

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. When two infectious parasites infect the
51 copepods, the copepods' activity increases, and so does the predation risk for the copepod.
52 However, when the copepods are infected by one infectious and one noninfectious parasite,
53 their interests clash, and the infectious parasite wins.



Figure 1: Who is in control? Schistocephalus eggs, hatch into microscopically tiny swimming larvae. These larvae are eaten by copepods, where they develop to the second larval stage. However, the copepod is only the first intermediate host. The larvae are then eaten by sticklebacks, reaching the third larval stage and growing prominently in size and weight. For the parasite to successfully reach its final host, a warm-blooded animal like a bird, it manipulates its intermediate hosts. The presence of multiple parasites in the same host can lead to competition and strategic decisions pertaining to investment in manipulation and growth. Indeed, a stickleback can be infected by numerous parasites, all vying for control, as shown above and photographed by Martin Kalbe (Kalbe et al., 2002).

54 Theoretical work that considers multiple infections often focuses on the evolution of viru-
55 lence (van Baalen and Sabelis, 1995; Alizon et al., 2013; Alizon and van Baalen, 2008; Choisy
56 and de Roode, 2010; Alizon, 2012), while host manipulation in trophically transmitted par-
57 asites receives less attention. Even though host manipulation and virulence correlate with
58 parasite transmission, there are subtle differences, such that virulence implies an addition to
59 the natural mortality rate of the infected host, whereas manipulation links to the immediate
60 death of the intermediate host due to predation. Host manipulation in trophically transmitted
61 parasites, therefore, strongly affects the entire predator-prey dynamics. Theoretical studies
62 regarding host manipulation rarely consider multiple infections. Studies incorporating this
63 feature neglect the predator-prey dynamics, which will likely have important feedback on the
64 evolution of host manipulation (Parker et al., 2003; Vickery and Poulin, 2009). Moreover,
65 these models assume that transmission from definitive hosts to intermediate hosts is due to
66 direct contact between the two types of hosts (Rogawa et al., 2018; Hadeler and Freedman,
67 1989; Fenton and Rands, 2006). This is often not the case in nature, as parasites are re-
68 leased from the definitive hosts into the environment. Transmission thus happens only when
69 intermediate hosts have contact with this free-living parasite pool. The inclusion of this free-
70 living stage could have a profound effect on the dynamics of the whole predator-prey-parasite
71 system.

72 Our study addresses the gap in the theoretical work on host manipulation in trophically
73 transmitted parasites. We include multiple infections and consider the dynamics of the free-
74 living parasite pool. Our compartment model helps illustrate a parasite's complex life cycle
75 with two host species: an intermediate host preyed upon by a definitive host. Transmission
76 from the intermediate host to the definitive host occurs when predation on infected interme-
77 diate hosts happens. Reproduction only happens in the definitive hosts. New parasites then
78 enter the environment, where the cycle continues. We focus on the intermediate host manip-
79 ulation, such that the parasite increases the uptake of the intermediate host by the definitive
80 host to increase its transmission rate. We then analyse the effect of host manipulation on

81 the ecological dynamics in the predator-prey-parasite system. We found that sabotage in
82 host manipulation almost always pushes the dynamical system toward bistability, provided
83 the reproduction in a single infection is sufficiently small. The bistable nature suggests that
84 the predator-prey parasite system is finely balanced and susceptible to extinction via ecolog-
85 ical disturbances. Initially surprising, we showed that cooperation in host manipulation and
86 enhanced reproduction in co-infecting parasites is not always beneficial and might expose
87 the parasite population to the risk of extinction.

88 **Model**

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

95 For simplicity, we assume that hosts can be infected by one (single infection) or, at most,
96 two parasites (double infections). Thus, while I_s and D_s are the susceptible intermediate
97 and definitive hosts, their singly and doubly infected counterparts are denoted by I_w and D_w
98 and I_{ww} and D_{ww} respectively. Our model is, therefore, more relevant to the macroparasitic
99 system. Figure (2) illustrates the transmission dynamics, and details of the model's variables
100 and parameters are shown in Table 1. Note that multiple infections in nature often involve
101 more than two parasites. Typically, the number of parasites in multiple infections follows a
102 negative binomial distribution, i.e. most hosts are infected with a few parasites while very
103 few hosts are infected with many parasites (Wilson et al., 1996). However, since we use a
104 compartmental model, enabling binomial distribution would mean infinitely many differential
105 equations, making it impossible to formulate and analyze the model. Instead, we focus on
106 another aspect of multiple infections, that is, co-transmission, which has been shown to

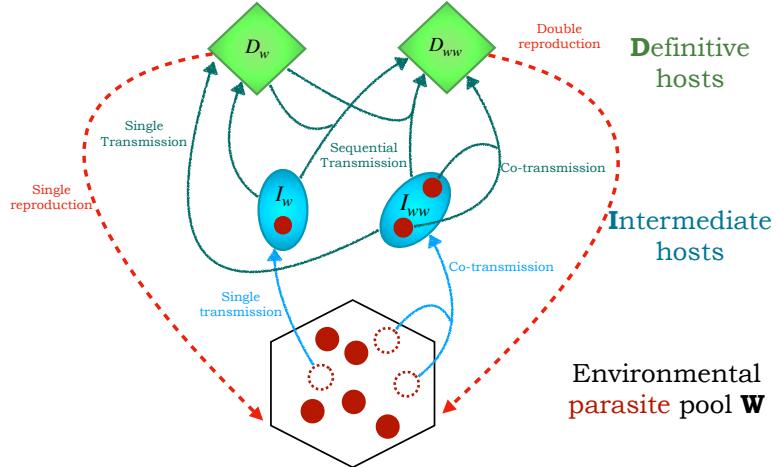


Figure 2: Schematics of the transmission routes. Blue ovals represent the intermediate hosts, while green diamonds represent the definitive hosts. The hexagon represents the parasite pool compartment, with the red circles illustrating the free-living individual parasites. The parasites infect the intermediate 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 \mathbf{W} (red dashed arrows).

affect the evolutionary trajectories of parasites in infectious disease (Alizon, 2012). Given that infection occurs, the probability that two parasites from the parasite pool co-transmit to an intermediate host is denoted by p . Thus, $1 - p$ is the probability that a single parasite enters an intermediate host. When a definitive host consumes an intermediate host infected by two parasites, there is a probability q that the parasites co-transmit to the definitive host. With probability $1 - q$, only one parasite successfully transmits. This formulation assumes that infection always happens when intermediate hosts encounter free-living parasites and when definitive hosts consume infected intermediate hosts (Figure 2). The dynamics of a complex life cycle parasite that requires two host species is described by the following system

116 of equations, firstly for the intermediate host as,

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

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

133 For the definitive hosts, we have,

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

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

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

144 where f_w and f_{ww} are the reproduction rates of parasites in single and double infection,
145 respectively, and parasites die naturally at a rate δ .

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

₁₅₂ definitive hosts. Therefore, a singly infected definitive host can be further infected by another
₁₅₃ parasite if it consumes infected intermediate hosts.

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
α_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
γ	Transmission rate of parasites in the environment to intermediate hosts
μ	Natural death rate of definitive hosts
σ_i	Additional death rate of definitive hosts due to infection by a single parasite ($i = w$) or two parasites ($i = ww$)
σ_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
β_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 ($i = ww$)
δ	Natural death rate of parasites in the environment
h	Probability that the parasites successfully established inside the definitive host

₁₅₄ Basic reproduction ratio R_0 of the parasites

₁₅₅ The basic reproduction ratio R_0 (or basic reproduction number as often used in epidemiology)
₁₅₆ indicates parasite fitness. It can be understood as the expected number of offspring a parasite

157 produces during its lifetime when introduced to a susceptible host population. We calculate
 158 the basic reproduction ratio R_0 using the next-generation method (Diekmann et al., 1990,
 159 2009; Hurford et al., 2010) (See SI1 for details).

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

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

170 If $R_0 > 1$, a parasite spreads when introduced into the disease-free equilibrium of prey and
 171 predator. Intuitively, the higher the density of susceptible intermediate and definitive hosts,
 172 the larger the value of R_0 as the infection reservoir is more extensive. In contrast, regardless
 173 of the explicit form of the predation function, the higher the predation rate P_w and P_{ww} , the
 174 lower the value of R_0 given the smaller reservoir of intermediate hosts. The effect of host
 175 manipulation on the value of R_0 is more complex; as host manipulation becomes efficient,
 176 the transmission rate from the intermediate host to the definitive host increases, but so does
 177 the predation rate. A higher predation rate results in a smaller intermediate host reservoir
 178 for the parasites to infect. To understand the effect of manipulation on parasites' fitness

179 and the system's ecological dynamics, we next specify the predation functions. We consider
180 linear functions for predation to begin with,

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

181 where ρ is the baseline capture rate of the predator on the prey. If an intermediate host is
182 infected, it is captured by the definitive hosts with rate $\rho + \beta_w$ if it is singly infected and with
183 rate $\rho + \beta_{ww}$ if it is doubly infected. Zero values for β_w and β_{ww} suggest no manipulation,
184 and predation is at the baseline value ρ .

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

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

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

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

197 **Birth function of intermediate hosts**

198 The simplest form of the prey's birth rate is a linear function, in which case the disease-free
199 equilibrium is always in a cyclic regime (see SI 2). This follows from the Lotka-Volterra
200 system using linear functions for prey birth and predation ([Lotka, 1920](#)). Since the disease-
201 free dynamics is cyclic, it is difficult to analyse the spread of a parasite using the basic
202 reproduction ratio, which is evaluated when the disease-free state is stable. Here, $R_0 > 1$
203 happens when γ , the transmission rate from the environment to intermediate hosts, and the
204 reproduction rates f_w, f_{ww} are quite large (as compared to the theoretical threshold shown
205 by the mathematical conditions in SI3). However, even when this condition is satisfied, the
206 parasite may not be able to spread and persist in cyclic susceptible host dynamics (Figure
207 SI1). This result agrees with the conclusion in ([Ripa and Dieckmann, 2013](#)), which suggests
208 that it is difficult for a mutant to invade a cyclic resident population. In our case, it is not
209 the invasion of a mutant in a resident population but the invasion of a parasite in a cyclic
210 disease-free host population; the argument, however, remains valid in both cases. This issue
211 deserves a more thorough investigation, which is out of the scope of this article. Therefore,
212 we choose a non-linear birth function of the intermediate hosts to obtain a stable disease-free
213 state and focus on the effect of host manipulation on the ecological dynamics (Figure 3).

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

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

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

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

216 This equilibrium is positive and stable if components of the parasite, such as reproduction
217 and transmission, are sufficiently small; details of the condition can be found in section SI

218 4. Here, as reproduction and transmission value of the parasite are not sufficient, it goes
 219 extinct (Figure 3A, B), leaving the predator-prey dynamics attaining equilibrium (Figure 3C,
 220 D)

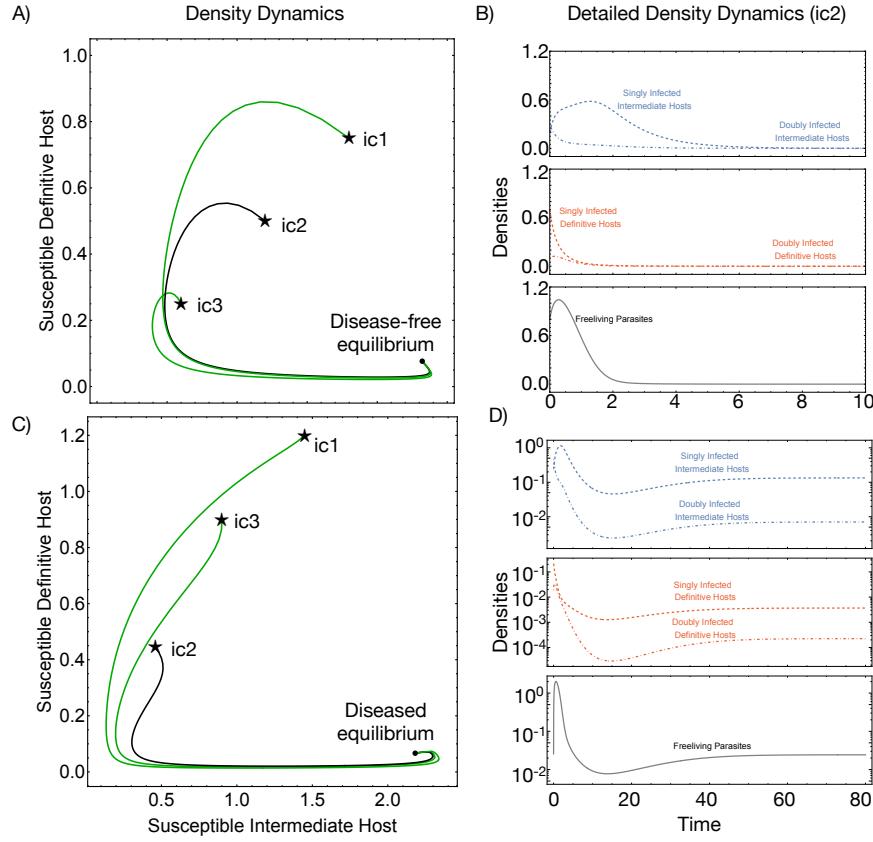


Figure 3: Ecological dynamics of the predator-prey-parasite system. On the left, we show the density dynamics of the susceptible intermediate and definitive hosts at different initial conditions (ic1, ic2, and ic3). The detailed dynamics of infected compartments are further shown for specific initial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

221 When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio
 222 $R_0 > 1$ (Figure 4). Since the expression is complicated, we could only obtain analytical

223 solutions for this inequality with assumptions. We assume the same parasite virulence,
 224 $\alpha_w = \alpha_{ww}$, $\sigma_w = \sigma_{ww}$, and reproduction in double infection as a linear function concerning
 225 reproduction in single infections, $f_{ww} = \epsilon f_w$. When $\epsilon > 1$, reproduction in double infections
 226 is enhanced compared to in single infections, whereas $\epsilon \leq 1$, reproduction in double infections
 227 is suppressed or equal to reproduction in single infections. We found that the parasite can
 228 establish if its reproduction value in a single infection f_w is more significant than a threshold
 229 (Figure 5, see section SI 5 and Eq. (SI.19)).

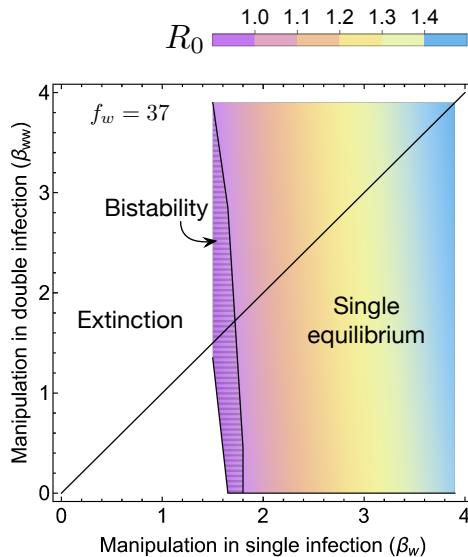


Figure 4: Effect of manipulation in single and double infections on the reproduction ratio R_0 . R_0 values increase with more efficient manipulation in single and double infection. The hatched area indicates the bistable region. As manipulation in single infection increases, the system only has one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

230 Our numerical results show that the parasite reproduction is substantial compared to
 231 other parameters (Figure 5A). For instance, in the parameter set used to generate Figure
 232 5, to spread in the predator-prey system, the value of parasite reproduction (f_w) has to
 233 be at least 20 times the value of intermediate host reproduction $r = 2.5$, given that both

234 these parameters represent the *per capita* growth rate of the parasite and the intermediate
235 host population. This observation suggests that trophically transmitted parasites should
236 release many offspring into the environment to persist. Interestingly, bistability occurs if
237 the reproduction rate of the parasite in double infections is enhanced. Bistability suggests
238 that the parasite population is vulnerable to extinction. Specifically, if sufficient parasites are
239 introduced into the disease-free predator-prey populations, the parasite population persists
240 and reaches a stable equilibrium . In contrast, if only a few parasites are introduced into the
241 disease-free populations, or if sufficient disturbance occurs when the parasite population is
242 already established, the parasite population could go extinct (Figure 5C).

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

244 Host manipulation can be cooperative; two parasites increase the predation rate on interme-
245 diate hosts, or $\beta_{ww} > \beta_w$. However, it can also be uncooperative; the predation rate on
246 doubly-infected intermediate hosts is lower than that on singly-infected ones or $\beta_{ww} < \beta_w$.
247 Cooperation in parasite manipulation increases the parasite's basic reproduction ratio R_0 ,
248 but the manipulation in a single infection substantially affects the value of R_0 (Figure 4).
249 Intuitively, if the manipulation in a single infection is minor, there is not enough transmission,
250 and the parasite goes extinct. However, we could suppose that the ability to manipulate the
251 host in a single infection is enough for the parasite population to escape extinction. In that
252 case, the system is in a bistable state where intermediate cooperation in host manipulation
253 cannot guarantee a single equilibrium (Hatched area Figure 4). In the bistable region, the
254 basic reproduction ratio can be less than one, implying that the parasite with manipulative
255 values within this range, i.e. weak manipulation ability, cannot spread. When the system
256 encounters bistability, the parasite population risks extinction if there is a disturbance in the
257 community. In the following parts, we will explore scenarios where bistability may occur.

258 Besides manipulation, co-infecting parasites can influence each other in different life his-
259 tory traits. Parasites can have an enhanced reproduction rate in coinfections, i.e. $f_{ww} > f_w$

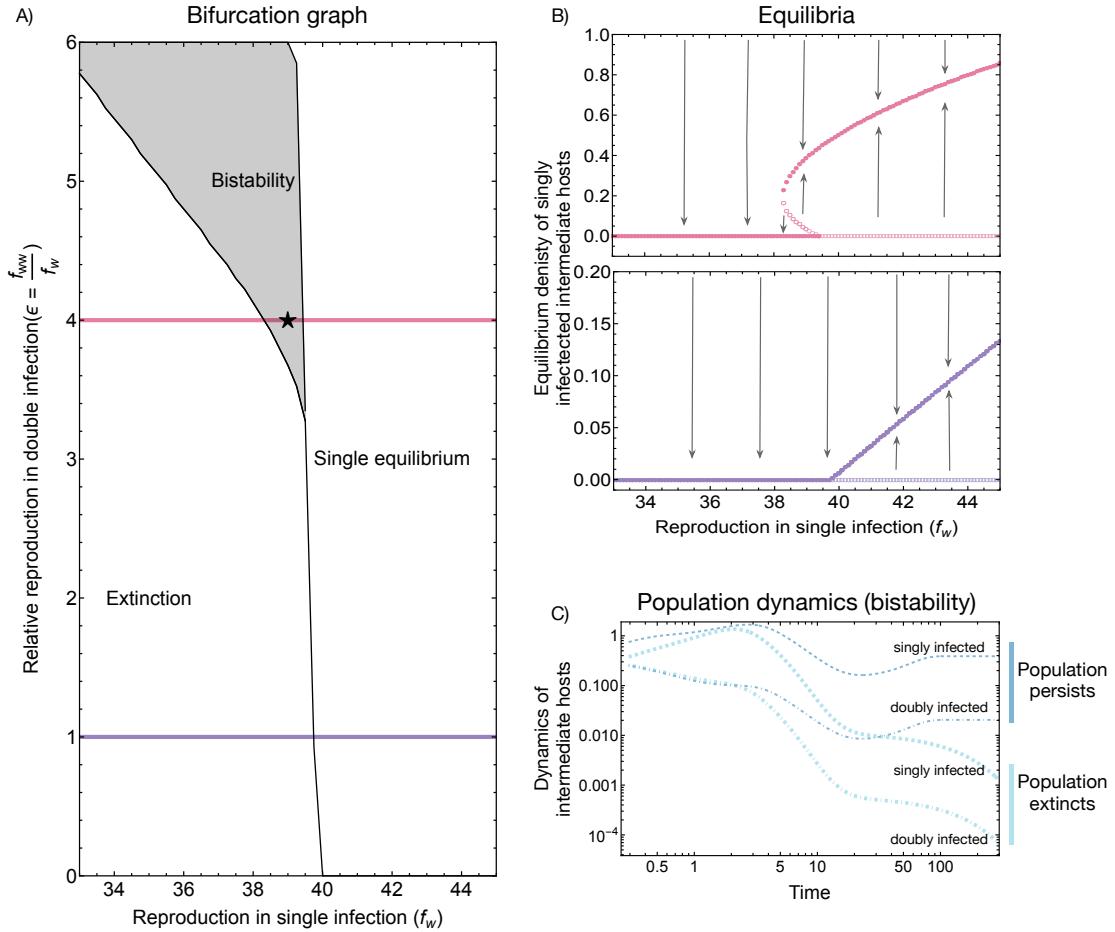


Figure 5: Effect of parasite reproduction on the ecological dynamics. A) A bifurcation graph for different values of reproduction in single infection and double infections. B) Equilibrium values of intermediate host when $\epsilon = 4$ when bistability occur at high values of f_w (in pink), and $\epsilon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

(upper part of the horizontal line in all panels Figure 6). Likewise, they can compete for resources, so reproduction in double infection is suppressed compared to single infection (lower

parts of the horizontal lines in all panels Figure 6). Without any assumption on the link between manipulative ability and reproduction, 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 quadrants in all panels Figure 6), ii, parasites cooperate to increase manipulation and enhance reproduction – coordination (top right quadrants in all panels Figure 6), iii, parasites cooperate in manipulation but suppress reproduction – reproductive incoordination (bottom right quadrants in all panels Figure 6), and iv, parasites sabotage manipulation and suppress reproduction – discordance (bottom left quadrants in all panels Figure 6).

If coinfected 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. We often expect intermediate levels of coordination where a bistable area could occur (top left quadrant in Figure 6A, 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 so that sufficiently large reproduction in a single infection can guarantee single equilibrium when parasites coordinate (Figure 6 B). In contrast, slightly reducing values of 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 enhanced. Interestingly, reproductive incoordination, i.e. depressed reproduction and manipulative cooperation, always leads to a single equilibrium of the system (Figure 5B, 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.

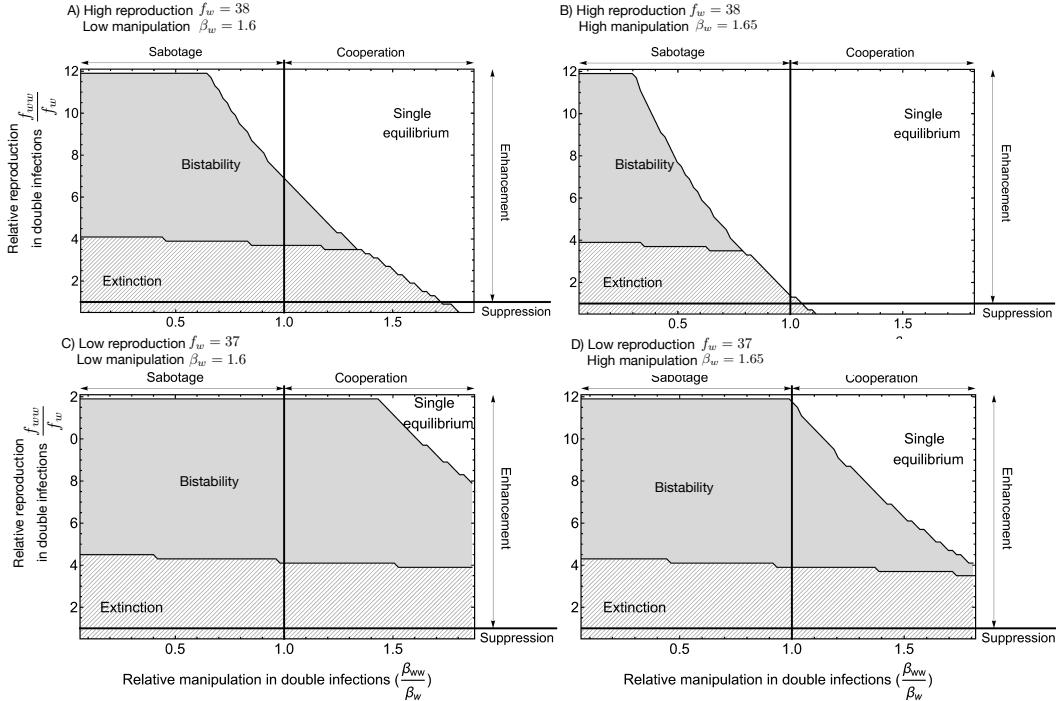


Figure 6: Effect of manipulation and reproduction on bistability. The bistability area (shaded areas) reduces as the reproduction rate (f_w) and manipulation (β_w) in single infection increases. Reproduction in single infection decreases from the upper panels (A, B) to the lower panels (C, D) while manipulation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

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

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

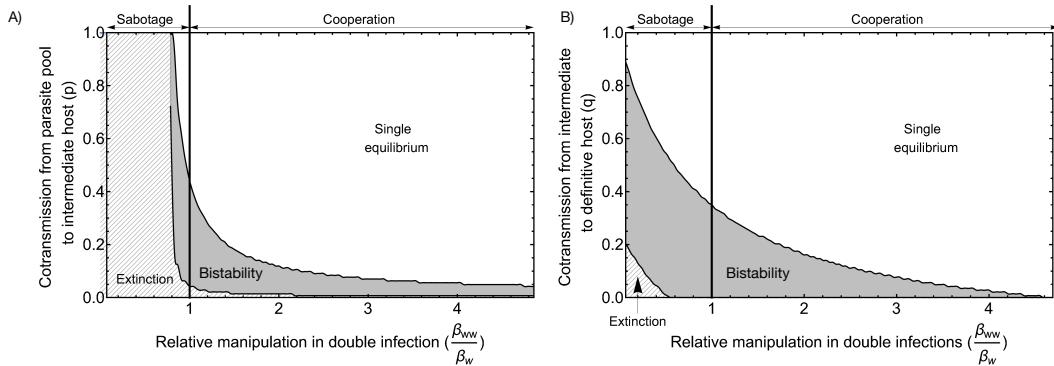


Figure 7: A) Effect of cotransmission from parasite pool to intermediate host. B) Effect of co-transmission from intermediate to the definitive host. On the left side of the vertical line, $\beta_{ww} > \beta_w$, indicating cooperation, whereas on the right side of the vertical line, $\beta_{ww} < \beta_w$,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

309 **Discussion & Conclusion**

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

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

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

336 Another study on host manipulation, Iritani and Sato (2018), showed that manipulative
337 parasites persist if they switch from suppressing to boosting predation rate. This theoretical
338 work modelled the ability to change the manipulative strategy of a single parasite inside
339 a host, which can be equal to introducing the developmental state of a parasite, where a
340 suppressed predation rate protects the parasites that are not ready to transmit. That is
341 why decreasing manipulative ability is beneficial and prevents parasite extinction. In our
342 model, sabotaging manipulation also reduces manipulative ability, which only reduces the
343 basic reproduction ratio R_0 and makes the system bistable, exposing the parasite to the risk
344 of extinction. This result contrasts with Iritani and Sato (2018) because in our model, the
345 parasite cannot switch its manipulative strategy, and sabotage decreases the transmission
346 rate from intermediate to definitive host and does not benefit the parasite in any way.

347 In our study, population dynamics exhibit bistability under certain circumstances. This
348 is very likely due to the introduction of co-transmission, which has been shown to result in
349 bistable population dynamics in plant virus Allen et al. (2019) and infectious diseases Gao
350 et al. (2016). In this bistability region, if the system is disturbed (e.g. migration of the
351 intermediate or definitive hosts or predation of intermediate hosts by other predators), then
352 the density of the infected hosts may crash, leading to parasite extinction. The bistability
353 region widens as parasites show enhanced reproduction but sabotage manipulation. This
354 extension is because the density of the doubly infected hosts is always much smaller than
355 the singly infected hosts, limited by sequential transmission and a small probability of co-
356 transmission. If manipulation in a single infection is insufficient, then the transmission of
357 the parasites depends mainly on the doubly infected hosts, which is rare. So, extinction is
358 possible if manipulation in double infections is low.

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

363 introduce more realistic features compared to previous models, such as a free-living parasite
364 pool and multiple infections, regardless of some simplifications, such as multiple infections
365 being limited to at most two parasites. In this way, we can obtain analytical results of the
366 reproduction ratio and mathematical expressions for the existing condition of the parasite.
367 Our model serves as a groundwork for future exploration into more complex and realistic
368 systems, where numerical simulation may be the only possible approach. Moreover, the
369 results of our ecological model are a baseline for further investigation of the evolution of host
370 manipulation, where introducing the parasite pool may create interesting eco-evolutionary
371 feedback to the system.

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