

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
19 a parasite needs to traverse to complete its life cycle. This complex life cycle results in
20 the evolution of various strategies that enable successful 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 its host's morphology and/or behaviour to
23 enhance its transmission to the next host (Hughes et al., 2012). Host manipulation has been
24 shown in many host-parasite systems, from parasites with simple life cycles to those with a
25 complex life cycle that involves more than one host species (Hughes et al., 2012; Molyneux
26 and Jefferies, 1986). For instance, sand flies infected by *Leishmania* parasites bite more and
27 take more time for a blood meal from mammals (the definitive host of *Leishmania*) compared
28 to their uninfected counterparts (Rogers and Bates, 2007). Copepods infected by cestode
29 parasites are more active and accessible to sticklebacks (the cestodes' definitive hosts) than
30 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 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

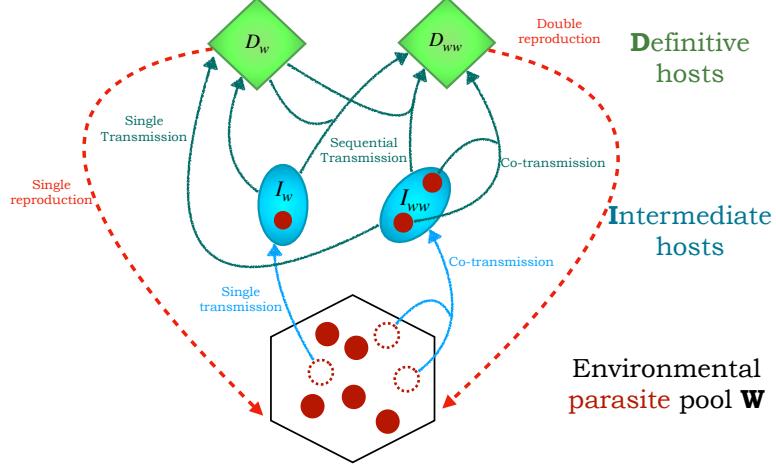


Figure 2: Schematics of the transmission routes. Blue ovals represent the intermediate hosts, while green diamonds represent the definitive hosts. The hexagon represents the parasite pool compartment, with the red circles illustrating the free-living individual parasites. The parasites infect the intermediate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

affect the evolutionary trajectories of parasites in infectious disease (Alizon, 2012). Given an infection, 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} . We assume no specific relationship between β_w and β_{ww} to
 126 explore all possible ecological outcomes of the system. The force of infection by parasites in
 127 the environment is denoted by $\eta = \gamma W$, where γ represents the infection rate of free-living
 128 parasites. The force of infection is a term often used in epidemiology, which represents
 129 the rate at which a host gets infected by the parasites. Since parasites can manipulate
 130 intermediate and definitive hosts, whenever we mention host manipulation, it specifically
 131 refers to the manipulation in intermediate hosts, which correlates to the predation rate.

132 For the definitive hosts, we have,

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

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

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

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

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

153 Basic reproduction ratio R_0 of the parasites

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

Table 1: Description of variables and parameters

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

¹⁵⁶ produces during its lifetime when introduced to a susceptible host population. We calculate
¹⁵⁷ the basic reproduction ratio R_0 using the next-generation method (Diekmann et al., 1990,

158 2009; Hurford et al., 2010) (See SI1 for details).

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

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

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

179 linear functions for predation to begin with,

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

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

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

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

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

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

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

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

196 **Birth function of intermediate hosts**

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

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

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

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

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

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

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

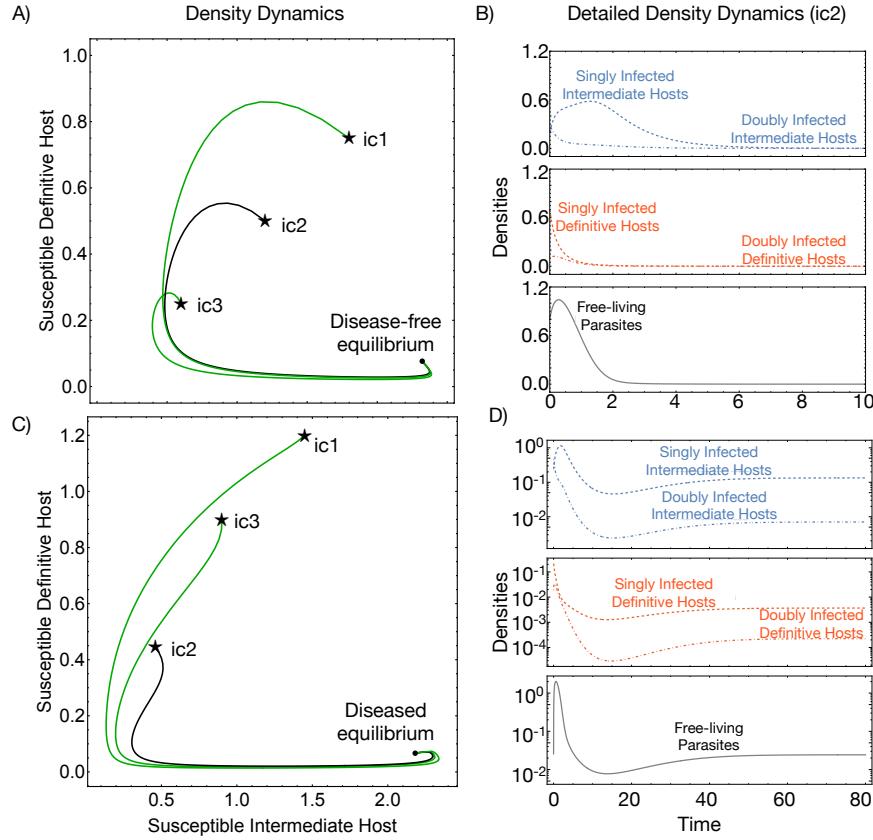


Figure 3: Ecological dynamics of the predator-prey-parasite system. On the left, we show the density dynamics of the susceptible intermediate and definitive hosts at different initial conditions (ic1, ic2, and ic3). The detailed dynamics of infected compartments are further shown for specific initial 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 has the same parameter values except for higher host manipulation $\beta_w = \beta_{ww} = 4.5$ and parasite reproduction $f_w = f_{ww} = 45$

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

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

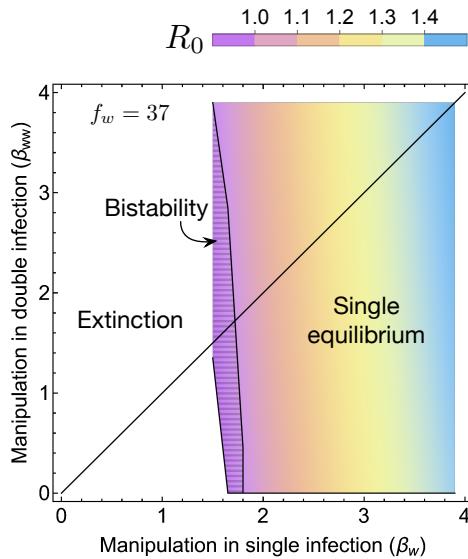


Figure 4: Effect of manipulation in single and double infections on the reproduction ratio R_0 . R_0 values increase with more efficient manipulation in single and double infection. The hatched area indicates the bistable region. As manipulation in single infection increases, the system only has one 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: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $f_w = 37$, $\epsilon = 4.5$, $h = 0.6$.

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

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

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

243 Host manipulation can be cooperative; two parasites increase the predation rate on interme-
244 diate hosts, or $\beta_{ww} > \beta_w$ (Hafer and Milinski, 2015). However, it can also be uncooperative;
245 the predation rate on doubly-infected intermediate hosts is lower than that on singly-infected
246 ones or $\beta_{ww} < \beta_w$ (Hafer and Milinski, 2015). Cooperation in parasite manipulation increases
247 the parasite's basic reproduction ratio R_0 , but the manipulation in a single infection substan-
248 tially affects the value of R_0 (Figure 4, see section SI 6 for analytical results). Intuitively,
249 if the manipulation in a single infection is minor, there is not enough transmission, and the
250 parasite goes extinct. However, we could suppose that the ability to manipulate the host in
251 a single infection is enough for the parasite population to escape extinction. In that case,
252 the system is in a bistable state where intermediate cooperation in host manipulation cannot
253 guarantee a single equilibrium (Hatched area Figure 4). In the bistable region, the basic
254 reproduction ratio can be less than one, implying that the parasite with manipulative values
255 within this range, i.e. weak manipulation ability, cannot spread. When the system encounters
256 bistability, the parasite population risks extinction if there is a disturbance in the community.
257 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-

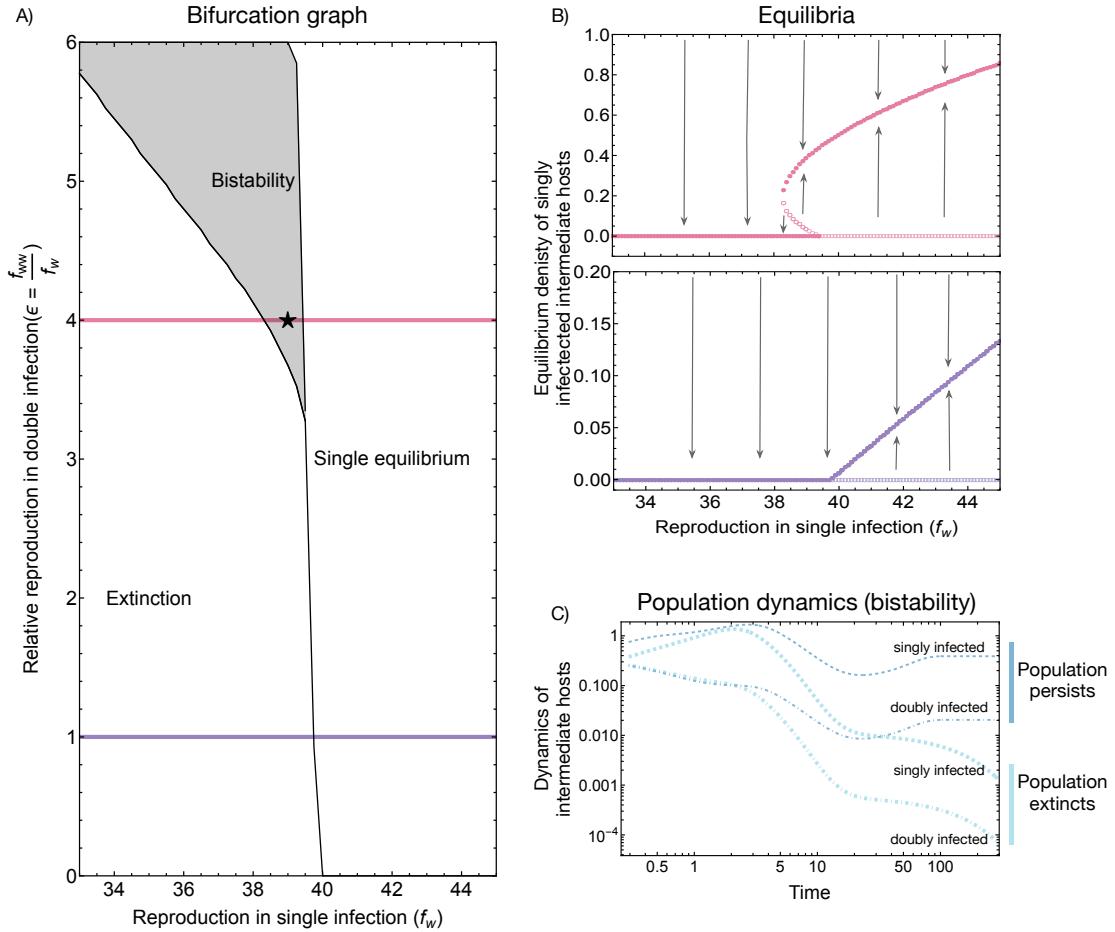


Figure 5: Effect of parasite reproduction on the ecological dynamics. A) A bifurcation graph for different reproduction values in single and double infections. B) Equilibrium density of intermediate host when $\epsilon = 4$ when bistability occurs at high values of f_w (in pink), and $\epsilon = 4$ when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

259 tory traits. Parasites can have an enhanced reproduction rate in coinfections, i.e. $f_{ww} > f_w$
 260 (upper part of the horizontal line in all panels Figure 6). Likewise, they can compete for re-
 261 sources, 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 (bottom left quadrants 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 (top right quadrants Figure 6A-D). 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 right 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, D). 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, with depressed reproduction and sufficient manipulative cooperation, always leads to a single equilibrium of the system (bottom

right quadrants Figure 6A, B). 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.

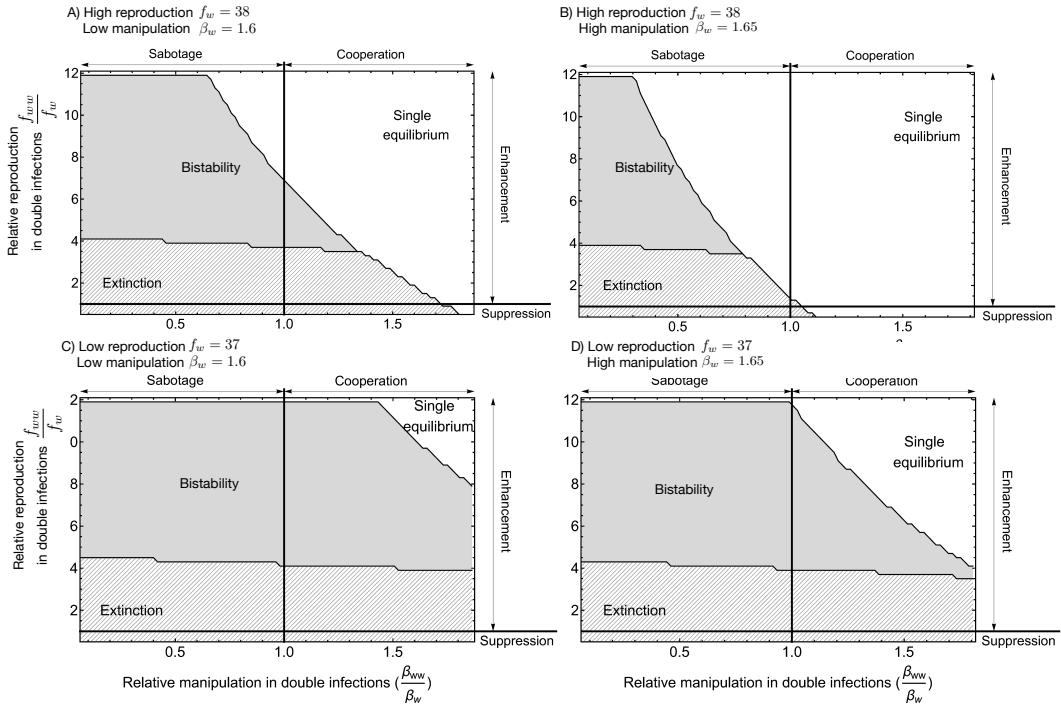


Figure 6: Effect of manipulation and reproduction on bistability. The bistability area (shaded areas) reduces as the reproduction rate (f_w) and manipulation (β_w) in single infection increases. Reproduction in single infection decreases from the upper panels (A, B) to the lower panels (C, D) while manipulation 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 parasite pool to the intermediate host, p , compared to varying levels of co-transmission from the intermediate host to the definitive host, q . For exceptionally high levels of cooperation and intermediate values of p and q , the predator-prey-parasite system will always persist with one stable equilibrium. However, limitations and trade-offs are often unavoidable, and such high values of cooperation may be impossible, putting the system in the parameter space where bistability likely occurs. When the parasite sabotages manipulation, the bistable area decreases with increasing p and q . However, this bistable area disappears with high values of q but not with high values of p . When parasites cooperate in manipulation, reducing p almost always leads to bistability, whereas reducing q can lead to a single equilibrium if cooperation is sufficiently large. Bistability indicates vulnerability to disturbance, so cooperation in manipulation may be beneficial when q , the co-transmission from the intermediate host to the definitive host, decreases. However, cooperation in manipulation may still harm the population by reducing p , the co-transmission from the parasite pool to the intermediate host.

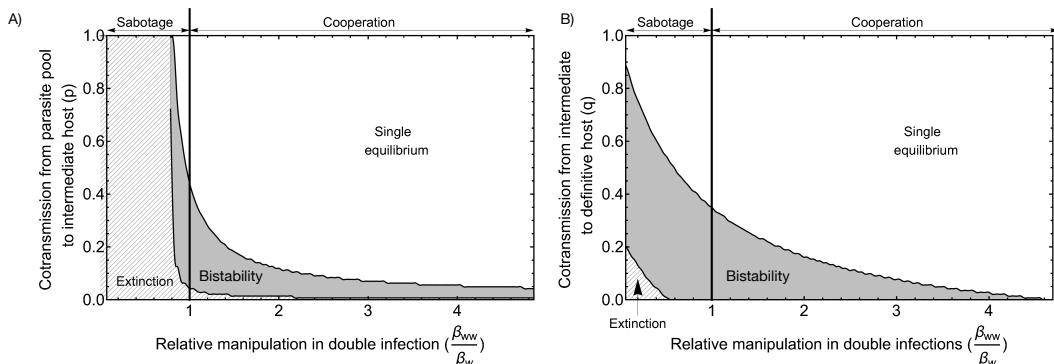


Figure 7: A) Effect of cotransmission from parasite pool to intermediate host. B) Effect of cotransmission from intermediate to the definitive host. On the left side of the vertical line, $\beta_{ww} > \beta_w$, indicating cooperation, whereas on the right side of the vertical line, $\beta_{ww} < \beta_w$,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

310 **Discussion & Conclusion**

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

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

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

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

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

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

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

373 **References**

- 374 Alizon, S., 2012. Parasite co-transmission and the evolutionary epidemiology of virulence.
375 Evolution 67:921–933. URL <https://doi.org/10.1111/j.1558-5646.2012.01827.x>.
- 376 Alizon, S. and M. van Baalen, 2008. Multiple infections, immune dynamics, and the evolution
377 of virulence. The American Naturalist 172:E150–E168. URL <https://doi.org/10.1086/590958>.
- 379 Alizon, S., J. C. de Roode, and Y. Michalakis, 2013. Multiple infections and the evolution of
380 virulence. Ecology Letters 16:556–567. URL <https://doi.org/10.1111/ele.12076>.
- 381 Allen, L. J. S., V. A. Bokil, N. J. Cunniffe, F. M. Hamelin, F. M. Hilker, and M. J. Jeger, 2019.
382 Modelling Vector Transmission and Epidemiology of Co-Infecting Plant Viruses. Viruses
383 11:1153. URL <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6950130/>.
- 384 van Baalen, M. and M. W. Sabelis, 1995. The dynamics of multiple infection and the
385 evolution of virulence. The American Naturalist 146:881–910. URL <https://doi.org/10.1086/285830>.

- 387 Benesh, D. P., 2016. Autonomy and integration in complex parasite life cycles. *Parasitology*
388 143:1824 – 1846.
- 389 Choisy, M. and J. C. de Roode, 2010. Mixed infections and the evolution of virulence: Effects
390 of resource competition, parasite plasticity, and impaired host immunity. *The American*
391 *Naturalist* 175:E105–E118. URL <https://doi.org/10.1086/651587>.
- 392 Diekmann, O., J. Heesterbeek, and J. Metz, 1990. On the definition and the computation
393 of the basic reproduction ratio r_0 in models for infectious diseases in heterogeneous
394 populations. *Journal of Mathematical Biology* 28. URL <https://doi.org/10.1007/bf00178324>.
- 396 Diekmann, O., J. A. P. Heesterbeek, and M. G. Roberts, 2009. The construction of next-
397 generation matrices for compartmental epidemic models. *Journal of The Royal Society*
398 *Interface* 7:873–885. URL <https://doi.org/10.1098/rsif.2009.0386>.
- 399 Fenton, A. and S. A. Rands, 2006. The impact of parasite manipulation and predator
400 foraging behavior on predator - prey communitites. *Ecology* 87:2832–2841. URL [https://doi.org/10.1890/0012-9658\(2006\)87\[2832:tiopma\]2.0.co;2](https://doi.org/10.1890/0012-9658(2006)87[2832:tiopma]2.0.co;2).
- 402 Gandon, S., 2018. Evolution and manipulation of vector host choice. *The American Naturalist*
403 192:23–34. URL <https://doi.org/10.1086/697575>.
- 404 Gao, D., T. C. Porco, and S. Ruan, 2016. Coinfection dynamics of two diseases in a single
405 host population. *Journal of Mathematical Analysis and Applications* 442:171–188. URL
406 <https://www.sciencedirect.com/science/article/pii/S0022247X16300841>.
- 407 Hadeler, K. P. and H. I. Freedman, 1989. Predator-prey populations with parasitic infec-
408 tion. *Journal of Mathematical Biology* 27:609–631. URL <https://doi.org/10.1007/bf00276947>.
- 410 Hafer, N. and M. Milinski, 2015. When parasites disagree: evidence for parasite-induced
411 sabotage of host manipulation. *Evolution* 69:611 – 620.

- 412 Hosack, G. R., P. A. Rossignol, and P. van den Driessche, 2008. The control of vector-borne
413 disease epidemics. *Journal of Theoretical Biology* 255:16–25. URL <https://doi.org/10.1016/j.jtbi.2008.07.033>.
- 415 Hughes, D. P., J. Brodeur, and F. Thomas, 2012. *Host Manipulation by Parasites*. Oxford
416 University Press, London, England.
- 417 Hurford, A., D. Cownden, and T. Day, 2010. Next-generation tools for evolutionary invasion
418 analyses. *Journal of The Royal Society Interface* 7:561–571.
- 419 Iritani, R. and T. Sato, 2018. Host-manipulation by trophically transmitted parasites: The
420 switcher-paradigm. *Trends in Parasitology* 34:934–944. URL <https://doi.org/10.1016/j.pt.2018.08.005>.
- 422 Kalbe, M., K. M. Wegner, and T. B. H. Reusch, 2002. Dispersion patterns of parasites in
423 0+ year three-spined sticklebacks: a cross population comparison. *Journal of Fish Biology*
424 60:1529–1542.
- 425 Lotka, A. J., 1920. Analytical note on certain rhythmic relations in organic systems. *Proceedings of the National Academy of Sciences* 6:410–415. URL <https://doi.org/10.1073/pnas.6.7.410>.
- 428 Molyneux, D. H. and D. Jefferies, 1986. Feeding behaviour of pathogen-infected vectors.
429 *Parasitology* 92:721–736.
- 430 Parker, G. A., J. C. Chubb, G. N. Roberts, M. Michaud, and M. Milinski, 2003. Optimal
431 growth strategies of larval helminths in their intermediate hosts. *Journal of Evolutionary
432 Biology* 16:47–54. URL <https://doi.org/10.1046/j.1420-9101.2003.00504.x>.
- 433 Ripa, J. and U. Dieckmann, 2013. Mutant invasions and adaptive dynamics in variable
434 environments. *Evolution* 67:1279–1290. URL <https://onlinelibrary.wiley.com/doi/abs/10.1111/evo.12046>.

- 436 Rogawa, A., S. Ogata, and A. Mougi, 2018. Parasite transmission between trophic levels
437 stabilizes predator–prey interaction. *Scientific Reports* 8. URL <https://doi.org/10.1038/s41598-018-30818-7>.
- 439 Rogers, M. E. and P. A. Bates, 2007. Leishmania manipulation of sand fly feeding behavior
440 results in enhanced transmission. *PLoS Pathogens* 3:e91. URL <https://doi.org/10.1371/journal.ppat.0030091>.
- 442 Roosien, B. K., R. Gomulkiewicz, L. L. Ingwell, N. A. Bosque-Pérez, D. Rajabaskar, and
443 S. D. Eigenbrode, 2013. Conditional vector preference aids the spread of plant pathogens:
444 Results from a model. *Environmental Entomology* 42:1299–1308. URL <https://doi.org/10.1603/en13062>.
- 446 Seppälä, O. and J. Jokela, 2008. Host manipulation as a parasite transmission strategy
447 when manipulation is exploited by non-host predators. *Biology Letters* 4:663–666. URL
448 <https://doi.org/10.1098/rsbl.2008.0335>.
- 449 Vickery, W. L. and R. Poulin, 2009. The evolution of host manipulation by parasites: a
450 game theory analysis. *Evolutionary Ecology* 24:773–788. URL <https://doi.org/10.1007/s10682-009-9334-0>.
- 452 Wedekind, C. and M. Milinski, 1996. Do three-spined sticklebacks avoid consuming cope-
453 pods, the first intermediate host of *Schistocephalus solidus*? - an experimental analysis
454 of behavioural resistance. *Parasitology* 112:371–383. URL <https://doi.org/10.1017/s0031182000066609>.
- 456 Wilson, K., B. T. Grenfell, and D. J. Shaw, 1996. Analysis of Aggregated Parasite Distribu-
457 tions: A Comparison of Methods. *Functional Ecology* 10:592.
- 458 Zimmer, C., 2001. *Parasite Rex: Inside the Bizarre World of Nature's Most Dangerous*
459 *Creatures*. Atria Books.