enough transmission, and the parasite goes extinct. However, we could  
245 suppose that the ability to manipulate the host in a single infection is enough for the parasite  
246 population to escape extinction. In that case, the system is in a bistable state where interme-  
247 diate cooperation in host manipulation cannot guarantee a single equilibrium (Hatched area  
248 Figure 4). In the bistable region, the basic reproduction ratio can be less than one, implying  
249 that the parasite with manipulative values within this range, i.e. weak manipulation ability,  
250 cannot spread. This is due to the Allee effect (?) where the parasite spreads and persists  
251 if, initially, there are sufficient parasites in the free-living pool as well as in the intermediate  
252 and definitive hosts. On the contrary, if the initial populations of parasites are insufficient,  
253 the parasite will perish (Figure SI.2). In addition, when the system encounters bistability,  
254 the parasite population risks extinction if there is a disturbance in the community. In the  
255 following parts, we will explore scenarios where bistability may occur.

256 Besides manipulation, co-infecting parasites can influence each other in different life his-  
257 tory traits. Parasites can have an enhanced reproduction rate in coinfections, i.e.  $f_{ww} > f_w$   
258 (upper part of the horizontal line in all panels Figure 6). Likewise, they can compete for re-  
259 sources, so reproduction in double infection is suppressed compared to single infection (lower  
260 parts of the horizontal lines in all panels Figure 6). Without any assumption on the link be-  
261 tween manipulative ability and reproduction, and a linear relationship between manipulation  
262 in single and double infections, we explore all possible combinations of cooperation-sabotage  
263 range in manipulation and suppressed-enhanced range in reproduction. This results in four

264 scenarios of parameter combinations: i, parasites sabotage manipulation but have enhanced  
265 reproduction – manipulative incoordination (top left quadrants in all panels Figure 6), ii,  
266 parasites cooperate to increase manipulation and enhance reproduction – coordination (top  
267 right quadrants in all panels Figure 6), iii, parasites cooperate in manipulation but suppress  
268 reproduction – reproductive incoordination (bottom right quadrants in all panels Figure 6),  
269 and iv, parasites sabotage manipulation and suppress reproduction – discordance (bottom  
270 left quadrants in all panels Figure 6).

271 If coinfecting parasites are discordant, i.e. uncooperative in manipulations and show sup-  
272 pressed reproduction, they cannot persist (bottom left quadrants Figure 6A-D). On the other  
273 extreme, where they are highly cooperative in manipulation and show enhanced reproduction,  
274 i.e., an extreme level of coordination, there is a guaranteed single equilibrium for parasite  
275 existence (top right quadrants Figure 6A-D). Note that this happens at the combination  
276 of  $\beta_{ww}/\beta_w \rightarrow \infty$  and  $f_{ww}/f_w \rightarrow \infty$ , a scenario that is rather impossible in reality. We  
277 often expect intermediate levels of coordination where a bistable area could occur (top right  
278 quadrant in Figure 6A, C, D). However, the size of this area is sensitive to the value of  
279 reproduction and manipulation in a single infection. In particular, higher values of these  
280 two parameters reduce the bistability area so that sufficiently large reproduction in a single  
281 infection can guarantee single equilibrium when parasites coordinate (Figure 6 B, D). In  
282 contrast, slightly reducing values of either reproduction or manipulation in a single infection  
283 increases the bistability area (Figure 6A, C, D). If the parasites sabotage each other, the  
284 system is highly prone to bistability and only has a single equilibrium when reproduction is  
285 enhanced. Interestingly, reproductive incoordination, with depressed reproduction and suffi-  
286 cient manipulative cooperation, always leads to a single equilibrium of the system (bottom  
287 right quadrants Figure 6, note that if we extend the relative manipulation in Figure C and  
288 D, we also obtain single equilibrium in this area). While a single equilibrium guarantees  
289 the existence of a parasite population, bistability indicates that a disturbance of the system  
290 may likely lead to the extinction of the parasite population. This suggests that the benefits

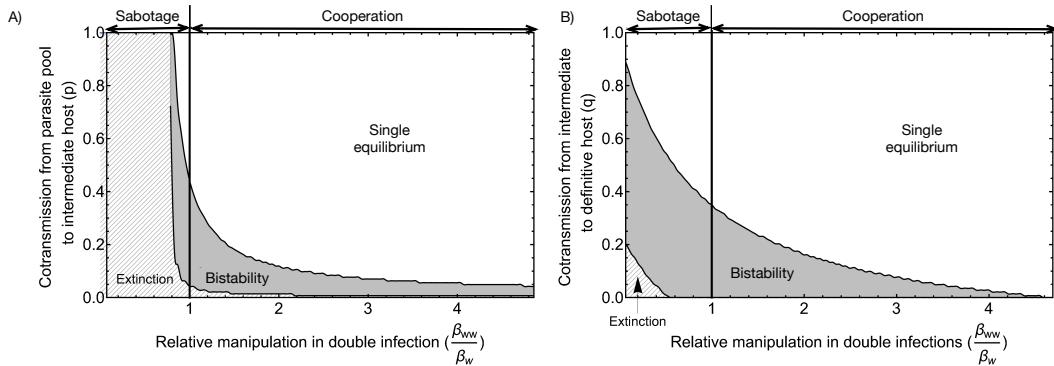
291 of coordination in reproduction and manipulation are context-dependent. Coordinating is  
 292 advantageous if no significant tradeoffs and reproduction or manipulation in single infections  
 293 are large enough.



**Figure 6: Effect of manipulation and reproduction on bistability.** The bistability area (shaded areas) reduces as the reproduction rate ( $f_w$ ) and manipulation ( $\beta_w$ ) in single infection increases. Reproduction in single infection decreases from the upper panels (A, B) to the lower panels (C, D) while manipulation in single infection increases from the left panels (A, C) to the right panels (B, D). Manipulation and reproduction levels are equal between single and double infection on the vertical and horizontal lines. On the left side of the vertical line,  $\beta_{ww} > \beta_w$ , indicating cooperation, whereas on the right side of the vertical line,  $\beta_{ww} < \beta_w$ , indicating sabotage. On the upper part of the horizontal line,  $f_{ww} > f_w$ , indicating enhanced reproduction, whereas, on the lower part of the horizontal line,  $f_{ww} < f_w$ , indicating suppressed reproduction. Common parameter:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\beta_w = 1.65$ ,  $h = 0.6$ .

294 We now explore the effect of co-transmission probability on the bistability of the system  
 295 (Figure 7). First, extinction is more likely with varying levels of co-transmission from the

296 parasite pool to the intermediate host,  $p$ , compared to varying levels of co-transmission from  
 297 the intermediate host to the definitive host,  $q$ . For exceptionally high levels of cooperation  
 298 and intermediate values of  $p$  and  $q$ , the predator-prey-parasite system will always persist with  
 299 one stable equilibrium. However, limitations and trade-offs are often unavoidable, and such  
 300 high values of cooperation may be impossible, putting the system in the parameter space  
 301 where bistability likely occurs. When the parasite sabotages manipulation, the bistable area  
 302 decreases with increasing  $p$  and  $q$ . However, this bistable area disappears with high values  
 303 of  $q$  but not with high values of  $p$ . When parasites cooperate in manipulation, reducing  
 304  $p$  almost always leads to bistability, whereas reducing  $q$  can lead to a single equilibrium if  
 305 cooperation is sufficiently large. Bistability indicates vulnerability to disturbance, so cooper-  
 306 ation in manipulation may be beneficial when  $q$ , the co-transmission from the intermediate  
 307 host to the definitive host, decreases. However, cooperation in manipulation may still harm  
 308 the population by reducing  $p$ , the co-transmission from the parasite pool to the intermediate  
 309 host.



**Figure 7:** A) Effect of cotransmission from parasite pool to intermediate host. B) Effect of co-transmission from intermediate to the definitive host. On the left side of the vertical line,  $\beta_{ww} > \beta_w$ , indicating cooperation, whereas on the right side of the vertical line,  $\beta_{ww} < \beta_w$ , indicating sabotage. Common parameters:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\epsilon = 4.5$ ,  $\beta_w = 1.45$ ,  $f_w = 38$ ,  $h = 0.6$ .

310 **Discussion & Conclusion**

311 Host manipulation is a ubiquitous phenomenon suggested to affect predator-prey dynamics in  
312 trophically transmitted parasites. In particular, manipulation of infected intermediate hosts  
313 to increase the predation rate of definitive hosts may result in a heavy burden of predators  
314 on the intermediate host population. This pressure can make parasites more vulnerable to  
315 extinction (??).

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

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

337 Another study on host manipulation, ?, showed that manipulative parasites persist if they  
338 switch from suppressing to boosting predation rate. This theoretical work modelled the ability  
339 to change the manipulative strategy of a single parasite inside a host, which can be equal to  
340 introducing the developmental state of a parasite, where a suppressed predation rate protects  
341 the parasites that are not ready to transmit. That is why decreasing manipulative ability  
342 is beneficial and prevents parasite extinction. In our model, sabotaging manipulation also  
343 reduces manipulative ability, which only reduces the basic reproduction ratio  $R_0$  and makes  
344 the system bistable, exposing the parasite to the risk of extinction. This result contrasts with  
345 ? because in our model, the parasite cannot switch its manipulative strategy, and sabotage  
346 decreases the transmission rate from intermediate to definitive host and does not benefit the  
347 parasite in any way.

348 In our study, population dynamics exhibit bistability under certain circumstances. This  
349 is very likely due to the introduction of co-transmission, which has been shown to result in  
350 bistable population dynamics in plant virus ? and infectious diseases ?. In this bistability  
351 region, if the system is disturbed (e.g. migration of the intermediate or definitive hosts or  
352 predation of intermediate hosts by other predators), then the density of the infected hosts  
353 may crash, leading to parasite extinction. The bistability region widens as parasites show  
354 enhanced reproduction but sabotage manipulation. This extension is because the density of  
355 the doubly infected hosts is always much smaller than the singly infected hosts, limited by  
356 sequential transmission and a small probability of co-transmission. If manipulation in a single  
357 infection is insufficient, then the transmission of the parasites depends mainly on the doubly  
358 infected hosts, which is rare. So, extinction is possible if manipulation in double infections  
359 is low.

360 Our study specifically focuses on the ecological dynamics of a trophically transmitted  
361 parasite between two host species. In nature, parasites with complex life cycles can have more  
362 than two hosts. However, our model of a single intermediate host species already includes  
363 enough complexity to discuss the relationship between transmission and manipulation. In

addition, we consider multiple infections of the same parasite species. Although, in nature, a host can be co-infected by multiple parasites of the same or different species, the results of our model stay valid as the key aspect in host manipulation is the alignment or conflict of interest between co-infecting parasites. Here, we introduce more realistic features compared to previous models, such as a free-living parasite pool and multiple infections, regardless of some simplifications, such as multiple infections being limited to at most two parasites. In this way, we can obtain analytical results of the reproduction ratio and mathematical expressions for the existing condition of the parasite.

Our model serves as a groundwork for future exploration into more complex and realistic systems, where numerical simulation may be the only possible approach. Given that few studies considered measuring different parameters of trophically transmitted parasites (??), our model calls for additional empirical work to measure relevant parameters, especially those from the parasite's perspective. For instance, comparing the reproduction rate of parasites in single versus multiple infections (parameters  $f_w$  and  $f_{ww}$ ) sheds light on parasite cooperation in definitive hosts. Studying the distribution of parasites in the environment (the variable  $W$ ) informs us about feeding strategies and reflects the distribution of parasites within intermediate hosts. Finally, comparing conditions of host (parameters  $\alpha_w$ ,  $\alpha_{ww}$ ,  $\sigma_w$ , and  $\sigma_{ww}$ ) between no infection, single and multiple infections illustrates the magnitude of parasite virulence. Although parasite virulence has been quantified in some studies, none have examined differences between single and multiple infections. Eventually, the results of our ecological model are a baseline for further investigation of the evolution of host manipulation, where introducing the parasite pool may create interesting eco-evolutionary feedback to the system.