

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

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

31 Theoretical studies have long attempted to understand the ecological and evolutionary  
32 consequences of host manipulation. Roosien et al. (2013) and Hosack et al. (2008) showed  
33 that manipulative parasites could increase the disease prevalence in an epidemic. Gandon  
34 (2018) studied the evolution of the manipulative ability of infectious disease parasites, show-  
35 ing different evolutionary outcomes depending on whether the pathogen can control its vector  
36 or host. Hadeler and Freedman (1989); Fenton and Rands (2006) and Rogawa et al. (2018)  
37 showed that host manipulation could stabilise or destabilise the predator-prey dynamics de-  
38 pending on how manipulation affects the predation response function and the reproduction of  
39 the infected definitive host. Seppälä and Jokela (2008) showed that host manipulation could  
40 evolve even when it increases the risk of the intermediate host being eaten by a non-host  
41 predator, given that the initial predation risk is sufficiently low.

42 Most studies mentioned above have not explicitly considered a crucial aspect of parasite  
43 dynamics – multiple infections (Kalbe et al., 2002) i.e. the presence of multiple individual  
44 parasites within a single host. Multiple infections are a norm rather than an exception in  
45 parasitism. They result in the coinfection of more than one parasite inside a host, which may  
46 alter the manipulative outcomes (figure 1). An alignment of interest between coinfecting  
47 parasites may enhance manipulation, while a conflict of interest may reduce the manipulative  
48 effect. Indeed, Hafer and Milinski (2015) showed that copepods infected by two cestode  
49 parasites reduce the activity of copepods when both parasites are at the same noninfectious  
50 stage, i.e. both parasites are not ready to transmit. ~~Thus, the reduction in mobility is~~  
51 ~~suggested to reduce the predation rate by the definitive hosts.~~ When two infectious parasites  
52 infect the copepods, the copepods' activity increases, and so does the predation risk for the  
53 copepod. However, when the copepods are infected by one infectious and one noninfectious  
54 parasite, their interests clash, and ~~one—the infectious~~ parasite wins.

55 Theoretical work that considers multiple infections often focuses on the evolution of viru-  
56 lence (van Baalen and Sabelis, 1995; Alizon et al., 2013; Alizon and van Baalen, 2008; Choisy  
57 and de Roode, 2010; Alizon, 2012), while host manipulation in trophically transmitted par-  
58 asites receives less attention. Even though host manipulation and virulence ~~both~~ correlate  
59 with parasite transmission, there are subtle differences, such that virulence implies an ad-  
60 dition to the natural mortality rate of the infected host, whereas manipulation links to the  
61 immediate death of the intermediate host due to predation. Host manipulation in trophically  
62 transmitted parasites, therefore, ~~not only affects the intermediate host population but also~~  
63 ~~the~~ strongly affects the entire predator-prey dynamics. Theoretical studies regarding host  
64 manipulation rarely consider multiple infections. Studies incorporating this feature neglect  
65 the predator-prey dynamics, which will likely have important feedback on the evolution of  
66 host manipulation (Parker et al., 2003; Vickery and Poulin, 2009). Moreover, these models  
67 assume that transmission from definitive hosts to intermediate hosts is due to direct contact  
68 between the two types of hosts (Rogawa et al., 2018; Hadeler and Freedman, 1989; Fenton



**Figure 1: Who is in control? Who is in control?** Schistocephalus eggs, which overwinter at the bottom of bodies of water, hatch into microscopically tiny 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, 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 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 decisions pertaining to investment in manipulation and growth. And indeedIndeed, a stickleback can be infected by numerous tapewormsparasites, all vying for control, as shown above and photographed by Martin Kalbe (Kalbe et al., 2002).

and Rands, 2006). This is often not the case in nature, as parasites are released from the definitive hosts into the environment. Transmission thus happens only when intermediate hosts have contact with this free-living parasite pool. The inclusion of this free-living stage could have a profound effect on the dynamics of the whole predator-prey-parasite system.

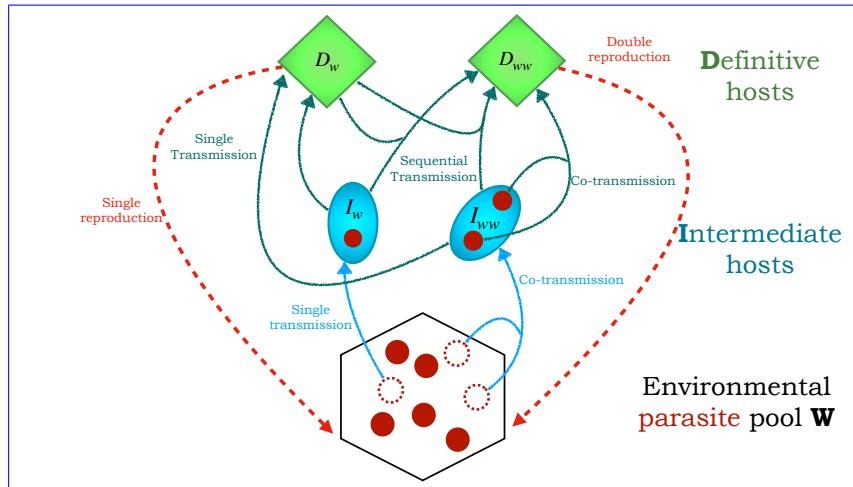
Our study addresses the gap in the theoretical work on host manipulation in trophically transmitted parasites. We include multiple infections and consider the dynamics of the free-living parasite pool. Our compartment model helps illustrate a parasite's complex life cycle with two host species: an intermediate 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

79 enter the environment, where the cycle continues. We focus on the intermediate host manip-  
80 ulation, such that the parasite increases the uptake of the intermediate host by the definitive  
81 host to increase its transmission rate. We then analyse the effect of host manipulation on  
82 the ecological dynamics in the predator-prey-parasite system. We found that sabotage in  
83 host manipulation almost always pushes the dynamical system toward bistability, provided  
84 the reproduction in a single infection is sufficiently small. The bistable nature suggests that  
85 the predator-prey parasite system is finely balanced and susceptible to extinction via ecolog-  
86 ical disturbances. Initially surprising, we showed that cooperation in host manipulation and  
87 enhanced reproduction in co-infecting parasites is not always beneficial and might expose  
88 the parasite population to the risk of extinction.

## 89 Model

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

96 For simplicity, we assume that hosts can be infected by one (single infection) or, at most,  
97 two parasites (double infections). Thus, while  $I_s$  and  $D_s$  are the susceptible intermediate  
98 and definitive hosts, their singly and doubly infected counterparts are denoted by  $I_w$  and  $D_w$   
99 and  $I_{ww}$  and  $D_{ww}$  respectively. Our model is, therefore, more relevant to the macroparasitic  
100 system. [Figure \(2\) illustrates the transmission dynamics, and details of the model's variables](#)  
101 [and parameters are shown in Table 1. Note that multiple infections in nature often involve](#)  
102 [more than two parasites. Typically, the number of parasites in multiple infections follows a](#)  
103 [negative binomial distribution, i.e. most hosts are infected with a few parasites while very](#)  
104 [few hosts are infected with many parasites \(Wilson et al., 1996\). However, since we use a](#)



**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).

105 compartmental model, enabling binomial distribution would mean infinitely many differential  
 106 equations, making it impossible to formulate and analyze the model. Instead, we focus on  
 107 another aspect of multiple infections, that is, co-transmission, which has been shown to  
 108 affect the evolutionary trajectories of parasites in infectious disease (Alizon, 2012). Given  
 109 that infection occurs, the probability that two parasites from the parasite pool co-transmit  
 110 to an intermediate host is denoted by  $p$ . Thus,  $1 - p$  is the probability that a single parasite  
 111 enters an intermediate host. When a definitive host consumes an intermediate host infected  
 112 by two parasites, there is a probability  $q$  that the parasites co-transmit to the definitive  
 113 host. With probability  $1 - q$ , only one parasite successfully transmits. This formulation  
 114 assumes that infection always happens when hosts encounter parasites—intermediate hosts  
 115 encounter free-living parasites and when definitive hosts consume infected intermediate hosts  
 116 (Figure 2). The dynamics of a complex life cycle parasite that requires two host species is

117 described by the following system of equations, firstly for the intermediate host as,

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

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

134 For the definitive hosts, we have,

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

135 where  $B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww}) - B(D_{\text{total}}, I_{\text{total}})$  represents the birth rate of definitive  
136 hosts. The birth rates depend on the density of both intermediate and definitive hosts,  
137 infected or uninfected. The natural mortality rate of definitive hosts is represented by  $\mu$ ,  
138 and parasites induce additional mortality rates  $\sigma_w$  and  $\sigma_{ww}$  in single and double infection,  
139 respectively. The force of infection that corresponds respectively to singly infected inter-  
140 mediate host ( $I_w$ ) and doubly infected intermediate hosts ( $I_{ww}$ ) is denoted respectively by  
141  $\lambda_w = h(\rho + \beta_w)I_w$  and  $\lambda_{ww} = h(\rho + \beta_{ww})I_{ww}$ , where  $\rho$  is the baseline predation rate, i.e.  
142 the basic constitutive level of predation, and  $h$  is the probability that the parasite successfully  
143 establishes inside the host. Without manipulation, that is,  $\beta_w = \beta_{ww} = 0$ , the parasite is  
144 still transmitted via the baseline predation  $\rho$ . The dynamics of the free-living parasites in  
145 the environment are then given by

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

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

148 Here, we focus on manipulation that enhances transmission from intermediate hosts to  
149 definitive hosts; we thus simplify the transmission from the parasite pool to intermediate hosts  
150 so that no sequential infection occurs. This assumption is motivated given that because the  
151 prey life cycle is often shorter than the predator's. A prey likely encounters the free-living  
152 parasite pool once and then dies due to predation, making sequential transmission less likely

153 at this state. Sequential infection can happen when parasites transmit from intermediate  
 154 hosts to definitive hosts. Therefore, a singly infected definitive host can be further infected by  
 155 another parasite if it consumes infected intermediate hosts. ~~Figure (2) illustrates the system's~~  
 156 ~~dynamics, and Table (1) contains the different parameters and variables used.~~ ~~Schematic of~~  
 157 ~~the model.~~ Blue ovals represent the intermediate hosts, while the green diamonds represent  
 158 the definitive hosts. The hexagon represents the parasite pool compartment, with the red  
 159 circles illustrating the free-living individual parasites. The parasites infect the intermediate  
 160 hosts singly ( $I_w$ , top) or doubly ( $I_{ww}$ , bottom). These intermediate hosts are then predated  
 161 upon by the definitive hosts, thus moving the parasites to the final host (either as  $D_w$  or  
 162  $D_{ww}$ ) where they can reproduce and reenter the free-living stage in the environmental pool  
 163 **W.**

## 164 **Results****Basic reproduction ratio $R_0$ of the parasites**

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

166 The basic reproduction ratio  $R_0$  (or basic reproduction number as often used in epidemiology)  
 167 indicates parasite fitness. It can be understood as the expected number of offspring a parasite  
 168 produces during its lifetime when introduced to a susceptible host population. We calculate  
 169 the basic reproduction ratio  $R_0$  using the next-generation method (Diekmann et al., 1990,  
 170 2009; Hurford et al., 2010) (See SI1 for details).

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

171 where  $I_s^*$  and  $D_s^*$  are the densities of susceptible intermediate and definitive hosts at the  
 172 disease-free equilibrium. Here, the expression of  $R_0$  contains the possible reproduction routes

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

Parameters and Variables	Description
$I_i$	Density of intermediate hosts that are susceptible $i = s$ , singly infected $i = w$ , or doubly infected $i = ww$
$D_i$	Density of definitive hosts that are susceptible $i = s$ , singly infected $i = w$ , or doubly infected $i = ww$
$W$	Density of parasites released from definitive hosts into the environment
$d$	Natural death rate of intermediate hosts
$\alpha_i$	Additional death rate of intermediate hosts due to infection by a single parasite ( $i = w$ ) or two parasites ( $i = ww$ )
$p$	Probability that two parasites cotransmit from the environment to an intermediate host
$\gamma$	Transmission rate of parasites in the environment to intermediate hosts
$\mu$	Natural death rate of definitive hosts
$\sigma_i$	Additional death rate of definitive hosts due to infection by a single parasite ( $i = w$ ) or two parasites ( $i = ww$ )
$\sigma_i$	Additional death rate of the hosts due to being infected by a singly parasite ( $i = w$ ) or two parasites ( $i = ww$ )
$q$	Probability that two parasites cotransmit from intermediate hosts to definitive hosts
$\beta_i$	Transmission rate of parasites from intermediate hosts to definitive hosts
$f_i$	Reproduction rate of parasites in singly infected definitive hosts ( $i = w$ ) or doubly infected hosts ( $i = ww$ )
$\delta$	Natural death rate of parasites in the environment
$h$	Probability that the parasites successfully established inside the definitive host

173 of a parasite, which can be via double or single infections. The first component corresponds  
 174 to the double infections route, in which the focal parasite co-transmits with another parasite  
 175 into a susceptible intermediate host, then co-transmits into a susceptible definitive host and  
 176 reproduces. Here, parasites are so rare that only co-transmission matters and the compart-  
 177 ments with sequential infections are neglected. The second component corresponds to the  
 178 single infection route, wherein the focal parasite infects a susceptible intermediate host via  
 179 single or double infections. The parasite then transmits alone into the susceptible definitive

180 host and eventually reproduces.

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

$$P_s(D_s, D_w, D_{ww\text{total}}) = \rho D_{total}$$

$$P_w(D_{s\text{total}}, D_w, D_{ww}, \beta_w) = (\rho + \beta_w) D_{total}$$

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

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

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

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

198 where  $c$  is the efficiency of converting prey into predator's offspring, and  $I_{total} = I_s + I_w + I_{ww}$

199 ~~is the total density of the intermediate hosts.~~ It is important to note that host manipulation  
200 affects population dynamics via its influence on the predation rate, not the physiological  
201 aspect of the definitive host, i.e., the predator. The birth rate of the predators thus depends  
202 on the capture rate, but it is not affected by host manipulation; to our best knowledge, there  
203 is no supporting evidence to consider otherwise.

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

210 **Birth function of intermediate hosts**

211 **Birth function of intermediate hosts**

212 The simplest form of the prey's birth rate is a linear function, in which case the disease-  
213 free equilibrium is always ~~unstable. In particular, it has a cyclic behaviour because, at this~~  
214 ~~equilibrium, the jacobian matrix of the system (1, 2, 3) always has two pure imaginary~~  
215 ~~eigenvalues (see SI2 in a cyclic regime (see SI 2)).~~ This follows from the Lotka-Volterra  
216 system using linear functions for prey birth and predation (Lotka, 1920). Since the disease-  
217 free dynamics is cyclic, it is difficult to analyse the spread of a parasite using the basic  
218 reproduction ratio, which is evaluated when the disease-free state is stable. Here,  $R_0 > 1$   
219 happens when  $\gamma$ , the transmission rate from the environment to intermediate hosts, and the  
220 reproduction rates  $f_w, f_{ww}$  are quite large (as compared to the theoretical threshold shown  
221 by the mathematical conditions in SI3). However, even when this condition is satisfied, the  
222 parasite may not be able to spread and persist in cyclic susceptible host dynamics (Figure  
223 SI1). This result agrees with the conclusion in (Ripa and Dieckmann, 2013), which suggests

224 that it is difficult for a mutant to invade a cyclic resident population. In our case, it is not the  
225 invasion of a mutant in a resident population but the invasion of a parasite in a cyclic disease-  
226 free host population; the argument, however, remains valid in both cases. This issue deserves  
227 a more thorough investigation, which is out of the scope of this article. ~~Here~~Therefore, we  
228 choose a non-linear birth function of the intermediate hosts to obtain a stable disease-free  
229 state and focus on the effect of host manipulation on the ecological dynamics (Figure 3).

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

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

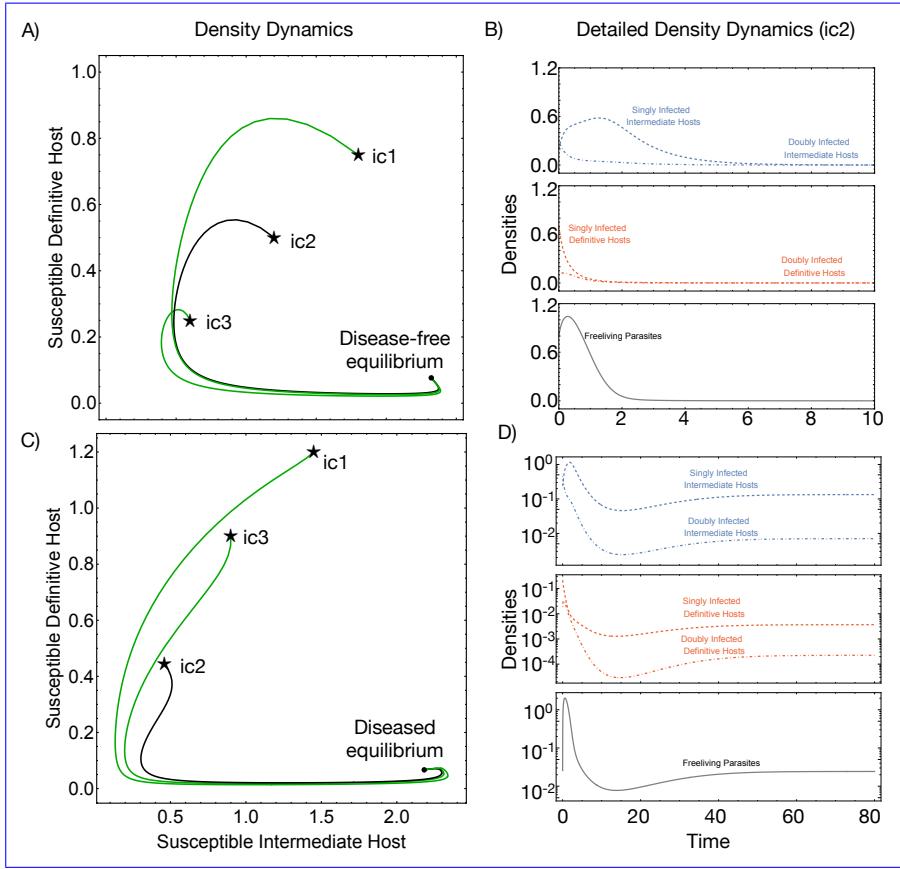
231 where  $k$  is the intraspecific competition coefficient. The disease-free equilibrium is as follows.3

232

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

233 This equilibrium is positive and stable if components of the parasite, such as reproduction  
234 and transmission, are sufficiently small; details of the condition can be found in section SI  
235 4. Here, ~~because as~~ reproduction and transmission value of the parasite are not sufficient, it  
236 goes extinct (Figure 3AB), leaving the predator-prey dynamics attaining equilibrium (Figure  
237 3BC~~D~~)

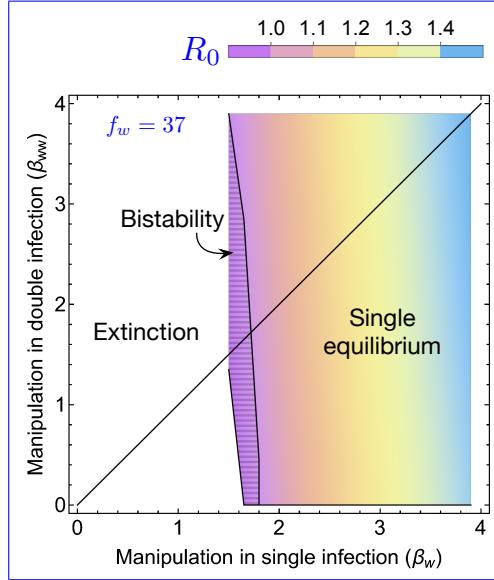
238 When a parasite appears in the disease-free equilibrium, it spreads if its reproduction  
239 ratio  $R_0 > 1$  (Figure 3C~~D~~4). Since the expression is complicated, we could only obtain  
240 analytical solutions for this inequality with assumptions. We assume the same parasite  
241 virulence,  $\alpha_w = \alpha_{ww}$ ,  $\sigma_w = \sigma_{ww}$ , and reproduction in double infection as a linear function  
242 concerning reproduction in single infections,  $f_{ww} = \epsilon f_w$ . When  $\epsilon > 1$ , reproduction in  
243 double infections is enhanced compared to in single infections, whereas  $\epsilon \leq 1$ , reproduction  
244 in double infections is suppressed or equal to reproduction in single infections. We found that  
245 the parasite can establish if its reproduction value in a single infection  $f_w$  is more significant



**Figure 3: Ecological dynamics of the predator-prey-parasite system.** **A)** Ecological trajectories of infected hosts and free-living parasite when parasites cannot persist. On the left, **B)** Phase plane we show the density dynamics of the susceptible intermediate and definitive hosts under disease free scenario. **C**) different initial conditions (ic1, ic2, and ic3). Ecological trajectories. The detailed dynamics of infected hosts and compartments are further shown for specific initial conditions (ic2), including the free-living parasite when parasites persist. **D**) **A-B)** Phase plane A case of susceptible and definitive host under disease circulating scenario 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 have the same parameter values except for higher host manipulation  $\beta_w = \beta_{ww} = 4.5$  and parasite reproduction  $f_w = f_{ww} = 45$

246 than a threshold (Figure 5, see section SI 5 and Eq. (SI.19)).

247 Our numerical results show that the parasite reproduction is substantial compared to other



**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:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $g = 0$ .

parameters (Figure 5A). For instance, in the parameter set used to generate Figure 5B, 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 ~~must release a large amount of~~ should release many offspring into the environment to persist. Interestingly, bistability occurs if the reproduction rate of the parasite in double infections is enhanced (Figure 5A). The parasite population can reach ~~a~~ 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~~ a stable equilibrium ~~in the bistable region if the initial density is large enough~~. In contrast, ~~with~~

259 sufficient disturbance if only a few parasites are introduced into the disease-free populations,  
260 or if sufficient disturbance occurs when the parasite population is already established, the  
261 parasite population could go extinct (Figure 5C).

## 262 The effect of host manipulation on ecological dynamics

263 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  
264 doubly-infected intermediate hosts is lower than that on singly-infected ones or  $\beta_{ww} < \beta_w$ .  
265 Cooperation in parasite manipulation increases the parasite's basic reproduction ratio  $R_0$ ,  
266 but the manipulation in a single infection substantially affects the value of  $R_0$  (Figure 64).  
267 Intuitively, if the manipulation in a single infection is minor, there is not enough transmission,  
268 and the parasite goes extinct. However, suppose we could suppose that the ability to manipulate  
269 the host in a single infection is merely enough for the parasite population to escape  
270 extinction. In that case, the system is in a bistable state where intermediate cooperation  
271 in host manipulation cannot guarantee a single equilibrium (Hatched area Figure 6 Left4).  
272 In the bistable region, the basic reproduction ratio can be less than one, implying that the  
273 parasite with manipulative values within this range, i.e. weak manipulation ability, cannot  
274 spread. When the system encounters bistability, the parasite population risks extinction if  
275 there is a disturbance in the community. In the following parts, we will explore scenarios  
276 where bistability may occur.

277 Besides manipulation, co-infecting parasites can influence each other in different life history traits. Parasites can have an enhanced reproduction rate in coinfections, i.e.  $f_{ww} > f_w$   
278 (upper part of the horizontal line in Figure 6 Right all panels Figure 6). Likewise, they can  
279 compete for resources, so reproduction in double infection is suppressed compared to single  
280 infection (lower part parts of the horizontal line in Figure 6 Right lines in all panels Figure  
281 6). Without any assumption on the relationship link between manipulative ability and re-  
282 production, and a linear relationship between manipulation in single and double infections,

we explore all possible combinations of cooperation-sabotage range in manipulation and suppressed-enhanced range in reproduction. This results in four scenarios of parameter combinations: i, parasites sabotage manipulation but have enhanced reproduction – manipulative incoordination (top left ~~quadrant in Figure 6 Right~~quadrants in all panels Figure 6), ii, parasites cooperate to increase manipulation and enhance reproduction – coordination (top right ~~quadrant in Figure 6 Right~~quadrants in all panels Figure 6), iii, parasites cooperate in manipulation but suppress reproduction – reproductive incoordination (bottom right ~~quadrant in Figure 6 Right~~quadrants in all panels Figure 6), and iv, parasites sabotage manipulation and suppress reproduction – discordance (bottom left ~~quadrant in Figure 6 Right~~quadrants in all panels Figure 6).

If coinfecting parasites are discordant, i.e. uncooperative in manipulations and show suppressed reproduction, they cannot persist (Figure 6A-D). On the other extreme, where they are highly cooperative in manipulation and show enhanced reproduction, i.e., an extreme level of coordination, there is a guaranteed single equilibrium for parasite existence. Note that this happens at the combination of  $\beta_{ww}/\beta_w \rightarrow \infty$  and  $f_{ww}/f_w \rightarrow \infty$ , a scenario that is rather impossible in reality. ~~Very often, we~~ We often expect intermediate levels of coordination where a bistable area could occur (top left quadrant in Figure 6 at  $f_w = (37, 37.5)$  A, C, D). 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 ~~to the point so~~ that sufficiently large reproduction in a single infection can guarantee single equilibrium when parasites coordinate (~~top left quadrant in Figure 6 Left at  $f_w = (38)$ , Figure SI.2~~ Figure 6 B). In contrast, slightly reducing values of ~~reproduction and manipulation in single infection increase either reproduction or manipulation in a single infection increases~~ the bistability area (Figure 6A, C, D). If the parasites sabotage each other, the system is highly prone to bistability and only has a single equilibrium when reproduction is ~~especially enhanced(left side of vertical line in Figure 6 Left)enhanced~~. Interestingly, ~~sufficiently high reproduction enhancement leads to bistability (i.e.  $f_{ww}$  is at least four times  $f_w$ )~~, and re-

productive incoordination, i.e. depressed reproduction and manipulative cooperation, always leads to a single equilibrium of the system (Figure 5A, and bottom right quadrant in Figure 6LeftB, D). 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 is advantageous if no significant tradeoffs and reproduction or manipulation in single infections are large enough.

We now explore the effect of co-transmission probability on the bistability of the system (Figure 7). First, extinction is more likely with varying levels of co-transmission from the parasite pool to the intermediate host,  $p$ , compared to varying levels of co-transmission from the intermediate host to the definitive host,  $q$ . For exceptionally high levellevels of cooperation and not very small values of bothintermediate values of  $p$  and  $q$ , the predator-prey-parasite system will always persist with one stable equilibrium. However, limitations and trade-offs are often unavoidable, and such high values of cooperation may be impossible, putting the system in the parameter space where bistability likely occurs. When the parasite sabotages manipulation, the bistable area decreases with increasing  $p$  and  $q$ . However, this bistable area disappearsdisappears with high values of  $q$  but not with high values of  $p$ . When parasites cooperate in manipulation, reducing  $p$  almost always lead to bistability where asleads to bistability, whereas reducing  $q$  can lead to a single equilibrium if cooperation is sufficiently large. Bistability indicates vulnerability to disturbance, andso cooperation in manipulation may be beneficial when  $q$ , the co-transmission from the intermediate host to the definitive host, decreases. However, cooperation in manipulation may still harm the population with by reducing  $p$ , the co-transmission from the parasite pool to the intermediate host.

## Discussion & Conclusion

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

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

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

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

362 Another study on host manipulation, Iritani and Sato (2018), showed that manipulative  
363 parasites persist if they switch from suppressing to boosting predation rate. This theoretical  
364 work modelled the ability to change the manipulative strategy of a single parasite inside

365 a host, which can be equal to introducing the developmental state of a parasite, where a  
366 suppressed predation rate protects the parasites that are not ready to transmit. That is  
367 why decreasing manipulative ability is beneficial and prevents parasite extinction. In our  
368 model, sabotaging manipulation also reduces manipulative ability, which only reduces the  
369 basic reproduction ratio  $R_0$  and makes the system bistable, exposing the parasite to the risk  
370 of extinction. This result contrasts with [Iritani and Sato \(2018\)](#) because in our model, the  
371 parasite cannot switch its manipulative strategy, and sabotage decreases the transmission  
372 rate from intermediate to definitive host and does not benefit the parasite in any way.

373 In our study, population dynamics exhibit bistability under certain circumstances. This  
374 is very likely due to the introduction of co-transmission, which has been shown to result in  
375 bistable population dynamics in plant virus [Allen et al. \(2019\)](#) and infectious diseases [Gao  
376 et al. \(2016\)](#). In this bistability region, if the system is disturbed (e.g. migration of the  
377 intermediate or definitive hosts or predation of intermediate hosts by other predators), then  
378 the density of the infected hosts may crash, leading to parasite extinction. The bistability  
379 region widens as parasites show enhanced reproduction but sabotage manipulation. This  
380 extension is because the density of the doubly infected hosts is always much smaller than  
381 the singly infected hosts, limited by sequential transmission and a small probability of co-  
382 transmission. If manipulation in a single infection is insufficient, then the transmission of  
383 the parasites depends mainly on the doubly infected hosts, which is rare. So, extinction is  
384 possible if manipulation in double infections is low.

385 Finally, our study focuses on the ecological dynamics of a trophically transmitted parasite  
386 between two host species. In nature, parasites with complex life cycles can have more than  
387 two hosts. However, our model of a single intermediate host species ~~can already provide~~  
388 already includes enough complexity to discuss the relationship between transmission and  
389 manipulation. Here, we introduce more realistic features compared to previous models, such  
390 as a free-living parasite pool and multiple infections, regardless of some simplifications, such  
391 as multiple infections being limited to at most two parasites. In this way, we can obtain

392 analytical results of the reproduction ratio and mathematical expressions for the existing  
393 condition of the parasite. Our model serves as a groundwork for future exploration into more  
394 complex and realistic systems, where numerical simulation may be the only possible approach.  
395 Moreover, the results of our ecological model are a baseline for further investigation of the  
396 evolution of host manipulation, where introducing the parasite pool may create interesting  
397 eco-evolutionary ~~feedbacks~~feedback to the system.

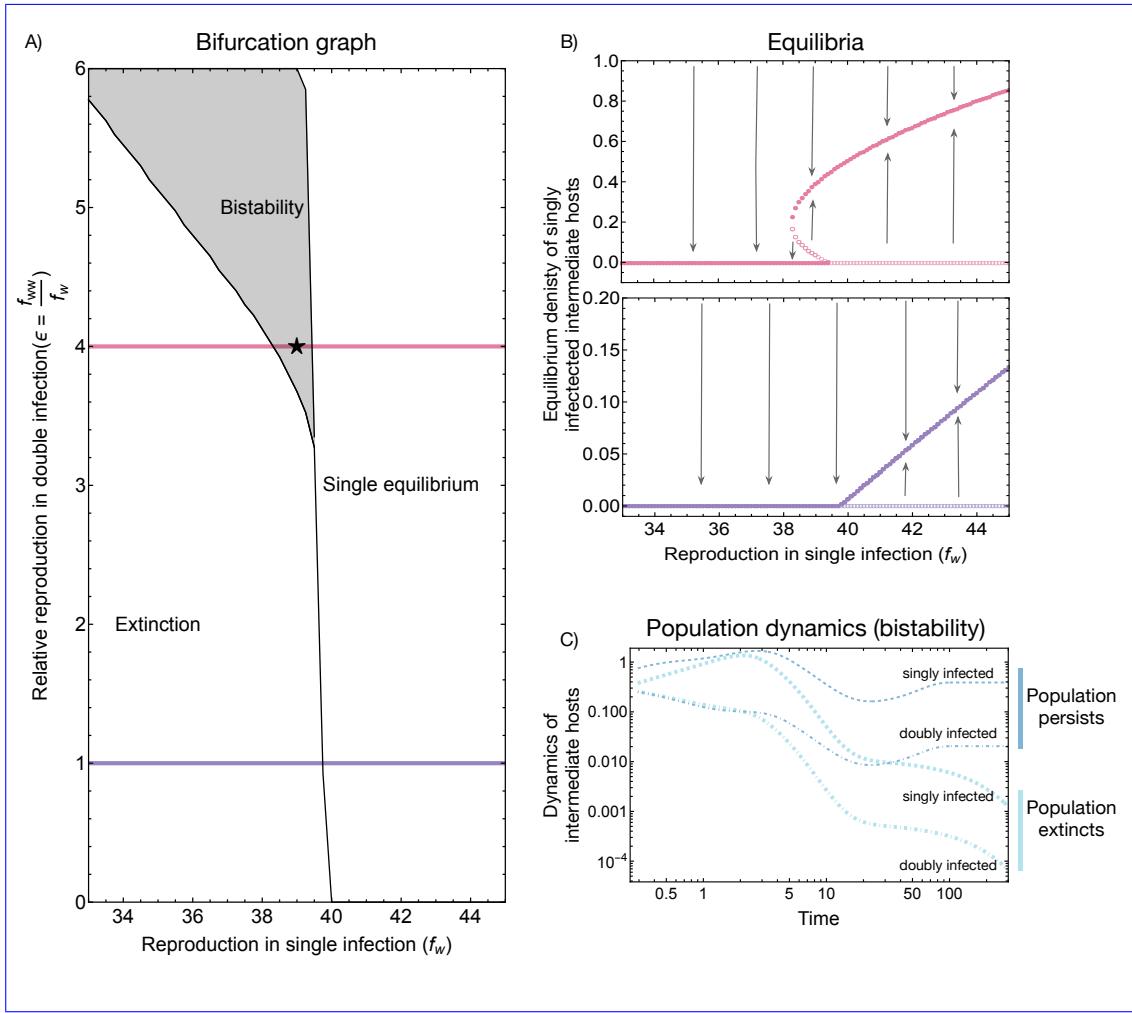
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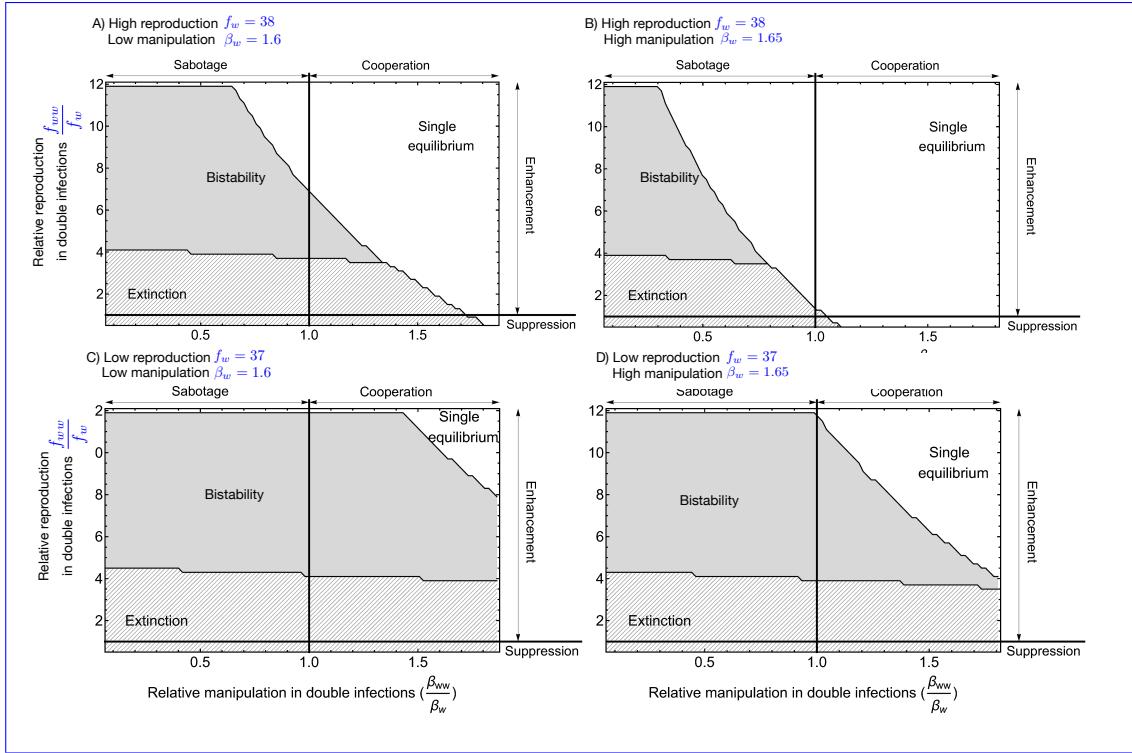
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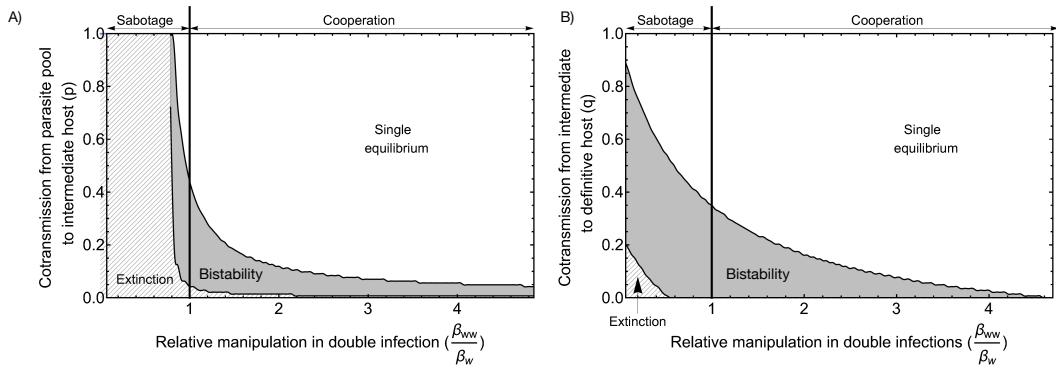
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**Figure 5: Effect of parasite reproduction on the ecological dynamics.** A) Enhanced A bifurcation graph for different values of reproduction in double and single infection leads to bistability, and double infections. B, C) Density Equilibrium values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



**Figure 6: Effect of manipulation and reproduction on bistability.** **Left:**  $R_0$  values increase with more efficient manipulation in single and double infection. The hatched bistability area indicates (shaded areas) reduces as the bistable region. As reproduction rate ( $f_w$ ) and manipulation ( $\beta_w$ ) in single infection increases, the system only has one stable equilibrium. On the black line, the manipulation level is equal between Reproduction in single and double infection ( $\beta_w = \beta_{ww}$ ). It decreases from the upper triangular area panels (A, parasites cooperate, and in B) to the lower triangular area, parasites sabotage. **Right:** Changes in the bistability area panels (shaded areas C, D) concerning different reproduction rates while manipulation in single infection increases from the left panels (different boundary styles 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:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\beta_w = 1.65$ ,  $h = 0.6$ .



**Figure 7:** Left: A) Effect of cotransmission from parasite pool to intermediate host. Right: B) Effect of eotransmission-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:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\epsilon = 4.5$ ,  $\beta_w = 1.45$ ,  $f_w = 38$ ,  $h = 0.6$ .