

On multiple infections by parasites with complex life cycles

Phuong L. Nguyen [†] and Chaitanya S. Gokhale ^{‡,*}

[†] Department of Biology, University of Fribourg,

Chemin du musée 15, Switzerland

[‡]Max Planck Institute for Evolutionary Biology, Department of Theoretical Biology

August-Thienemann-Straße 2, 24306 Plön, Germany

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

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

[†]linh.phuong.nguyen@evobio.eu

[‡]chaitanya.gokhale@uni-wuerzburg.de

Statement of Authorship

Both authors developed the theory.

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

Both authors wrote the manuscript.

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

15 **Introduction**

16 Parasites infect life on earth ubiquitously, and many of these parasites have complex life cycles
17 (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 the infectious parasite wins.

55 Theoretical work that considers multiple infections often focuses on the evolution of vir-
56 ulence (van Baalen and Sabelis, 1995; Alizon et al., 2013; Alizon and van Baalen, 2008;
57 Choisy and de Roode, 2010; Alizon, 2012), while host manipulation in trophically trans-
58 mitted parasites receives less attention. Even though host manipulation and virulence both
59 correlate with parasite transmission, there are subtle differences, such that virulence im-
60 plies an addition to the natural mortality rate of the infected host, whereas manipulation
61 links to the immediate death of the intermediate host due to predation. Host manipula-
62 tion in trophically transmitted parasites, therefore, not only affects the intermediate host
63 population but also 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?. 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 indeed, a stickleback can be infected by numerous tapeworms, as shown above by Martin Kalbe.

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

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

77 from the intermediate host to the definitive host occurs when predation on infected interme-
78 diate 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. Note that, multiple infections in nature often involve more than two parasites. Typ-
101 ically, the number of parasites in multiple infections follows a negative binomial distribution,
102 i.e. most hosts are infected with a few parasites while very few hosts are infected with many

103 parasites (ref). However, since we use a compartmental model, enabling binomial distribu-
 104 tion would mean infinite number of differential equations, making it impossible to formulate
 105 and analyze the model. Furthermore, what matters is the magnitude of the manipulative
 106 effect in multiple infections versus in single infection. Instead, we focus on another aspect of
 107 multiple infections, that is, co-transmission, which has shown to affect evolutionary trajec-
 108 tories of parasites in infectious disease (Alizon). Given that infection occurs, the probability
 109 that two parasites from the parasite pool co-transmit to an intermediate host is denoted by
 110 p . Thus, $1 - p$ is the probability that a single parasite enters an intermediate host. When
 111 a definitive host consumes an intermediate host infected by two parasites, there is a prob-
 112 ability q that the parasites co-transmit to the definitive host. With probability $1 - q$, only
 113 one parasite successfully transmits. This formulation assumes that infection always happens
 114 when intermediate hosts encounter free-living parasites, and when definitive hosts consume
 115 infected intermediate hosts. The dynamics of a complex life cycle parasite that requires two
 116 host species is described by the following system of equations, firstly for the intermediate
 117 host as,

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

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. Intermediate
 121 hosts die at a natural rate d , and parasites cause additional mortality rate α_w in single
 122 infection and α_{ww} in double infection. The predation function depends on the density of
 123 the definitive hosts and the manipulative strategies of parasites in the intermediate hosts.
 124 In particular, if a single parasite infects an intermediate host, the manipulation strategy is

125 β_w . However, if the intermediate host is co-infected, the manipulation strategy is β_{ww} . In
 126 the scope of this model, we assume no specific relationship between β_w and β_{ww} to explore
 127 all possible ecological outcomes of the system. The force of infection by parasites in the
 128 environment is denoted by $\eta = \gamma W$, where γ represents the infection rate of free-living
 129 parasites. The force of infection is a term often used in epidemiology, which represents
 130 the rate at which a host gets infected by the parasites. Since parasites can manipulate
 131 intermediate and definitive hosts, whenever we mention host manipulation, it specifically
 132 refers to the manipulation in intermediate hosts, which correlates to the predation rate.

133 For the definitive hosts, we have,

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

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

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

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

147 Here, we focus on manipulation that enhances transmission from intermediate hosts to
 148 definitive hosts; we thus simplify the transmission from the parasite pool to intermediate
 149 hosts so that no sequential infection occurs. This assumption is motivated given that the
 150 prey life cycle is often shorter than the predator's. A prey likely encounters the free-living
 151 parasite pool once and then dies due to predation, making sequential transmission less likely
 152 at this state. Sequential infection can happen when parasites transmit from intermediate
 153 hosts to definitive hosts. Therefore, a singly infected definitive host can be further infected
 154 by another parasite if it consumes infected intermediate hosts. Figure (2) illustrates the
 system's dynamics, and Table. (1) contains the different parameters and variables used.

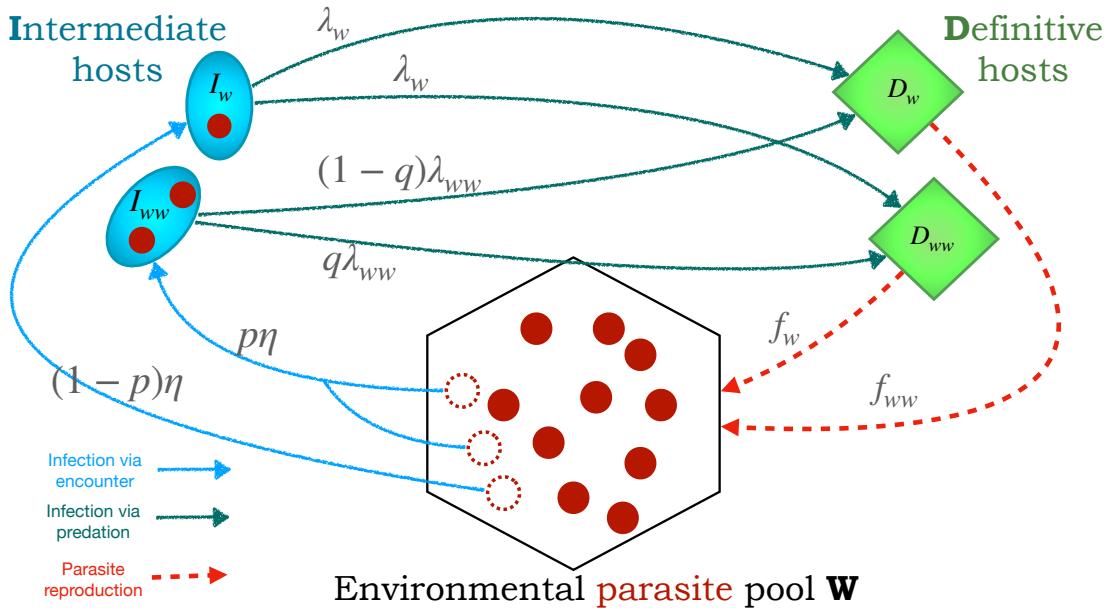


Figure 2: Schematic of the model. Blue ovals represent the intermediate hosts, while the green diamonds represent the definitive hosts. The hexagon represents the parasite pool compartment, with the red circles illustrating the free-living individual parasites. The parasites infect the intermediate hosts singly (I_w , top) or doubly (I_{ww} , bottom). These intermediate hosts are then predated upon by the definitive hosts, 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} .

155

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

156 **Results**

157 **Basic reproduction ratio R_0 of the parasites**

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

162 2009; Hurford et al., 2010) (See SI1 for details).

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

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

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

183 linear functions for predation to begin with,

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

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

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

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

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

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

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

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

202 **Birth function of intermediate hosts**

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

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

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

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

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

223 This equilibrium is positive and stable if components of the parasite, such as reproduction

and transmission, are sufficiently small; details of the condition can be found in section SI 4.
Here, because reproduction and transmission value of the parasite are not sufficient, it goes
extinct (Figure 3A), leaving the predator-prey dynamics attaining equilibrium (Figure 3B)

When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio
 $R_0 > 1$ (Figure 3C, D). Since the expression is complicated, we could only obtain analytical
solutions for this inequality with assumptions. We assume the same parasite virulence,
 $\alpha_w = \alpha_{ww}$, $\sigma_w = \sigma_{ww}$, and reproduction in double infection as a linear function concerning
reproduction in single infections, $f_{ww} = \epsilon f_w$. When $\epsilon > 1$, reproduction in double infections
is enhanced compared to in single infections, whereas $\epsilon \leq 1$, reproduction in double infections
is suppressed or equal to reproduction in single infections. We found that the parasite can
establish if its reproduction value in a single infection f_w is more significant than a threshold
(Figure 4, see section SI 5 and Eq. (SI.19)).

Our numerical results show that the parasite reproduction is substantial compared to other
parameters (Figure 4A). For instance, in the parameter set used to generate Figure (4B, 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 offspring into the environment to persist. Interestingly, bistability occurs if
the reproduction rate of the parasite in double infections is enhanced (Figure 4A). Bistability
suggests that parasite population is vulnerable to extinction. Specifically, if sufficient para-
sites are introduced into the disease-free predator-prey populations, the parasite population
persists and reach a stable equilibrium (Figure 4B, C). In contrast, if only a few parasites
are introduced into the disease-free populations, or if sufficient disturbance occur when the
parasite population already establishes, the parasite population could go extinct (Figure 4B,
C).

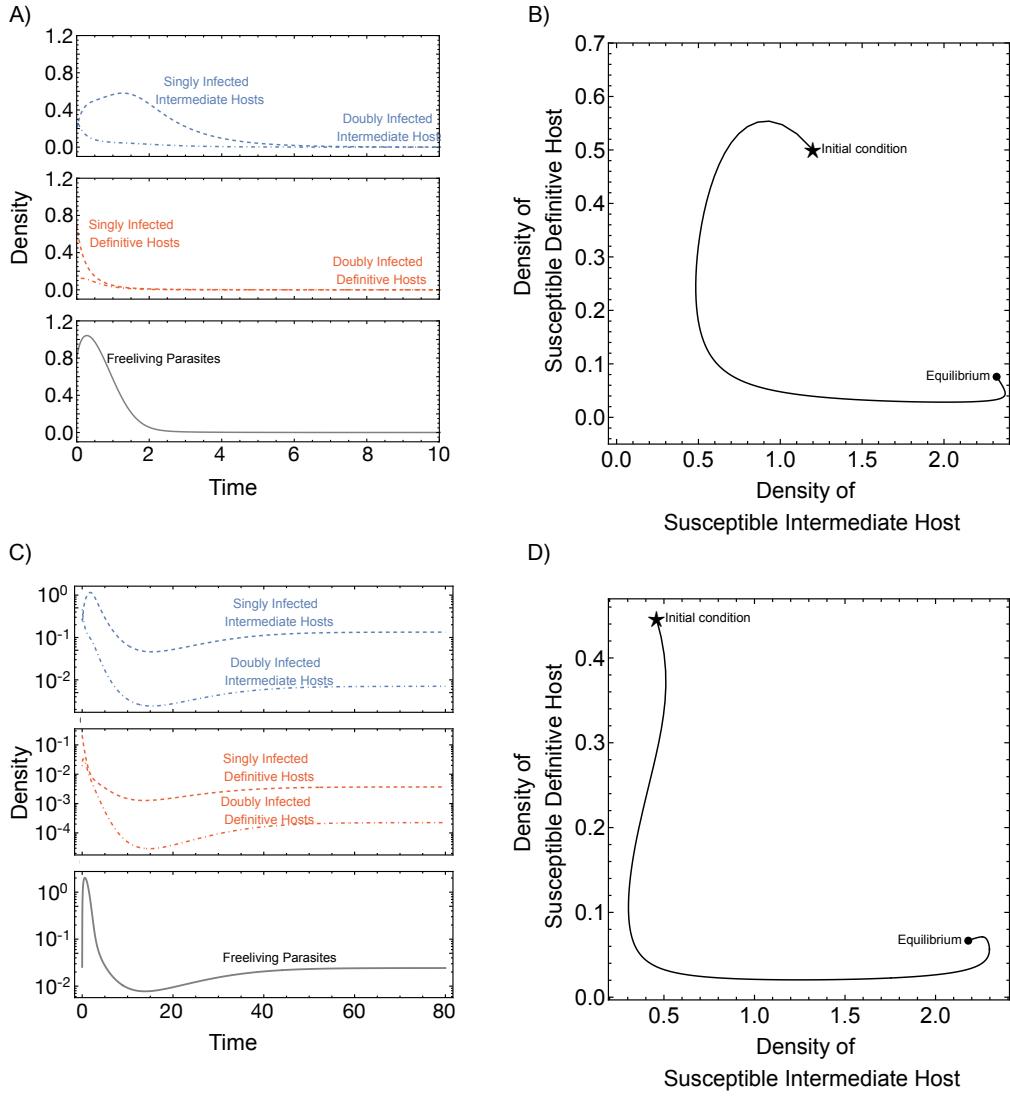


Figure 3: Ecological dynamics of the predator-prey-parasite system. A) Ecological trajectories of infected hosts and free-living parasite when parasites cannot persist, B) Phase plane of susceptible intermediate and definitive hosts under disease free scenario. C) Ecological trajectories of infected hosts and free-living parasite when parasites persist. D) Phase plane of susceptible and definitive host under disease circulating scenario. 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 $f_w = f_{ww} = 45$

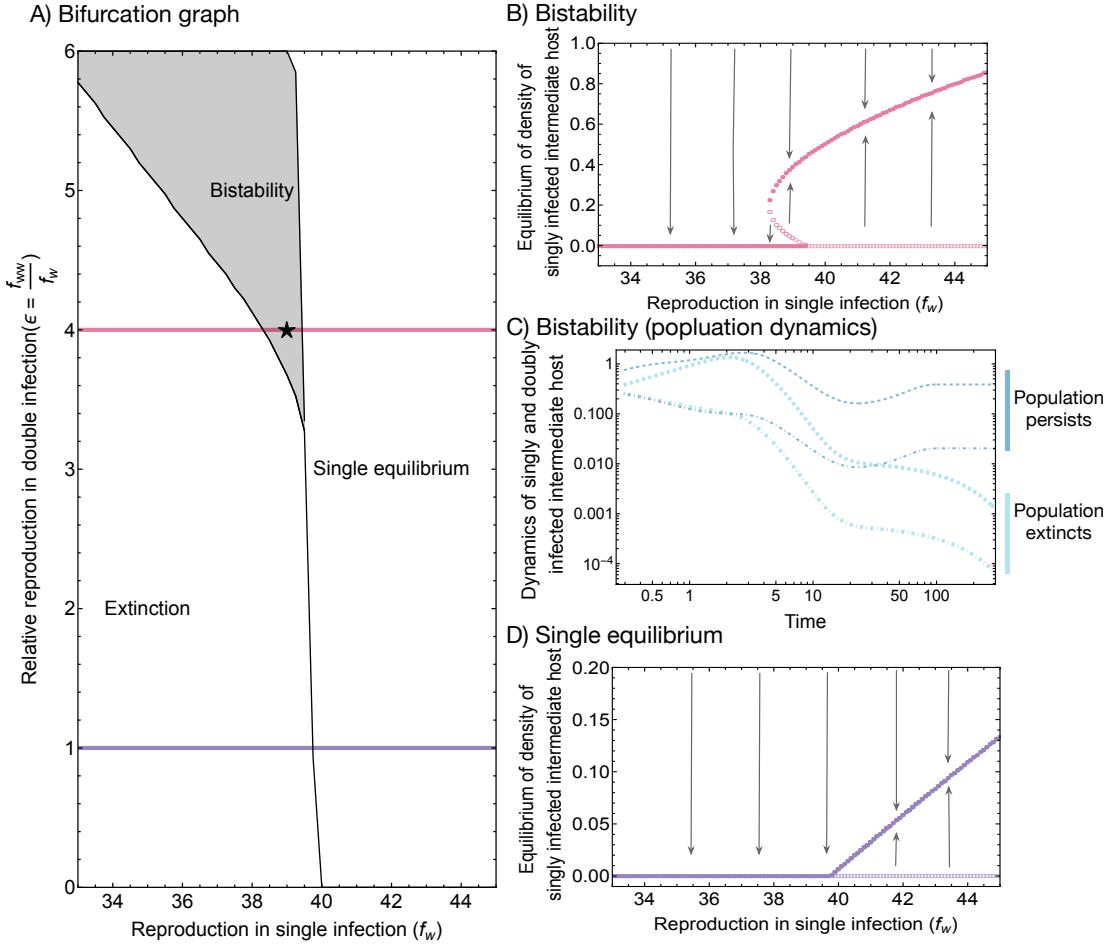


Figure 4: Effect of parasite reproduction on the ecological dynamics. A) Enhanced reproduction in double infection leads to bistability, suggesting vulnerability of parasite population to extinction. B) Stability of the equilibrium of singly infected host when $f_{ww} = 4f_w$, which correspond to the purple line in A). C) Parasite population dynamics in the case of bistability, which correspond to the star in A). If initial population is sufficiently large, the population persists, but if initial population is sufficiently small, the population goes extinct. D) Stability of the equilibrium of singly infected host when $f_{ww} = f_w$, which correspond to the pink line in A). Filled circles indicate stable equilibrium and open circles indicate unstable equilibrium. Parameter $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $\beta_w = 1.5$, $\beta_{ww} = 1.5$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $h = 0.6$.

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

251 Host manipulation can be cooperative; two parasites increase the predation rate on intermediate hosts, or $\beta_{ww} > \beta_w$. However, it can also be uncooperative; the predation rate on doubly-infected intermediate hosts is lower than that on singly-infected ones or $\beta_{ww} < \beta_w$.
252 Cooperation in parasite manipulation increases the parasite's basic reproduction ratio R_0 ,
253 but the manipulation in a single infection substantially affects the value of R_0 (Figure 6).
254 Intuitively, if the manipulation in a single infection is minor, there is not enough transmission,
255 and the parasite goes extinct. However, suppose the ability to manipulate the host in a single
256 infection is merely enough for the parasite population to escape extinction. In that case, the
257 system is in a bistable state where intermediate cooperation in host manipulation cannot
258 guarantee a single equilibrium (Hatched area Figure 6 Left). In the bistable region, the
259 basic reproduction ratio can be less than one, implying that the parasite with manipulative
260 values within this range, i.e. weak manipulation ability, cannot spread. When the system
261 encounters bistability, the parasite population risks extinction if there is a disturbance in the
262 community. In the following parts, we will explore scenarios where bistability may occur.

263 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. $f_{ww} > f_w$
264 (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
265 parts of the horizontal lines in all panels Figure 6). Without any assumption on the relationship
266 between manipulative ability and reproduction, we explore all possible combinations of
267 cooperation-sabotage range in manipulation and suppressed-enhanced range in reproduction.
268 This results in four scenarios of parameter combinations: i, parasites sabotage manipulation
269 but have enhanced reproduction – manipulative incoordination (top left quadrants in all panels
270 Figure 6), ii, parasites cooperate to increase manipulation and enhance reproduction –
271 coordination (top right quadrants in all panels Figure 6), iii, parasites cooperate in manipulation
272 but suppress reproduction – reproductive incoordination (bottom right quadrants in

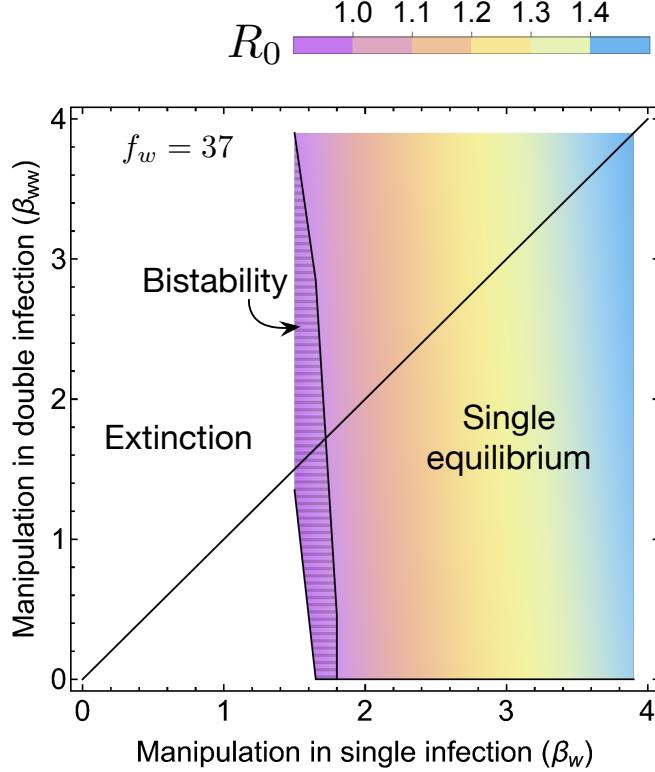


Figure 5: Effect of manipulation in single and double infections on the reproduction ratio R_0 . R_0 values increase with more efficient manipulation in single and double infection. The hatched area indicates the bistable region. As manipulation in single infection increases, the system only has one 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: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $f_w = 37$, $\epsilon = 4.5$, $h = 0.6$.

277 all panels Figure 6), and iv, parasites sabotage manipulation and suppress reproduction –
 278 discordance (bottom left quadrants in all panels Figure 6).

279 If coinfecting parasites are discordant, i.e. uncooperative in manipulations and show sup-
 280 pressed reproduction, they cannot persist (Figure 6A-D). On the other extreme, where they
 281 are highly cooperative in manipulation and show enhanced reproduction, i.e., an extreme
 282 level of coordination, there is a guaranteed single equilibrium for parasite existence. Note
 283 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 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 to the point that sufficiently large reproduction in single infection can guarantee single equilibrium when parasites coordinate (Figure 6 B). In contrast, slightly reducing values of either reproduction or manipulation in single infection increase 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. Interestingly, reproductive incoordination, i.e. depressed reproduction and manipulative cooperation, always leads to a single equilibrium of the system (Figure 4B, 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 intermediate host, p , compared to varying levels of co-transmission from intermediate host to definitive host, q . For exceptionally high level of cooperation and not very small values of both 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 disappears with high values of q but not with high values of p . When parasites cooperate in manipulation, reducing p almost always lead to bistability whereas reducing q can lead to single equilibrium if cooperation is sufficiently large. Bistability indicates vulnerability to disturbance, and so cooperation

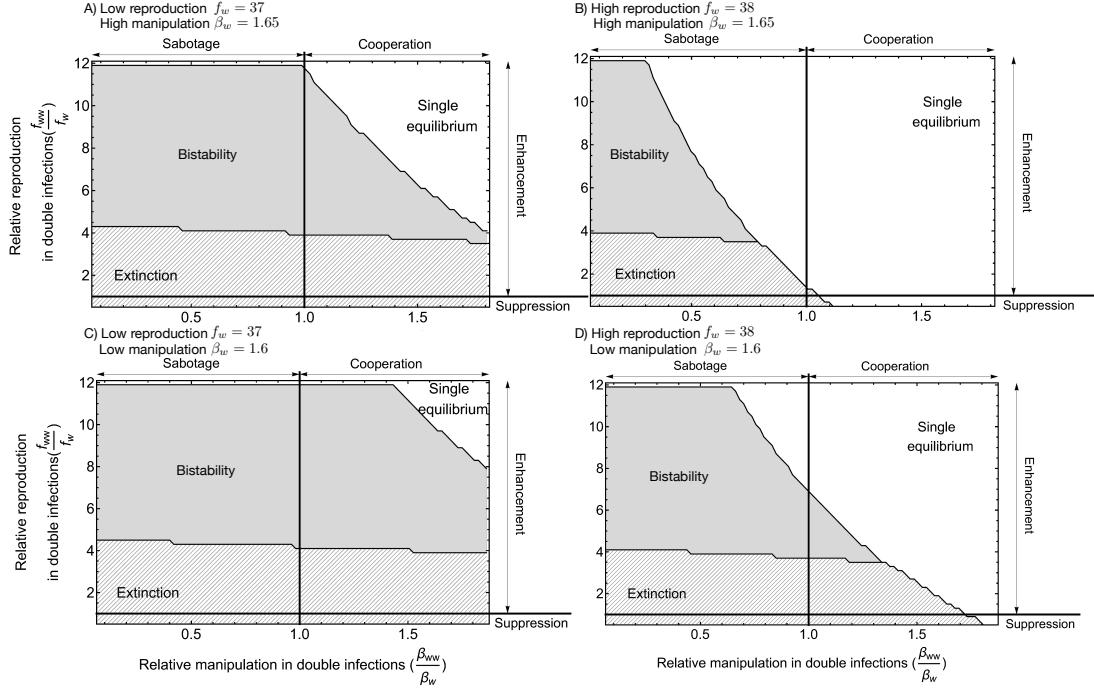


Figure 6: Effect of manipulation and reproduction on bistability. The bistability area (shaded areas) reduces as the reproduction rate in single infection f_w increases (different boundary styles). Manipulation and reproduction levels are equal between single and double infection on the vertical and 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: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $\beta_w = 1.65$, $h = 0.6$.

311 in manipulation may be beneficial when q , the co-transmission from the intermediate host
 312 to the definitive host, decreases. However, cooperation in manipulation may still harm the
 313 population with reducing p , the co-transmission from the parasite pool to intermediate host.

314 Discussion & Conclusion

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

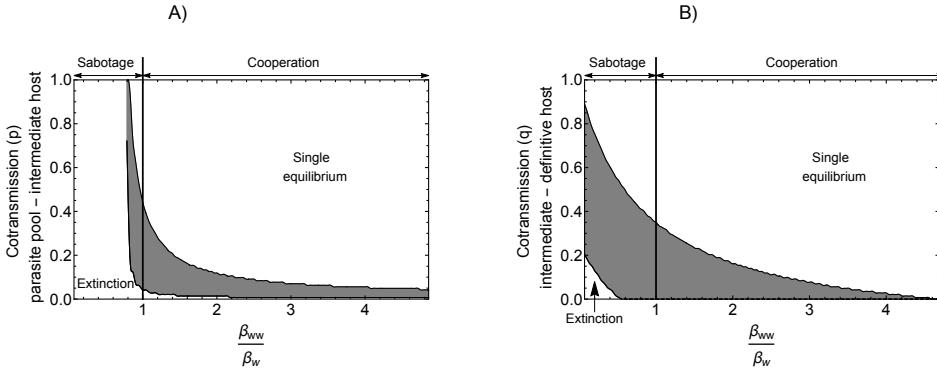


Figure 7: Left: Effect of cotransmission from parasite pool to intermediate host. Right: Effect of cotransmission from intermediate to definitive host. On the left side of the vertical line, $\beta_{ww} > \beta_w$, indicating cooperation, whereas on the right side of the vertical line, $\beta_{ww} <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

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

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

In the study by [Rogawa et al. \(2018\)](#), a non-manipulative parasite can invade a susceptible prey-predator population and cause the system to cycle. The system stops cycling and approaches a fixed point when the parasite becomes manipulative, and this stability increases with increased manipulation. In our model, non-manipulative parasites cannot persist in the

333 system, and the parasite never leads to cyclic dynamics. These results may contradict with
334 [Rogawa et al. \(2018\)](#), where non-manipulative parasites can still exist via cyclic behaviour.
335 We suggest that the different results may be due to our introduction of a parasite pool and
336 multiple infections, unlike the model of [Rogawa et al. \(2018\)](#). In their system, transmission
337 from the definitive host to the intermediate host was assumed to result from direct contact
338 between the two host species. Such immediate transmission could directly accelerate the
339 feedback loop between prey and predator. Hence, faster predator-prey dynamics occur,
340 which may lead to cyclic dynamics when parasites are introduced.

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

352 In our study, population dynamics exhibit bistability under certain circumstances. This
353 is very likely due to the introduction of co-transmission, which has been shown to result in
354 bistable population dynamics in plant virus [Allen et al. \(2019\)](#) and infectious diseases [Gao
355 et al. \(2016\)](#). In this bistability region, if the system is disturbed (e.g. migration of the
356 intermediate or definitive hosts or predation of intermediate hosts by other predators), then
357 the density of the infected hosts may crash, leading to parasite extinction. The bistability
358 region widens as parasites show enhanced reproduction but sabotage manipulation. This
359 extension is because the density of the doubly infected hosts is always much smaller than

360 the singly infected hosts, limited by sequential transmission and a small probability of co-
361 transmission. If manipulation in a single infection is insufficient, then the transmission of
362 the parasites depends mainly on the doubly infected hosts, which is rare. So, extinction is
363 possible if manipulation in double infections is low.

364 Finally, our study focuses on the ecological dynamics of a trophically transmitted para-
365 site between two host species. In nature, parasites with complex life cycles can have more
366 than two hosts. However, our model of a single intermediate host species can already pro-
367 vide enough complexity to discuss the relationship between transmission and manipulation.
368 Here, we introduce more realistic features compared to previous models, such as a free-living
369 parasite pool and multiple infections, regardless of some simplifications, such as multiple in-
370 fections being limited to at most two parasites. In this way, we can obtain analytical results
371 of the reproduction ratio and mathematical expressions for the existing condition of the para-
372 site. Our model serves as a groundwork for future exploration into more complex and realistic
373 systems, where numerical simulation may be the only possible approach. Moreover, the re-
374 sults of our ecological model are a baseline for further investigation of the evolution of host
375 manipulation, where introducing the parasite pool may create interesting eco-evolutionary
376 feedbacks to the system.

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