

On multiple infections by parasites with complex life cycles

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

24 **Introduction**

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

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

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

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



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

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

69 considered (Alizon and van Baalen, 2008), and a reduction in virulence is possible if parasites
70 are co-transmitted (Alizon, 2012). As host manipulation is suggested to affect transmission
71 rate, these studies also involve host manipulation to a certain extent even though they do
72 not explicitly consider the trait. Host manipulation in trophically transmitted parasite receive
73 less attention. Although manipulation is correlated with transmission rate in both trophically
74 transmitted parasites and infectious disease, there are subtlely difference. Host manipulation
75 influences the predation rate in trophically transmitted parasites, predominantly affecting
76 predator-prey dynamics. Theoretical studies on host manipulation in trophically transmitted
77 parasites with multiple infections are rare (Parker et al., 2003; Vickery and Poulin, 2009).
78 Moreover, they do not consider the prey-predator dynamics, which will likely have important
79 feedback on the evolution of host manipulation. A few studies considering the prey-predator
80 dynamics do not incorporate multiple infections (Rogawa et al., 2018; Iritani and Sato, 2018;
81 Hadeler and Freedman, 1989; Fenton and Rands, 2006). More importantly, they assume that
82 transmission from definitive hosts to intermediate hosts is due to direct contact between the
83 two types of hosts. This is often not the case, as parasites are released from the definitive
84 hosts into the environment. Transmission happens only when intermediate hosts have
85 contact with this free-living parasite pool.

86 Our study addresses the gap in the theoretical work on host manipulation in trophically
87 transmitted parasites. We include multiple infections and consider the dynamics of the free-
88 living parasite pool. Our compartment model helps illustrate a parasite's complex lifecycle
89 with two hosts: an intermediate host preyed upon by a definitive host. Transmission from the
90 intermediate host to the definitive host occurs when predation on infected intermediate hosts
91 happens. Reproduction only happens in the definitive hosts. New parasites are then released
92 into the environment, where they again have contact with the intermediate hosts to complete
93 their lifecycle. We focus on the manipulation of the intermediate hosts, such that the parasite
94 increases the predation rate on the intermediate host by the definitive host to increase
95 its transmission rate. We then analyse the effect of host manipulation on the ecological
96 dynamics in the prey-predator-parasite system. In contrast to the examples mentioned above
97 our model consists of a single intermediate host as it already provides enough complexity
98 to discuss between transmission and manipulation. We found that cooperation in host
99 manipulation leads to bistability in the predator-prey system, given that reproduction from
100 multiple infections is sufficiently high. This finding suggests that the predator-prey parasite
101 system is finely balanced and susceptible to extinction via ecological disturbances. Initially
102 surprising, we show that cooperation in multiple infections is not always beneficial and might
103 make the parasite population vulnerable to extinction.

104 **Model and Results**

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

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

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

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

133 For the definitive hosts we have,

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

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 force of infection that corresponds respectively to singly infected intermediate host (I_w)
137 and doubly infected intermediate hosts (I_{ww}) is denoted respectively by $\lambda_w = h_1(\rho + \beta_w)I_w$
138 and $\lambda_{ww} = h_2(\rho + \beta_{ww})I_{ww}$, where ρ is the based line predation rate and h_1 and h_2
139 are the probability that the parasite successfully established inside the host. If there is no
140 manipulation, that is, $\beta_w = \beta_{ww} = 0$, the parasite is still transmitted via the based line
141 predation. The dynamics of the free-living parasites in the environment are then given by,

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

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

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

152 Basic reproduction ratio R_0 of the parasites

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

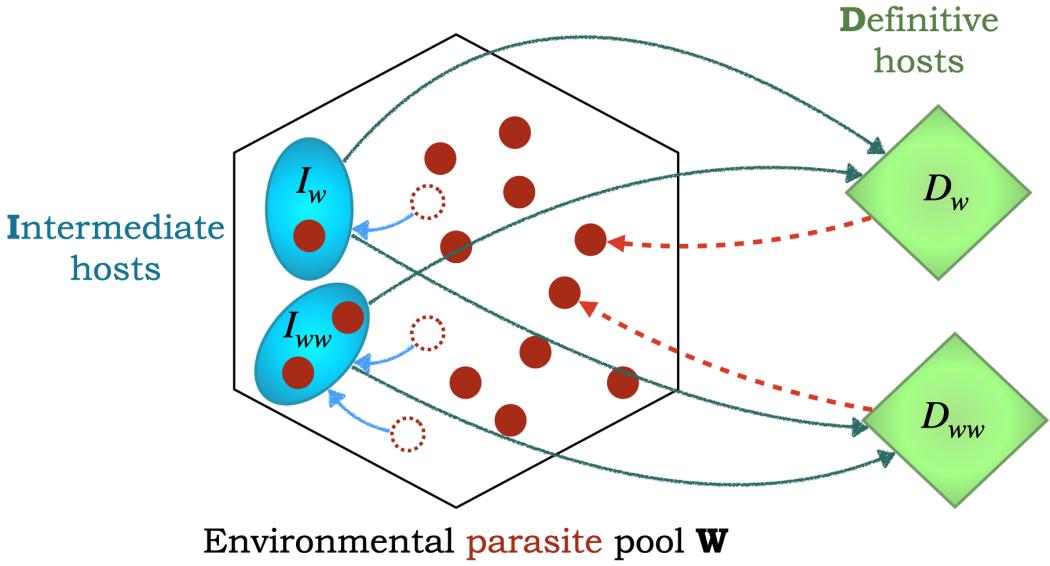


Figure 2: Schematic of the model. Blue ovals represent intermediate host compartment, green diamonds represent definitive host compartment, and the transparent hexagon represents the parasite pool compartment with red circles illustrating individual parasites.

¹⁵⁷ 2009; Hurford et al., 2010) (See SI1 for details).

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

¹⁵⁸ where I_s^* and D_s^* are the densities of susceptible intermediate and definitive hosts at the
¹⁵⁹ disease-free equilibrium. Here, the expression of R_0 contains the possible reproduction routes
¹⁶⁰ of a parasite, which can be via double or single infections. The first component corresponds
¹⁶¹ to the double infections route, in which the focal parasite co-transmits with another par-
¹⁶² asite into a susceptible intermediate host, then co-transmits into a susceptible definitive
¹⁶³ host and reproduces. Here, parasites are so rare that only co-transmission matters and the
¹⁶⁴ compartments with sequential infections are therefore neglected. The second component
¹⁶⁵ corresponds to the single infection route, wherein the focal parasite infects a susceptible
¹⁶⁶ intermediate host via single or double infections. The parasite then transmits alone into the

167 susceptible definitive host and eventually reproduces.

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

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

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

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

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

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

191 The explicit form of I_s^* and D_s^* , capturing the predator-prey dynamics, depends on the
192 precise form of all birth and predation functions B, R, P_s, P_w and P_{ww} . But, it does not
193 depend on the manipulation ability or any other parameter of the parasite. Given that the
194 birth rate of the predator and the predation rate are linear functions in prey and predator

195 density, the form of the birth rate R of the prey has a significant effect on the susceptible
196 intermediate and definitive host dynamics.

197 **Birth function of intermediate hosts**

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

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

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

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

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

218 This equilibrium is positive and stable if components of the parasite, such as reproduction
219 and transmission are sufficiently small, details of the condition can be found in SI4 (Figure
220 4B).

221 When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio
222 $R_0 > 1$. Since the expression is complicated, we could not obtain analytical solutions for
223 this inequality without assumptions. We assume the same parasite virulence, $\alpha_w = \alpha_{ww}$,

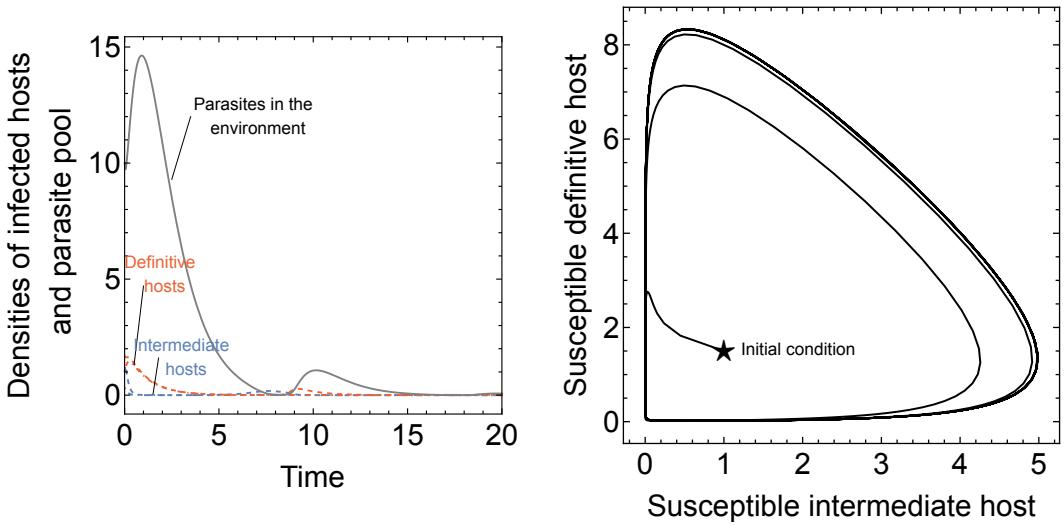


Figure 3: Disease-free equilibrium using linear birth function, where parasite goes extinct (left panel), and susceptible hosts demonstrate cyclic dynamics (right panel). Solid gray line indicate the density of free-living parasites, blue lines indicate infected intermediate hosts while red lines indicate infected definitive hosts. Dashed lines indicate singly infected hosts while dot-dashed lines indicate doubly infected hosts. Parameter values $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = \alpha_{ww} = 0$, $\beta_w = 1.5$, $\beta_{ww} = 1.5$, $p = 0.1$, $c = 1.4$, $\mu = 0.9$, $\sigma_w = \sigma_{ww} = 0$, $q = 0.01$, $f_w = 6.5$, $f_{ww} = 7.5$, $\delta = 0.9$, $h_1 = h_2 = 0.8$, $R_0 = 4.997$

224 $\sigma_w = \sigma_{ww}$, and reproduction in double infection as a linear function concerning reproduction
225 in single infections, $f_{ww} = \epsilon f_w$. When $\epsilon > 1$, reproduction in double infections is greater
226 than reproduction in a single infection, whereas $\epsilon \leq 1$, reproduction in double infections is
227 lower or equal to reproduction in a single infection. We found that the parasite can establish
228 if its reproduction value in a single infection f_w is more significant than a threshold (Figure
229 5, see S15).

230 Our numerical results show that the parasite reproduction is substantial compared to other
231 parameters (its value is nearly 40 times greater than other parameters). This observation
232 suggests that trophically transmitted parasites must release many offspring into the environ-
233 ment to persist. Interestingly, bistability occurs if the reproduction rate of the parasite in
234 double infections is greater than in the single infection state (Figure 5A, B). In the bistable
235 region, the parasite population can reach a stable equilibrium if the initial density is large
236 enough. In contrast, with sufficient disturbance, the parasite population could go extinct.

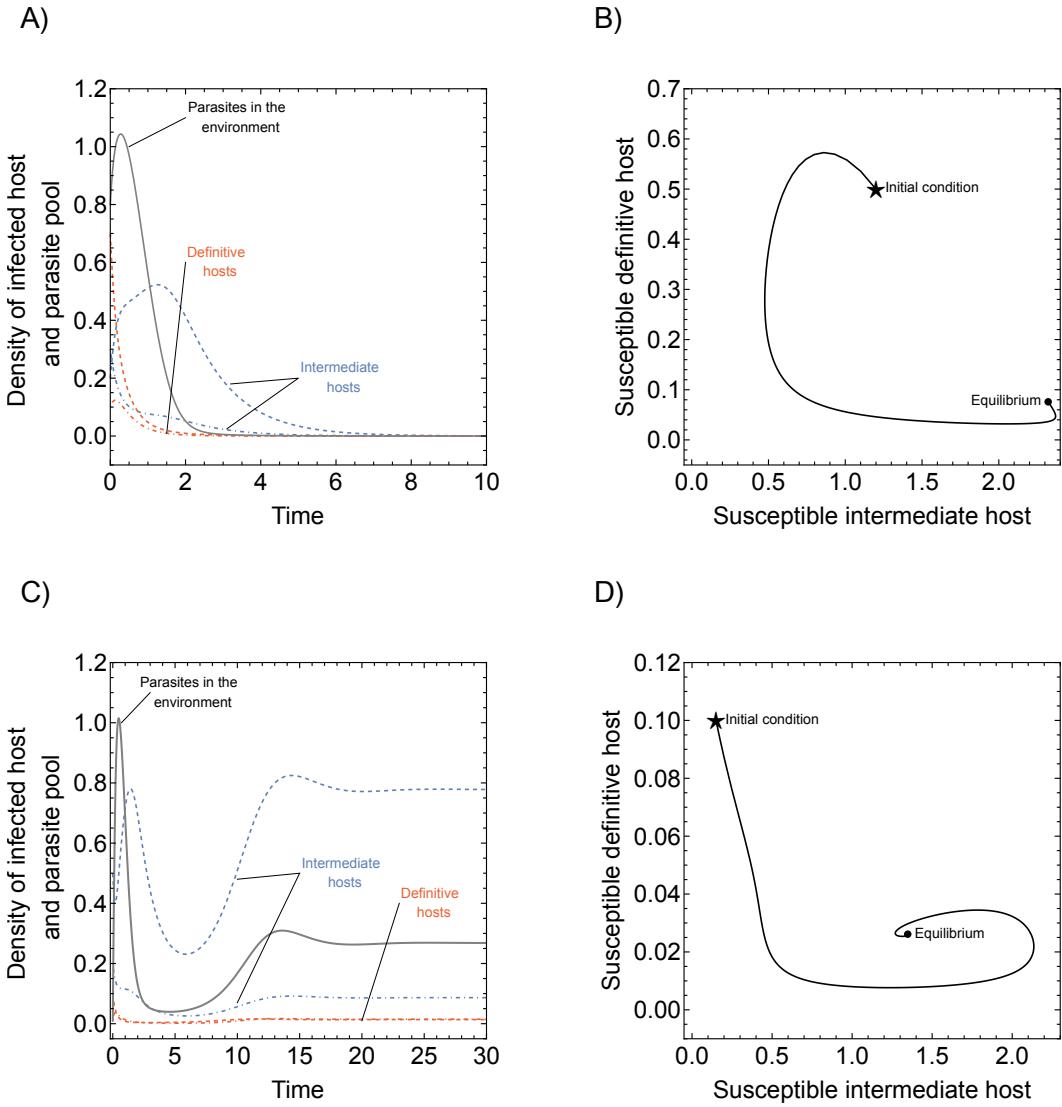


Figure 4: A, B) Disease free equilibrium where parasite cannot persist. C, D) Disease stable equilibrium. Annotations are the same in figure 3. Parameters for disease free equilibrium $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = \alpha_{ww} = 0$, $\beta_w = \beta_{ww} = 1.5$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = \sigma_{ww} = 0$, $q = 0.05$, $f_w = f_{ww} = 7.5$, $\delta = 0.9$, $k = 0.26$, $h_1 = h_2 = 0.8$. Disease stable equilibrium have the same parameter values except for higher host manipulation $\beta_w = \beta_{ww} = 4.5$ and parasite reproduction $f_w = f_{ww} = 45$

237 The effect of host manipulation on ecological dynamics

238 Host manipulation can be cooperative; two parasites increase the predation rate on interme-
 239 diate hosts, or $\beta_{ww} > \beta_w$. However, it can also be uncooperative; that is, the predation rate

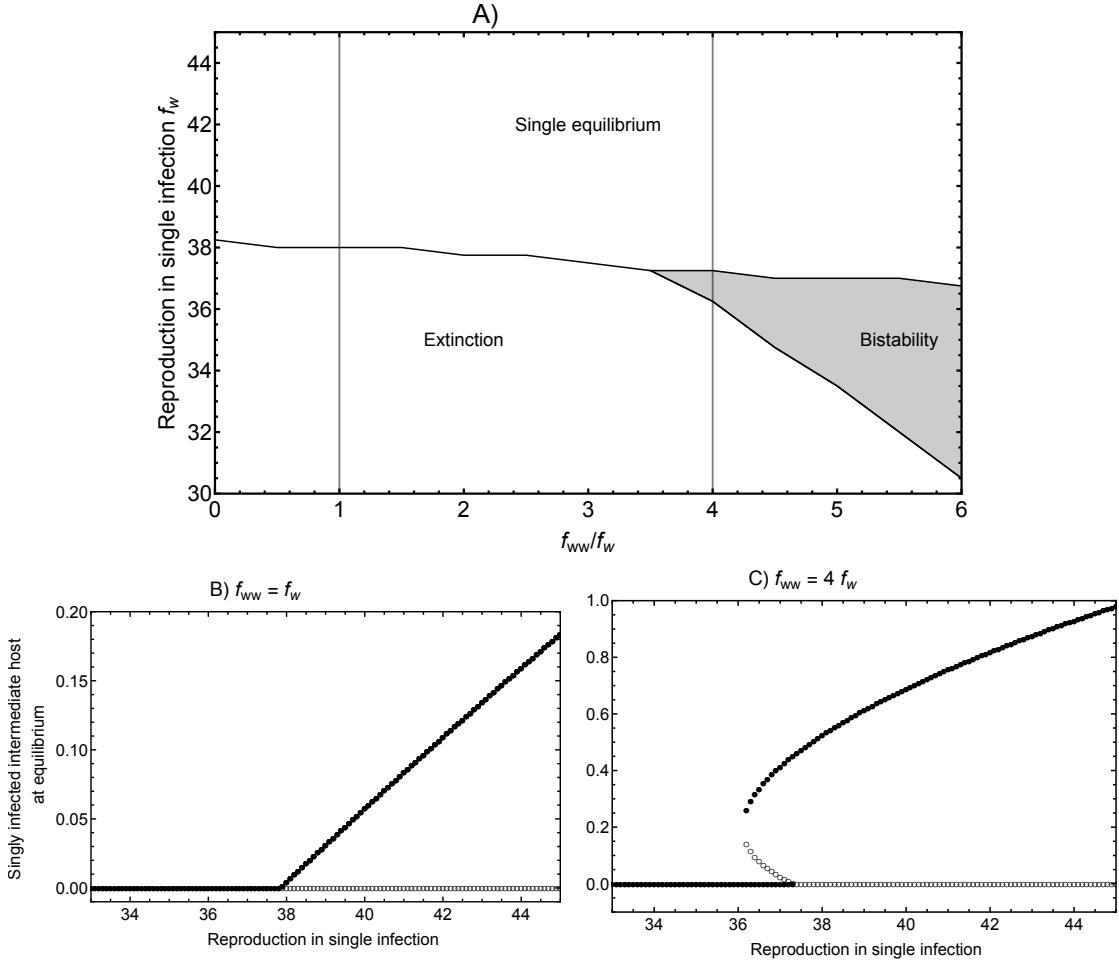


Figure 5: Effect of parasite reproduction on the ecological dynamics. A) Increasing reproduction in doubly infection leads to bistability, B, C) Density of singly infected host at equilibrium when reproduction of parasites are the same in singly and doubly infected hosts $f_{ww} = f_w$, and when reproduction of parasites in doubly infected hosts is four times greater than those in singly infected hosts $f_{ww} = 4 f_w$. Filled circles indicate stable equilibrium and open circles indicate unstable equilibrium. Parameter $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_w w = 0$, $\beta_w = 1.5$, $\beta_{ww} = 1.5$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $h_1 = h_2 = 0.6$

on doubly-infected intermediate hosts lower than that on singly-infected ones, or $\beta_{ww} < \beta_w$. Cooperation in parasite manipulation does increases the parasite's basic reproduction ratio R_0 but the manipulation in single infection has a stronger effect on the value of R_0 (Figure 6). Intuitively, if the manipulation in single infection is small, there is not enough transmission and the parasite goes extinct. However, suppose the ability to manipulate the host in a

245 single infection is just enough for the parasite population to escape extinction but not strong.
246 In that case, cooperation in host manipulation leads to a bistable state of the system. Within
247 the bistable region, the basic reproduction ratio can be less than one, suggesting that the
248 parasite cannot spread when its manipulative values are within this area of weak manipulation
249 when coinfecte

250 Cooperation between parasites need not be limited to host manipulation. Parasites can
251 cooperate to have a higher reproduction rate in co-infections, i.e. $f_{ww} > f_w$. Likewise,
252 they can compete for resources, so reproduction in double infection is smaller than in single
253 infection. Without any assumption on the relationship between manipulative ability and
254 reproduction, we explore all possible combinations of cooperation and sabotage in both
255 manipulation and reproduction. If parasites are uncooperative in both reproduction and
256 manipulation, they can not persist (Figure 7). In contrast, if they are highly cooperative
257 in both aspects (i.e. $f_{ww}/f_w^- > \infty$ and $\beta_{ww}/\beta_w^- > \infty$), there is one guaranteed single
258 equilibrium for parasite existence.

259 For intermediate cooperation of both reproduction and manipulation, the bistable area
260 could occur. However, the size of this area is highly sensitive to the value of reproduction
261 and manipulation in single infection. In particular, higher values of these two parameters re-
262 duce the bistability area, whereas larger values increase the bistability area (Figure 7, Figure
263 SI1). If the parasites sabotage in host manipulation, the system is highly prone to bistability,
264 and only has a single equilibrium when cooperation in reproduction is extremely high. Inter-
265 estingly, sufficiently high cooperation in reproduction leads to bistability (i.e. f_{ww} is at least
266 4 times f_w), and noncooperation in reproduction always lead to single equilibrium of the
267 system (Figure 7). While a single equilibrium guarantees existence of parasite population,
268 bistability indicates that a disturbance of the system may likely lead to extinction of parasite.
269 This suggests that whether or not cooperation benefits the population is context dependent.
270 Clearly, if there is no limitation to cooperation, and if reproduction or manipulation in single
271 infection is large enough, cooperation always hold an advantage. On the other hand, co-
272 operation in one aspect or insufficient cooperation in both aspects may make the parasite
273 population more vulnerable to extinction than systems with less cooperative parasites.

274 Co-transmission probability from the parasite pool to intermediate hosts p has opposite
275 effect on the bistable area compared to co-transmission probability q from intermediate hosts
276 to intermediate hosts (Figure 8). In particular, when parasite sabotage the manipulation,
277 increasing p enlarge the bistable area whereas increasing q reduce it. In contrast, when
278 parasites cooperate in manipulation, reducing p decreases the bistable area while reducing
279 q widen it. Evidently, if cooperation in manipulation is extremely high then regardless of
280 the co-transmission value, the population will always exist with one single stable equilibrium.

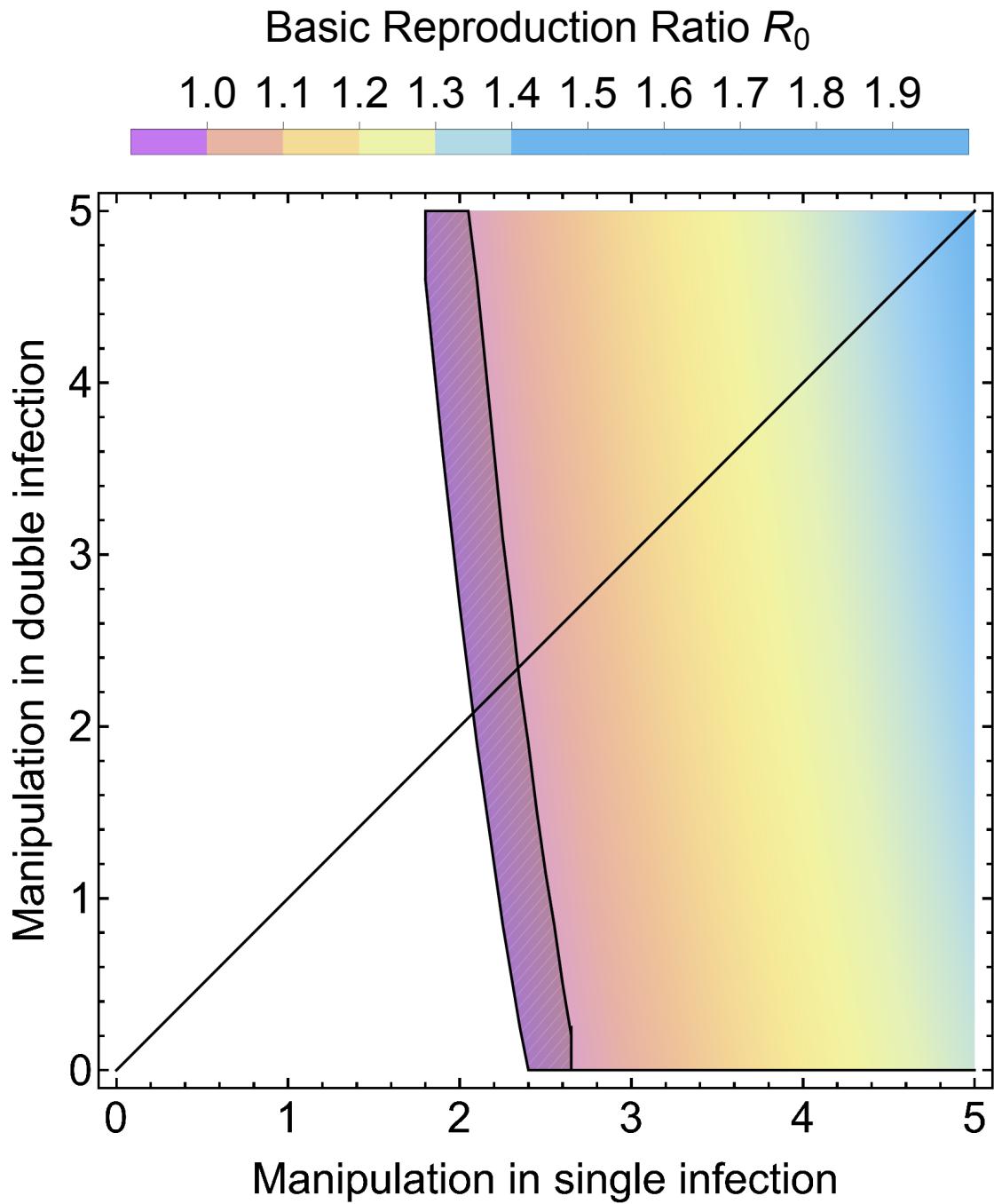


Figure 6: R_0 values increase with more efficient manipulation in both single and double infection. The parasite goes extinct if its manipulative ability is insufficient (white area). Bistability region occurs when cooperation in manipulation is intermediate (hatched area). As manipulation in single infection increases, the system only has one stable equilibrium. On the black line, manipulation is indifference between single infection and double infection ($\beta_w = \beta_{ww}$). Other parameters are the same as in Figure 6. $\epsilon = 4.5$, $f_w = 30$

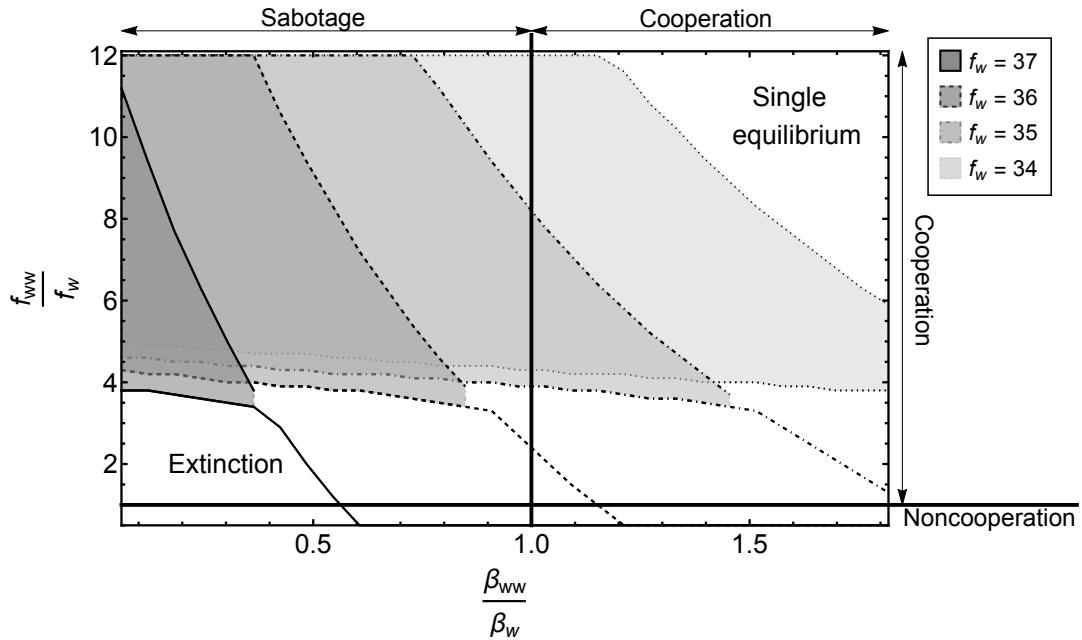


Figure 7: Changes of the bistability area (shaded areas) with respect to different reproduction rates in single infection (different boundary styles). Manipulation and reproduction is indifference between single infection and double infection on the vertical and horizontal lines respectively. Common parameter: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $\beta_w = 1.65$, $h_1 = h_2 = 0.6$.

281 However, as there are always limitation and trade-off in nature, extremely high values may
 282 not possible. Considering bistability indicates vulnerability to disturbance, this suggests that
 283 cooperation in manipulation may be beneficial when the co-transmission from the pool to
 284 intermediate host increases. However, cooperation in manipulation may be harmful to the
 285 population when the co-transmission from intermediate host to definitive host increases.

286 Discussion & Conclusion

287 Host manipulation is a ubiquitous phenomenon suggested to affect the prey-predator dynam-
 288 ics in trophically transmitted parasites. In particular, manipulation of infected intermediate
 289 hosts to increase the predation rate of definitive hosts may result in a heavy burden of preda-
 290 tors on the intermediate host population. This pressure can make parasites more vulnerable
 291 to extinction (Hadeler and Freedman, 1989; Fenton and Rands, 2006).

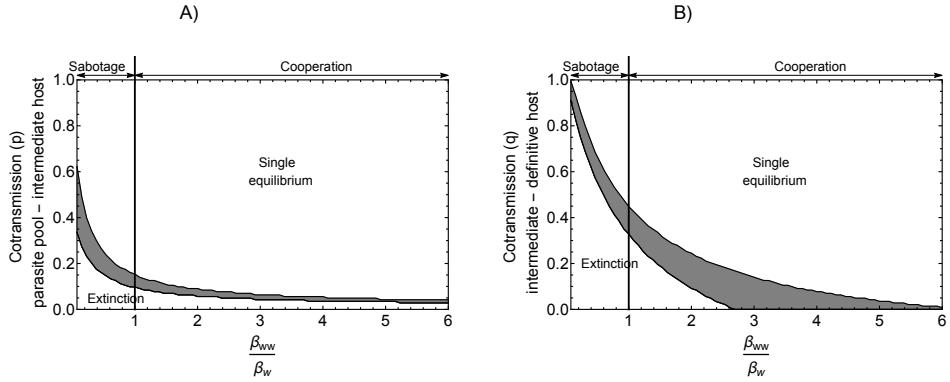


Figure 8: Common parameter: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $\epsilon = 4.5$, $\beta_w = 1.3$, $f_w = 35$, $h_1 = h_2 = 0.6$.

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. 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 causes the system to cycle. The system is stabilised when the parasite becomes manipulative, and the stability increases with the manipulative ability. In our model, non-manipulative parasites cannot persist in the system, and the parasite never leads the system into cyclic dynamics. These results may contradict with [Rogawa et al. \(2018\)](#), where non-manipulative parasites lead to a stable system, while increasing manipulation induces the cyclic behaviour of the population. We suggest that the different results may be due to our introduction of a parasite pool and multiple infections, unlike the model of [Rogawa et al. \(2018\)](#). In their system, transmission from the definitive host to the intermediate host was assumed to result from direct contact between the two hosts. Such immediate transmission could directly accelerate the feedback loop between prey and predator. Hence, faster predator-prey dynamics occur, which may lead to cyclic dynamics when parasites are introduced.

In our study, population dynamics exhibit bistability under certain circumstances. This

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

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

337 Finally, our study focuses on the ecological dynamics of the trophically transmitted para-
338 site. However, investigating the evolution of host manipulation is a natural extension beyond
339 the scope of a single manuscript, given the complexities that arise in the ecological dynam-
340 ics itself. Studying evolution of host manipulation taking into account free-living parasite
341 pool calls for thorough analyses, which itself could be a standalone study. In addition, the
342 occurrence of bistability in our model suggests that the evolution of host manipulation may
343 drive the parasite population to extinction simply because of the scarcity of the mutant and
344 the Allee effect in the population dynamics. The parasite can enhance both values if there
345 is no tradeoff between manipulation and reproduction. Nevertheless, our model shows that
346 this strategy, which seems to make the best of both worlds, can make the system even more
347 unstable. Evolutionary dynamics here depend mainly on the tradeoff between host manipu-
348 lation and other traits of the parasites, such as reproduction, virulence, and survivorship in
349 the parasite pool, to list a few. This extension deserves thorough analysis, and we will treat
350 it as a separate matter.

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352 Removed for review

353 **Statement of Authorship**

354 Removed for review

355 **Data and Code Availability**

356 All data and simulation codes for generating figures are available on <https://anonymous.4open.science/r/multipleinfections>

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443 **Tables**

Table 1: Description of variables and parameters

Parameters and Variables	Description
I_i	Density of intermediate hosts that are susceptible $i = s$, singly infected $i = w$, or doubly infected $i = ww$
D_i	Density of definitive hosts that are susceptible $i = s$, singly infected $i = w$, or doubly infected $i = ww$
W	Density of parasites released from definitive hosts into the environment
d	Natural death rate of intermediate hosts
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