

# On multiple infections by parasites with complex life cycles

1 Host manipulation is a common strategy of parasites of different complexity.  
2 Host manipulation directly affects predator-prey dynamics in trophically transmitted  
3 parasites, where parasite transmission requires predation. Theoretical studies  
4 suggest that manipulation that enhances predation often results in a heavy bur-  
5 den on the prey population. Consequently, the system is often destabilised,  
6 making parasites prone to extinction. Host manipulation, however, can also sup-  
7 press predation. Such suppression is possible if multiple parasites coinfect a host  
8 with conflicting interests in manipulation. The interests could be misaligned for  
9 various reasons, such as limited carrying capacity or parasitoid developmental  
10 stage. Multiple infections are a norm in parasite ecology but are often neglected  
11 in the theoretical assessment of host-parasite dynamics. We tease apart the  
12 effect of host manipulation of coinfecting parasites and manipulation interests  
13 via a mathematical model of a trophically transmitted parasite with a complex  
14 life cycle. The life cycle comprises a free-living state, an intermediate and a  
15 definitive host. With coinfection, we show that host manipulation that enhances  
16 predation need not permanently destabilise the predator-prey system. However,  
17 cooperation between coinfecting parasites leading to increased predation and can  
18 lead to bistability such that a slight disturbance in the system drives the parasite  
19 population to extinction. On the other hand, when coinfecting parasites sabo-  
20 tage the manipulative ability of one another, the stability of the predator-prey  
21 system is always guaranteed. Our study highlights the necessity and means of  
22 incorporating the reality of multiple parasites and their multi-trophic life cycles  
23 in a single system in studying parasite ecology.

24 **Introduction**

25 Parasites infect life on earth ubiquitously, and many of these parasites have complex life  
26 cycles (Zimmer, 2001). While a complex lifecycle can be defined as abrupt ontogenetic changes  
27 in morphology and ecology (Benesh, 2016), a complex parasitic lifecycle typically involves  
28 numerous hosts that a parasite needs to traverse to complete its life cycle. This complex  
29 lifecycle results in the evolution of various strategies that enable the success of parasite  
30 transmission from one host to another. One famous strategy that inspires many science  
31 fiction movies and novels is host manipulation, where a parasite can alter the morphology  
32 and/or behaviour of its host to enhance its transmission to the next host (Hughes et al.,  
33 2012). Host manipulation has been shown in many host-parasite systems, from parasites with  
34 simple life-cycle to those with complex life-cycle that involves more than one host (Hughes  
35 et al., 2012; Molyneux and Jefferies, 1986). For instance, sand flies infected by *Leishmania*  
36 parasites bite more and take more time for a blood meal from mammals (the definitive host of  
37 *Leishmania*) compared to their uninfected counterparts (Rogers and Bates, 2007). Copepods  
38 infected by cestode parasites are more active and accessible to sticklebacks (the definitive  
39 hosts of the cestodes) compared to uninfected copepods (Wedekind and Milinski, 1996).

40 Theoretical studies have long attempted to understand the ecological and evolutionary  
41 consequences of host manipulation. Roosien et al. (2013) and Hosack et al. (2008) showed  
42 that manipulative parasites could increase the disease prevalence in an epidemic. Gandon  
43 (2018) studied the evolution of the manipulative ability of infectious disease parasites, show-  
44 ing different evolutionary outcomes depending on whether the pathogen can control its vector  
45 or host. Hadeler and Freedman (1989); Fenton and Rands (2006) and Rogawa et al. (2018)  
46 showed that host manipulation could stabilise or destabilise the predator-prey dynamics de-  
47 pending on how manipulation affects the predation response function and the assumption  
48 on the fertility of the definitive infected host. Seppälä and Jokela (2008) showed that host  
49 manipulation could evolve even when it increases the risk of the intermediate host being  
50 eaten by a non-host predator, given that the initial predation risk is sufficiently low. These  
51 models, however, lack a crucial aspect of parasite dynamics, multiple infections (Kalbe et al.,  
52 2002)

53 Typical studies do not consider multiple infections, a phenomenon that is the norm rather  
54 than an exception in parasitism. Multiple infections result in the coinfection of more than one  
55 parasite inside a host, which may alter the manipulative outcomes (figure 1). An alignment of  
56 interest between coinfecting parasites may enhance manipulation, while a conflict of interest  
57 may reduce the manipulative effect. Indeed, Hafer and Milinski (2015) showed that copepods  
58 infected by two cestode parasites reduce the activity of copepods when both parasites are

59 at the same noninfectious stage, i.e. both parasites are not ready to transmit. Thus the  
60 reduction in mobility is suggested to reduce the predation rate by the definitive hosts. When  
61 two infectious parasites infect the copepods, the copepods' activity increases, and so does the  
62 predation risk for the copepod. However, when the copepods are infected by one infectious  
63 and one noninfectious parasite, their interests clash, and one parasite wins over the other.



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

64 Theoretical work that considers multiple infections often focuses on the evolution of vir-  
65 ulence (van Baalen and Sabelis, 1995; Alizon et al., 2013; Alizon and van Baalen, 2008;  
66 Choisy and de Roode, 2010; Alizon, 2012). They show that multiple infections can increase  
67 virulence (van Baalen and Sabelis, 1995; Choisy and de Roode, 2010). Evolutionary branch-  
68 ing of a less virulent and a hypervirulent parasite can occur when within-host dynamics are

69 considered (Alizon and van Baalen, 2008), and a reduction in virulence is possible if parasites  
70 are co-transmitted (Alizon, 2012). In epidemiological models, higher virulence often assumes  
71 a link with a higher transmission rate; virulence is therefore associated with host manipula-  
72 tion in such cases. Host manipulation influences the predation rate in trophically transmitted  
73 parasites, predominantly affecting predator-prey dynamics. Theoretical studies on host ma-  
74 nipulation in trophically transmitted parasites with multiple infections are rare (Parker et al.,  
75 2003; Vickery and Poulin, 2009). Moreover, they do not consider the prey-predator dynam-  
76 ics, which will likely have important feedback on the evolution of host manipulation. A few  
77 studies considering the prey-predator dynamics do not incorporate multiple infections (Ro-  
78 gawa et al., 2018; Iritani and Sato, 2018; Hadeler and Freedman, 1989; Fenton and Rands,  
79 2006). More importantly, they assume that transmission from definitive hosts to interme-  
80 diate hosts is due to direct contact between the two types of hosts. This is often not the  
81 case, as parasites are released from the definitive hosts into the environment. Transmission  
82 happens only when intermediate hosts have contact with this free-living parasite pool.

83 Our study addresses the gap in the theoretical work on host manipulation in trophically  
84 transmitted parasites. We include multiple infections and consider the dynamics of the free-  
85 living parasite pool. Our compartment model helps illustrate a parasite's complex lifecycle  
86 with two hosts: an intermediate host preyed upon by a definitive host. Transmission from the  
87 intermediate host to the definitive host occurs when predation on infected intermediate hosts  
88 happens. Reproduction only happens in the definitive hosts. New parasites are then released  
89 into the environment, where they again have contact with the intermediate hosts to complete  
90 their lifecycle. We focus on the manipulation of the intermediate hosts, such that the parasite  
91 increases the predation rate on the intermediate host by the definitive host to increase  
92 its transmission rate. We then analyse the effect of host manipulation on the ecological  
93 dynamics in the prey-predator-parasite system. In contrast to the examples mentioned above  
94 our model consists of a single intermediate host as it already provides enough complexity  
95 to discuss between transmission and manipulation. We found that cooperation in host  
96 manipulation leads to bistability in the predator-prey system, given that reproduction from  
97 multiple infections is sufficiently high. This finding suggests that the predator-prey parasite  
98 system is finely balanced and susceptible to extinction via ecological disturbances. Initially  
99 surprising, we show how sabotage in host manipulation guarantees a unique stable equilibrium  
100 in the system.

101 **Model and Results**

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

108 For simplicity, we assume that hosts can be infected by one (single infection) or, at most,  
109 two parasites (double infections). Our model is, therefore, more relevant to the macropara-  
110 sitic system. Given that infection occurs, the probability that two parasites from the parasite  
111 pool co-transmit to an intermediate host is denoted by  $p$ . Thus  $1 - p$  is the probability that a  
112 single parasite enters an intermediate host. When a definitive host consumes an intermediate  
113 host infected by two parasites, there is a probability  $q$  that the parasites co-transmit to the  
114 definitive host. With probability  $1 - q$ , only one parasite successfully transmits. This formu-  
115 lation assumes that infection always happens when hosts encounter parasites. The dynamics  
116 of a complex lifecycle parasite that requires two hosts is described by the following system  
117 of equations, firstly for the intermediate host as,

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

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

130 For the definitive hosts we have,

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

131 where  $B(D_s, D_w, D_{ww}, I_s, I_w, I_{ww})$  represents the birth rate of definitive hosts. The birth  
132 rates depend on the density of both intermediate and definitive hosts, infected or uninfected.  
133 The force of infection that corresponds respectively to singly infected intermediate host ( $I_w$ )  
134 and doubly infected intermediate hosts ( $I_{ww}$ ) is denoted respectively by  $\lambda_w = h_1(\rho + \beta_w)I_w$   
135 and  $\lambda_{ww} = h_2(\rho + \beta_{ww})I_{ww}$ , where  $\rho$  is the based line predation rate and  $h_1$  and  $h_2$   
136 are the probability that the parasite successfully established inside the host. If there is no  
137 manipulation, that is,  $\beta_w = \beta_{ww} = 0$ , the parasite is still transmitted via the based line  
138 predation. The dynamics of the free-living parasites in the environment are then given by,

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

139 Definitions of different parameters can be found in Table 1.

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 such that no sequential infection occurs at this transmission state. This assumption  
143 may not be implausible, given that the prey's lifecycle is often shorter than that of the  
144 predator. A prey likely encounters the free-living parasite pool once and then either die due  
145 to predation, making sequential transmission less likely at this state. Sequential infection  
146 can happen when parasites transmit from intermediate hosts to definitive hosts. Therefore,  
147 a singly infected definitive host can be further infected by another parasite if it consumes  
148 infected intermediate hosts. The system's dynamics are illustrated in figure (2).

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

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

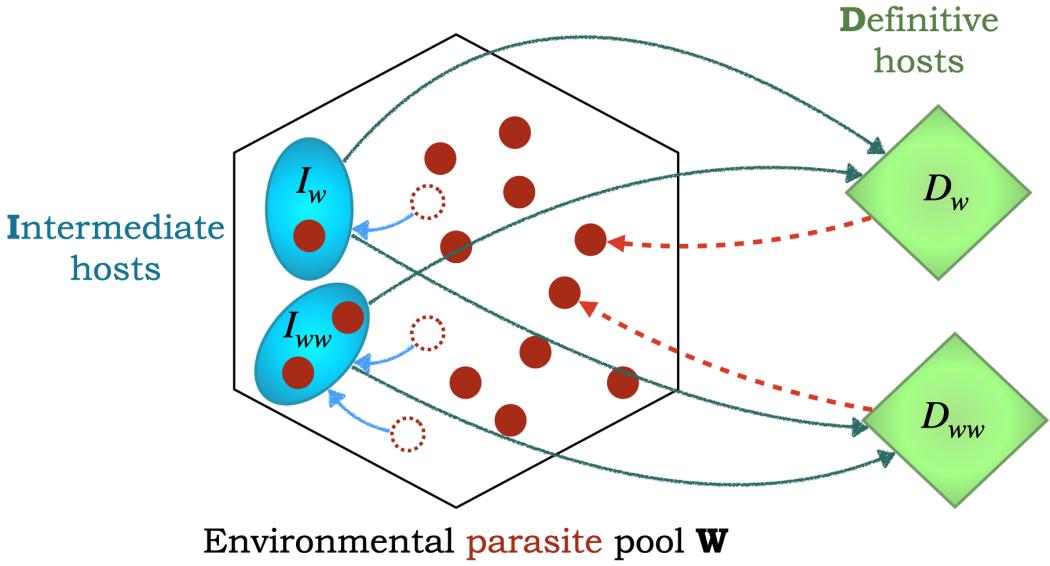


Figure 2: Schematic of the model. Blue ovals represent intermediate host compartment, green diamonds represent definitive host compartment, and the transparent hexagon represents the parasite pool compartment with red circles illustrating individual parasites.

<sup>154</sup> 2009; Hurford et al., 2010) (See SI1 for details).

$$R_0 = \overbrace{\gamma I_s^* \frac{2pqh(\rho + \beta_{ww})}{\alpha_{ww} + d + P_{ww}} \frac{D_s^*}{\mu + \sigma_{ww}} \frac{f_{ww}}{\delta + \gamma I_s^*} + \gamma I_s^* \left( \frac{(1-p)h(\rho + \beta_w)}{\alpha_w + d + P_w} + \frac{2p(1-q)h(\rho + \beta_{ww})}{\alpha_{ww} + d + P_{ww}} \right) \frac{D_s^*}{\mu + \sigma_w} \frac{f_w}{\delta + \gamma I_s^*}}^{\text{Double infections}} + \overbrace{\gamma I_s^* \left( \frac{(1-p)h(\rho + \beta_w)}{\alpha_w + d + P_w} + \frac{2p(1-q)h(\rho + \beta_{ww})}{\alpha_{ww} + d + P_{ww}} \right) \frac{D_s^*}{\mu + \sigma_w} \frac{f_w}{\delta + \gamma I_s^*}}^{\text{Single infection}} \quad (4)$$

<sup>155</sup> where  $I_s^*$  and  $D_s^*$  are the densities of susceptible intermediate and definitive hosts at the  
<sup>156</sup> disease-free equilibrium. Here, the expression of  $R_0$  contains the possible reproduction routes  
<sup>157</sup> of a parasite, which can be via double or single infections. The first component corresponds  
<sup>158</sup> to the double infections route, in which the focal parasite co-transmits with another par-  
<sup>159</sup> asite into a susceptible intermediate host, then co-transmits into a susceptible definitive  
<sup>160</sup> host and reproduces. Here, parasites are so rare that only co-transmission matters and the  
<sup>161</sup> compartments with sequential infections are therefore neglected. The second component  
<sup>162</sup> corresponds to the single infection route, wherein the focal parasite infects a susceptible  
<sup>163</sup> intermediate host via single or double infections. The parasite then transmits alone into the

164 susceptible definitive host and eventually reproduces.

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

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

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

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

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

181 where  $c$  is the efficiency of converting prey into predator's offspring, and  $I_{total} = I_s + I_w + I_{ww}$   
182 is the total density of the intermediate hosts. The birth rate of the predators depends on the  
183 capture rate, but it is not affected by host manipulation, as to our best knowledge, there is  
184 no supporting evidence to necessarily consider otherwise.

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

191 **Birth function of intermediate hosts**

192 The simplest form of the prey's birth rate is linear, in which case the disease free equilibrium  
193 is always unstable. In particular, it has a cyclic behaviour because, at this equilibrium, the  
194 jacobian matrix of the system (1, 2, 3) always has two pure imaginary eigenvalues (see SI  
195 2). This follows from the Lotka-Volterra system using linear functions for prey birth and  
196 predation ([Lotka, 1920](#)). Since the disease-free dynamics is cyclic, it is difficult to analyse  
197 the spread of a parasite using the basic reproduction ratio, which is evaluated when the  
198 disease-free state is stable. Here,  $R_0 > 1$  happens when  $\gamma$ , the transmission rate from  
199 the environment to intermediate hosts, and the reproduction rates  $f_w, f_{ww}$  are significantly  
200 large (the specific mathematical conditions can be found in SI2). However, even when this  
201 condition is satisfied, the parasite may not be able to spread and persist in cyclic susceptible  
202 host dynamics (Figure 3). This result agrees with the conclusion in ([Ripa and Dieckmann,  
203 2013](#)), which suggests that it is difficult for a mutant to invade a cyclic resident population.  
204 In our case, it is not the invasion of a mutant in a resident population but the invasion of  
205 a parasite in a cyclic disease-free host population; the argument, however, remains valid in  
206 both cases. This issue deserves a more thorough investigation, which is out of the scope of  
207 this article. Here, we choose a non-linear birth function of the intermediate hosts to obtain a  
208 stable disease circulation state and focus on the effect of host manipulation on the ecological  
209 dynamics.

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

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

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

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

212 This equilibrium is stable if the following three conditions are met,

- (i)  $r > d$
- (ii)  $\frac{2c\rho \left( \sqrt{\frac{-d+\mu+r}{\mu}} - 1 \right)}{r} \leq k < \frac{c\rho(r - d)}{\mu r}$
- (iii)  $\mu > \frac{4c^2\rho^2r - 4c^2d\rho^2}{4ck\rho r + k^2r^2}.$

213 The above conditions suggest that (i) the intrinsic reproduction of intermediate hosts  $r$  needs

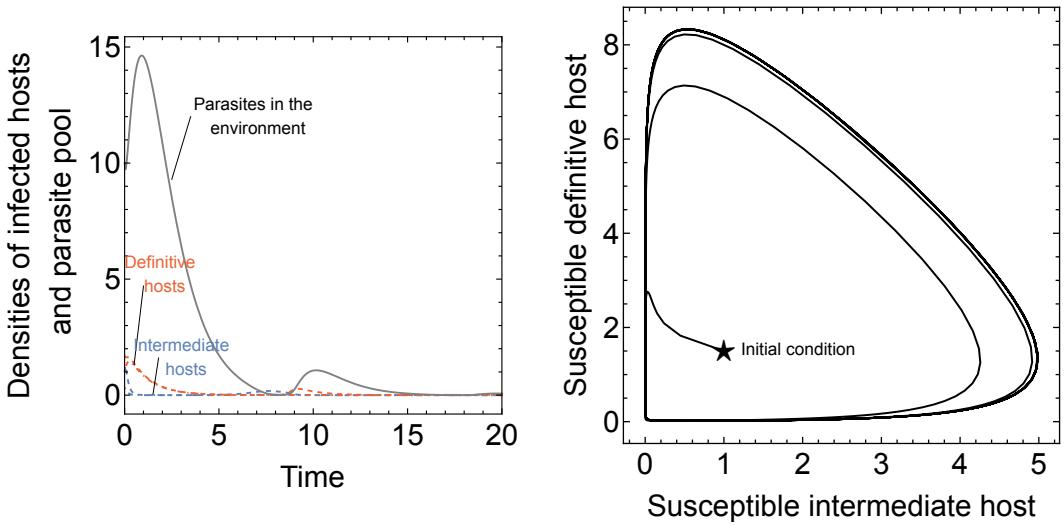


Figure 3: Disease-free equilibrium using linear birth function, where parasite goes extinct (left panel), and susceptible hosts demonstrate cyclic dynamics (right panel). Solid gray line indicate the density of free-living parasites, blue lines indicate infected intermediate hosts while red lines indicate infected definitive hosts. Dashed lines indicate singly infected hosts while dot-dashed lines indicate doubly infected hosts. Parameter values  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = \alpha_{ww} = 0$ ,  $\beta_w = 1.5$ ,  $\beta_{ww} = 1.5$ ,  $p = 0.1$ ,  $c = 1.4$ ,  $\mu = 0.9$ ,  $\sigma_w = \sigma_{ww} = 0$ ,  $q = 0.01$ ,  $f_w = 6.5$ ,  $f_{ww} = 7.5$ ,  $\delta = 0.9$ ,  $R_0 = 2.233$

to be greater than their natural mortality rate  $d$ . More importantly, (ii) the intraspecific competition coefficient has to be within a range allowing the population to survive. Finally, (iii) the definitive host's natural mortality rate must be sufficiently large. Satisfying such conditions, we obtain a stable disease-free equilibrium (Figure 4B).

When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio  $R_0 > 1$ . Since the expression is complicated, we could not obtain analytical solutions for this inequality without assumptions. We assume the same parasite virulence,  $\alpha_w = \alpha_{ww}$ ,  $\sigma_w = \sigma_{ww}$ , and reproduction in double infection as a linear function concerning reproduction in single infections,  $f_{ww} = \epsilon f_w$ . When  $\epsilon > 1$ , reproduction in double infections is greater than reproduction in a single infection, whereas  $\epsilon \leq 1$ , reproduction in double infections is lower or equal to reproduction in a single infection. 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 SI3).

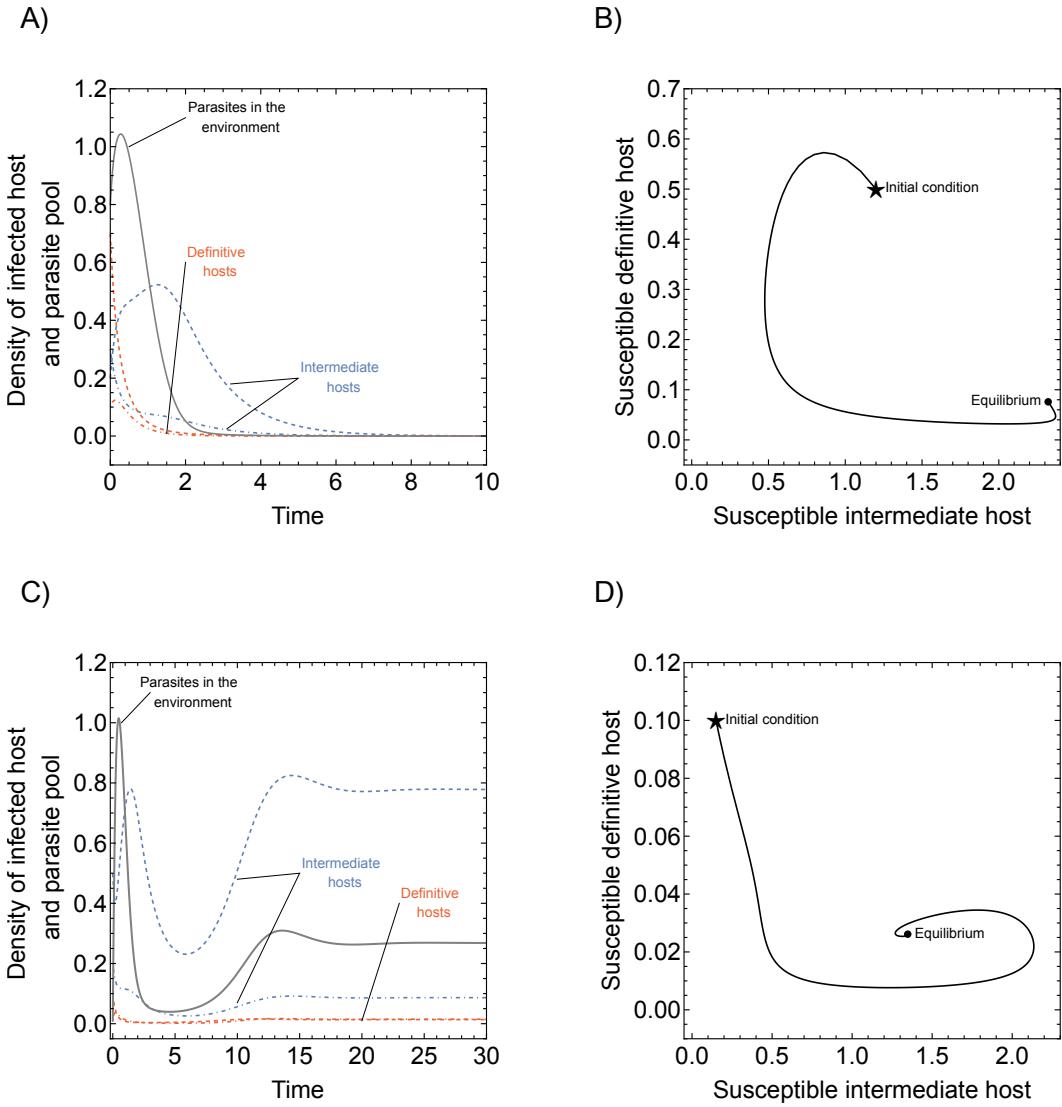


Figure 4: A, B) Disease free equilibrium where parasite cannot persist. C, D) Disease stable equilibrium. Annotations are the same in figure 3. Parameters for disease free equilibrium  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = \alpha_{ww} = 0$ ,  $\beta_w = \beta_{ww} = 1.5$ ,  $p = 0.1$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = \sigma_{ww} = 0$ ,  $q = 0.01$ ,  $f_w = f_{ww} = 7.5$ ,  $\delta = 0.9$ ,  $k = 0.26$ . Disease stable equilibrium have the same parameter values except for higher host manipulation  $\beta_w = \beta_{ww} = 4.5$  and parasite reproduction  $f_w = f_{ww} = 45$

227 Our numerical results show that the parasite reproduction is substantial compared to other  
 228 parameters (its value is nearly 40 times greater than other parameters). This observation  
 229 suggests that trophically transmitted parasites must release many offspring into the environ-

230 ment to persist. Interestingly, bistability occurs if the reproduction rate of the parasite in  
 231 double infections is greater than in the single infection state (Figure 5A, B).

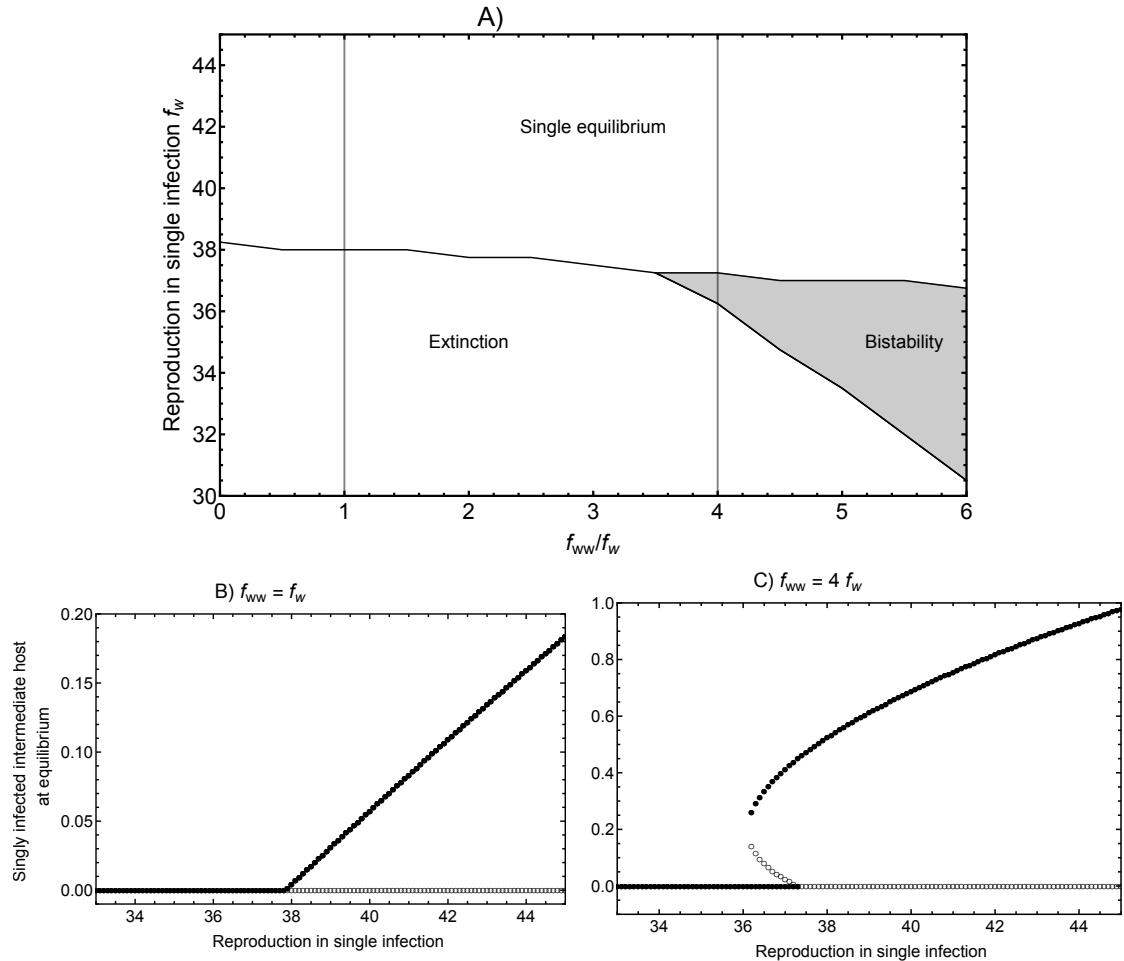


Figure 5: Effect of parasite reproduction on the ecological dynamics. A) Increasing reproduction in doubly infection leads to bistability, B, C) Density of singly infected host at equilibrium when reproduction of parasites are the same in singly and doubly infected hosts  $f_{ww} = f_w$ , and when reproduction of parasites in doubly infected hosts is four times greater than those in singly infected hosts  $f_{ww} = 4f_w$ . Filled circles indicate stable equilibrium and open circles indicate unstable equilibrium. Parameter  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $\beta_w = 1.5$ ,  $\beta_{ww} = 1.5$ ,  $p = 0.1$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.01$ ,  $\delta = 0.9$ ,  $k = 0.26$

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

233 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; that is, the predation rate  
234 on doubly-infected intermediate hosts lower than that on singly-infected ones, or  $\beta_{ww} < \beta_w$ . Cooperation in parasite manipulation does increases the parasite's basic reproduction ratio  
235  $R_0$  but the manipulation in single infection has a stronger effect on the value of  $R_0$  (Figure  
236 6). Intuitively, if the manipulation in single infection is small, there is not enough transmission  
237 and the parasite goes extinct. However, suppose the ability to manipulate the host in a single infection is just enough for the parasite population to escape extinction but not strong.  
238 In that case, cooperation in host manipulation leads to a bistable state of the system. Within  
239 the bistable region, the basic reproduction ratio can be less than one, suggesting that the parasite  
240 cannot spread when its manipulative values are within this area of weak manipulation when cointfected.

241 Cooperation between parasites need not be limited to host manipulation. Parasites can cooperate to have a higher reproduction rate in co-infections, i.e.  $f_{ww} > f_w$ . Likewise, they can  
242 compete for resources, so reproduction in double infection is smaller than in single infection.  
243 Without any assumption on the relationship between manipulative ability and reproduction,  
244 we explore all possible combinations of cooperation and sabotage in both manipulation and  
245 reproduction. Cooperation indicates that the value in double infections is larger than that in  
246 single infection, whereas the reverse suggests uncooperation. If parasites are uncooperative  
247 in both reproduction and manipulation, they can not persist. In contrast, if they are highly  
248 cooperative in both aspects, there is one guaranteed single equilibrium for parasite existence.  
249 For intermediate cooperation of both reproduction and manipulation, there could exist the  
250 area of bistability. However, the size of the bistability area is highly sensitive to the value of  
251 reproduction and manipulation in single infection. In particular, higher values of these two  
252 parameters reduce the bistability area, whereas larger values increase the bistability area. If  
253 the parasites only sabotage host manipulation, the system is highly prone to bistability, and  
254 only has a single equilibrium when cooperation in reproduction is extremely high. Interestingly,  
255 sufficiently high cooperation in reproduction leads to bistability (i.e.  $f_{ww}$  is at least 4 times  $f_w$ ), and noncooperation in reproduction always lead to single equilibrium of the  
256 system, given sufficiently large values of reproduction and manipulation in single infection  
257 (Figure 7). While a single equilibrium guarantees existence of parasite population, bistability  
258 indicates that a disturbance of the system may likely lead to extinction of parasite. This  
259 suggests that whether or not cooperation benefits the population is very context dependent.  
260 Clearly, if there is no limitation to cooperation, and if reproduction or manipulation in single  
261 infection is large enough, cooperation always hold an advantage. On the other hand, co-

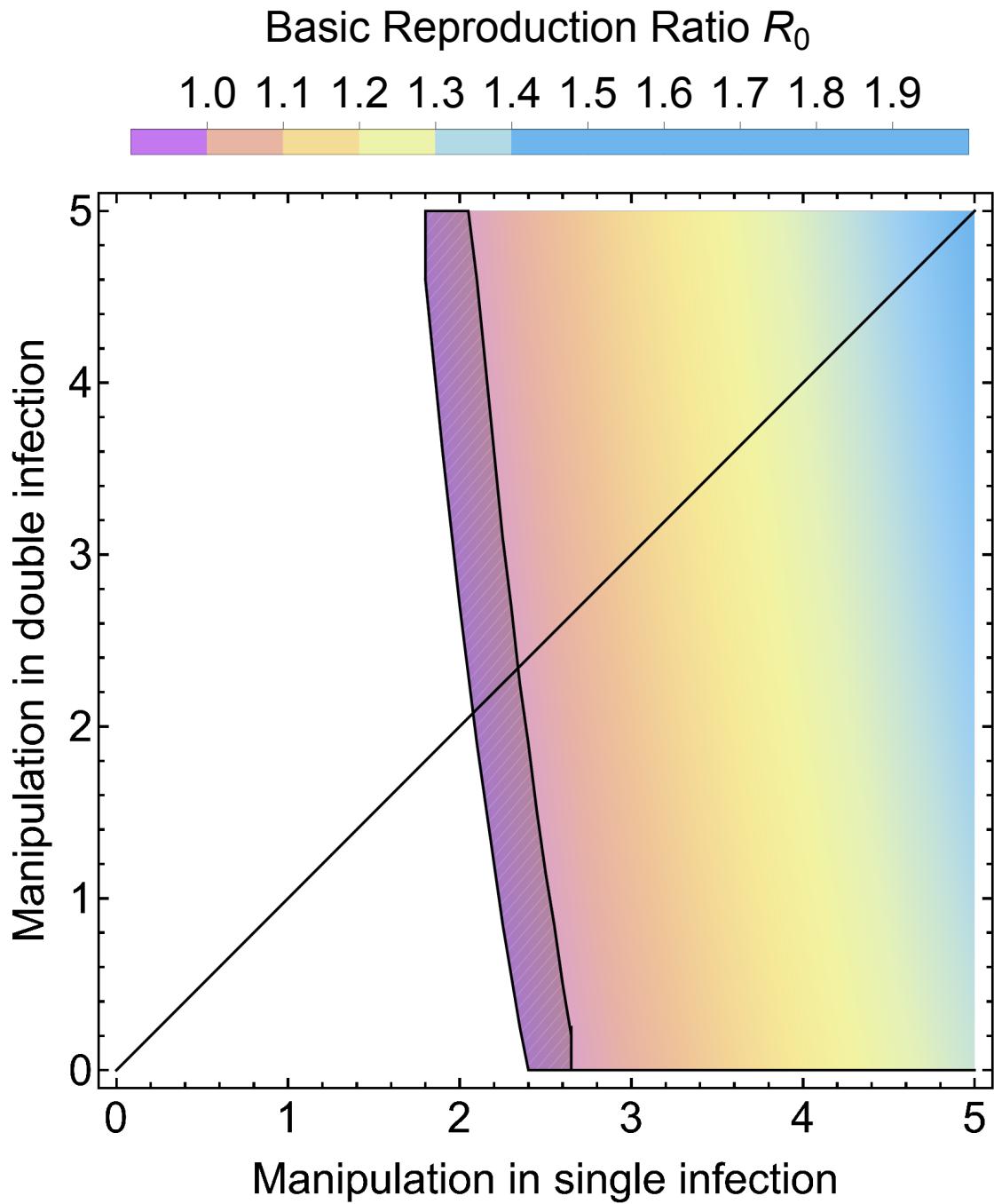


Figure 6:  $R_0$  values increase with more efficient manipulation in both single and double infection. The parasite goes extinct if its manipulative ability is insufficient (white area). Bistability region occurs when cooperation in manipulation is intermediate (hatched area). As manipulation in single infection increases, the system only has one stable equilibrium. On the black line, manipulation is indifference between single infection and double infection ( $\beta_w = \beta_{ww}$ ).

268 operation in one aspect or insufficient cooperation in both aspects may make the parasite  
 269 population more vulnerable to extinction than systems with less cooperative parasites.

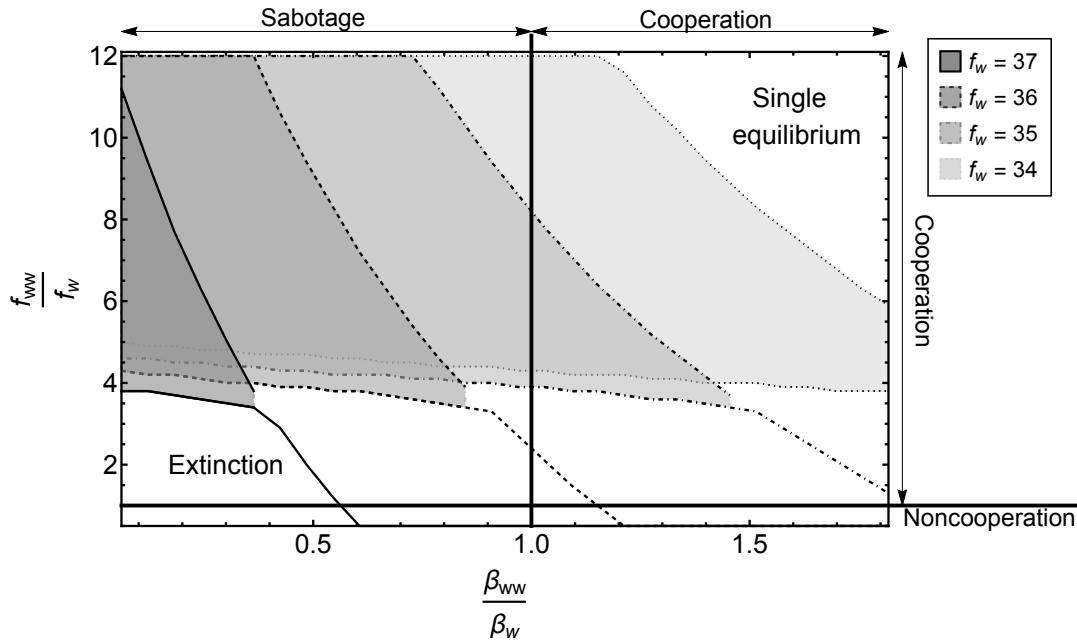


Figure 7: Changes of the bistability area (shaded areas) with respect to different reproduction rates in single infection (different boundary styles). Manipulation and reproduction is indifference between single infection and double infection on the vertical and horizontal lines respectively. Common parameter:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\epsilon = 0.5$ ,  $\beta_w = 1.5$ .

270 Co-transmission probability from the parasite pool to intermediate hosts  $p$  has opposite  
 271 effect on the bistable area compared to co-transmission probability  $q$  from intermediate hosts  
 272 to intermediate hosts. In particular, when parasite sabotage the manipulation, increasing  
 273  $p$  enlarge the bistable area whereas increasing  $q$  reduce it. In contrast, when parasites  
 274 cooperate in manipulation, reducing  $p$  decreases the bistable area while reducing  $q$  widen it.  
 275 Considering bistability indicates vulnerability to disturbance, this suggests that cooperation  
 276 in manipulation may be beneficial when the co-transmission from the pool to intermediate  
 277 host increases. However, cooperation in manipulation may be harmful to the population.  
 278 Evidently, if cooperation is extremely high, the population will always exist.

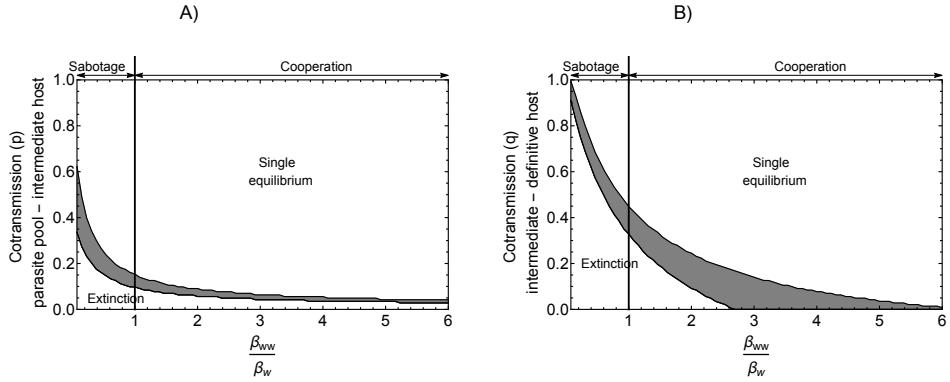


Figure 8: Common parameter:  $\rho = 1.2$ ,  $d = 0.9$ ,  $r = 2.5$ ,  $\gamma = 2.9$ ,  $\alpha_w = 0$ ,  $\alpha_{ww} = 0$ ,  $p = 0.05$ ,  $c = 1.4$ ,  $\mu = 3.9$ ,  $\sigma_w = 0$ ,  $\sigma_{ww} = 0$ ,  $q = 0.05$ ,  $\delta = 0.9$ ,  $k = 0.26$ ,  $\epsilon = 0.5$ ,  $\beta_w = 1.5$ .

## 279 Discussion & Conclusion

280 Host manipulation is a ubiquitous phenomenon suggested to affect the prey-predator dynamics  
 281 in trophically transmitted parasites. In particular, manipulation of infected intermediate  
 282 hosts to increase the predation rate of definitive hosts may result in a heavy burden of predators  
 283 on the intermediate host population. This pressure can make parasites more vulnerable  
 284 to extinction ([Hadeler and Freedman, 1989](#); [Fenton and Rands, 2006](#)).

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

294 In the study by [Rogawa et al. \(2018\)](#), a non-manipulative parasite can invade a susceptible  
 295 prey-predator population and causes the system to cycle. The system is stabilised when the  
 296 parasite becomes manipulative, and the stability increases with the manipulative ability.  
 297 In our model, non-manipulative parasites cannot persist in the system, and the parasite  
 298 never leads the system into cyclic dynamics. These results may contradict with [Rogawa  
 299 et al. \(2018\)](#), where non-manipulative parasites lead to a stable system, while increasing  
 300 manipulation induces the cyclic behaviour of the population. We suggest that the different

301 results may be due to our introduction of a parasite pool and multiple infections, unlike  
302 the model of [Rogawa et al. \(2018\)](#). In their system, transmission from the definitive host  
303 to the intermediate host was assumed to result from direct contact between the two hosts.  
304 Such immediate transmission could directly accelerate the feedback loop between prey and  
305 predator. Hence, faster predator-prey dynamics occur, which may lead to cyclic dynamics  
306 when parasites are introduced.

307 In our study, population dynamics exhibit bistability under certain circumstances. This  
308 is very likely due to the introduction of co-transmission, which has been shown to result in  
309 bistable population dynamics in plant virus [Allen et al. \(2019\)](#) and infectious disease [Gao](#)  
310 [et al. \(2016\)](#). In this bistability region, if the system is disturbed (e.g. migration of the  
311 intermediate or definitive hosts or predation of intermediate hosts by other predators), then  
312 the density of the infected hosts may crash, leading to parasite extinction. In other word, it  
313 is a way of destabilising the predator-prey system that is different from the result of [Rogawa](#)  
314 [et al. \(2018\)](#) (where destabilising means cyclic behaviour). In particular, the destabilisation  
315 of the system is possible due to the occurrence of bistability when parasite reproduction in  
316 coinfection is boosted. The bistability region widens as parasites cooperate in reproduction  
317 but sabotage in manipulation. This extension is because the density of the doubly infected  
318 hosts is always much smaller than the singly infected host density, limited by sequential  
319 transmission and a small probability of co-transmission. Suppose manipulation in a single  
320 infection is not sufficient. In that case, the transmission of the parasites depends mainly on  
321 the double infection hosts, which is rare. So extinction is possible if manipulation in double  
322 infection is not sufficiently high.

323 [Iritani and Sato \(2018\)](#) show that manipulative parasites can persist if they can alternate  
324 manipulation between enhancing and suppressing predation rate. In our model, the parasite  
325 cannot switch its manipulative strategy. However, we show that sabotage in manipulation  
326 when parasites are coinfecting always leads to a single stable equilibrium scenario when there  
327 is no cooperation in reproduction. This result suggests that manipulation suppression, either  
328 by alternating manipulative strategy or sabotaging, can be crucial in maintaining the parasite  
329 population.

330 Finally, our study focuses on the ecological dynamics of the trophically transmitted para-  
331 site. However, investigating the evolution of host manipulation is a natural extension beyond  
332 the scope of a single manuscript, given the complexities that arise in the ecological dynam-  
333 ics itself. Studying evolution of host manipulation taking into account free-living parasite  
334 pool calls for thorough analyses, which itself could be a standalone study. In addition, the  
335 occurrence of bistability in our model suggests that the evolution of host manipulation may  
336 drive the parasite population to extinction simply because of the scarcity of the mutant and

337 the Allee effect in the population dynamics. The parasite can enhance both values if there  
338 is no tradeoff between manipulation and reproduction. Nevertheless, our model shows that  
339 this strategy, which seems to make the best of both worlds, can make the system even more  
340 unstable. Evolutionary dynamics here depend mainly on the tradeoff between host manipu-  
341 lation and other traits of the parasites, such as reproduction, virulence, and survivorship in  
342 the parasite pool, to list a few. This extension deserves thorough analysis, and we will treat  
343 it as a separate matter.

## 344 **Acknowledgements**

345 Removed for review

## 346 **Statement of Authorship**

347 Removed for review

## 348 **Data and Code Availability**

349 All data and simulation codes for generating figures are available on [https://anonymous.  
350 4open.science/r/multipleinfections](https://anonymous.4open.science/r/multipleinfections)

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436 **Tables**

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