

On multiple infections by parasites with complex life cycles

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

24 **Introduction**

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

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

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

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



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

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

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

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

101 **Model and Results**

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

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

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

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

130 For the definitive hosts we have,

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

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

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

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

140 Here, we focus on manipulation that enhances transmission from intermediate hosts to
141 definitive hosts; we thus simplify the transmission from the parasite pool to intermediate
142 hosts such that no sequential infection occurs at this transmission state. Sequential infection
143 can happen when parasites transmit from intermediate hosts to definitive hosts. Therefore,
144 a singly infected definitive host can be further infected by another parasite if it consumes
145 infected intermediate hosts. The system's dynamics are illustrated in figure (2).

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

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

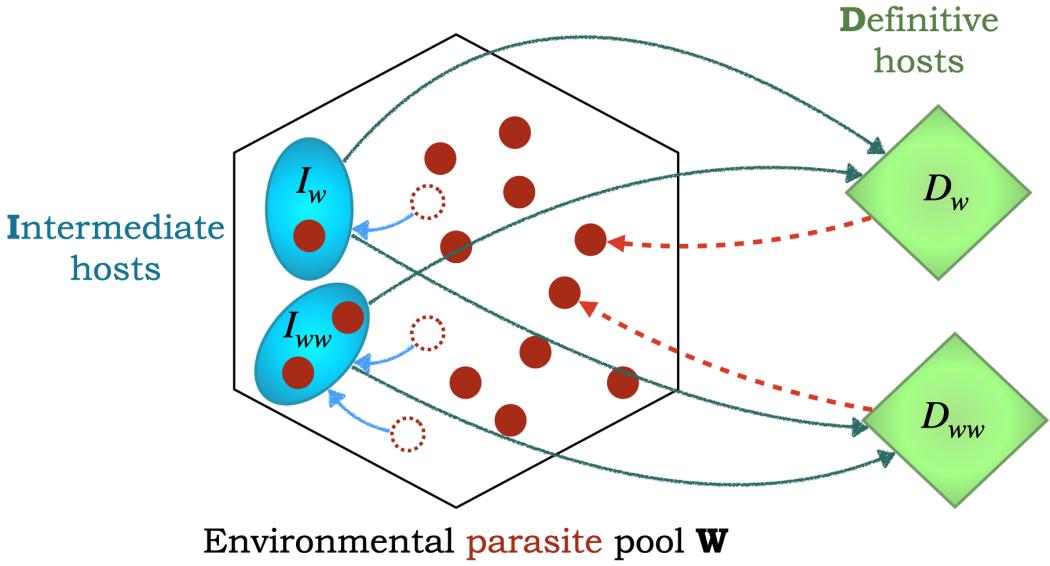


Figure 2: Schematic of the model. Blue ovals represent intermediate host compartment, green diamonds represent definitive host compartment, and the transparent hexagon represents the parasite pool compartment with red circles illustrating individual parasites.

¹⁵¹ 2009; Hurford et al., 2010) (See SI1 for details).

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

¹⁵² where I_s^* and D_s^* are the densities of susceptible intermediate and definitive hosts at the
¹⁵³ disease-free equilibrium. Here, the expression of R_0 contains the possible reproduction routes
¹⁵⁴ of a parasite, which can be via double or single infections. The first component corresponds
¹⁵⁵ to the double infections route, in which the focal parasite co-transmits with another par-
¹⁵⁶ asite into a susceptible intermediate host, then co-transmits into a susceptible definitive
¹⁵⁷ host and reproduces. Here, parasites are so rare that only co-transmission matters and the
¹⁵⁸ compartments with sequential infections are therefore neglected. The second component
¹⁵⁹ corresponds to the single infection route, wherein the focal parasite infects a susceptible
¹⁶⁰ intermediate host via single or double infections. The parasite then transmits alone into the

161 susceptible definitive host and eventually reproduces.

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

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

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

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

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

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

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

188 **Birth function of intermediate hosts**

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

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

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

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

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

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

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

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

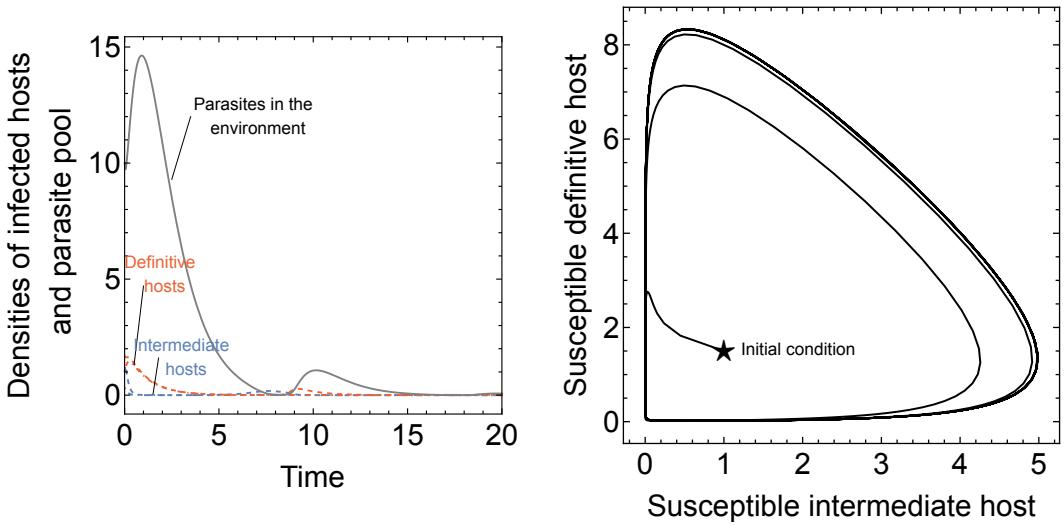


Figure 3: Disease-free equilibrium using linear birth function, where parasite goes extinct (left panel), and susceptible hosts demonstrate cyclic dynamics (right panel). Solid gray line indicate the density of free-living parasites, blue lines indicate infected intermediate hosts while red lines indicate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

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

When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio $R_0 > 1$. Since the expression is complicated, we could not obtain analytical solutions for this inequality without assumptions. We assume the same parasite virulence, $\alpha_w = \alpha_{ww}$, $\sigma_w = \sigma_{ww}$, and reproduction in double infection as a linear function concerning reproduction in single infections, $f_{ww} = \epsilon f_w$. When $\epsilon > 1$, reproduction in double infections is greater than reproduction in a single infection, whereas $\epsilon \leq 1$, reproduction in double infections is lower or equal to reproduction in a single infection. We found that the parasite can establish if its reproduction value in a single infection f_w is more significant than a threshold (Figure 5, see SI3).

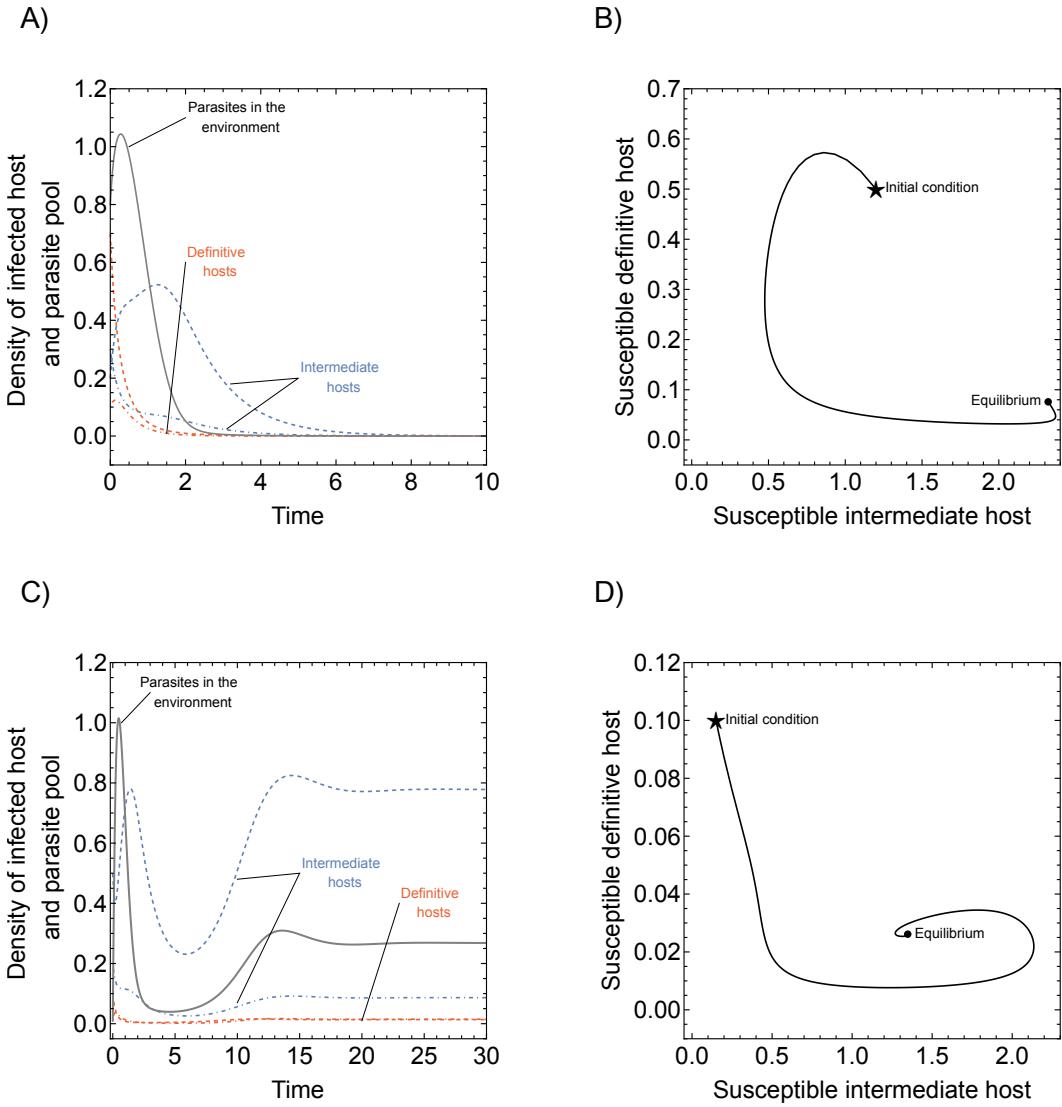


Figure 4: A, B) Disease free equilibrium where parasite cannot persist. C, D) Disease stable equilibrium. Annotations are the same in figure 3. Parameters for disease free equilibrium $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = \alpha_{ww} = 0$, $\beta_w = \beta_{ww} = 1.5$, $p =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

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

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

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

230 Host manipulation can be cooperative; two parasites increase the predation rate on interme-
231 diate hosts, or $\beta_{ww} > \beta_w$. However, it can also be uncooperative; that is, the predation rate
232 on doubly-infected intermediate hosts lower than that on singly-infected ones, or $\beta_{ww} < \beta_w$.
233 Cooperation in parasite manipulation does increases the parasite's basic reproduction ratio
234 R_0 but the manipulation in single infection has a stronger effect on the value of R_0 (Figure
235 6). However, suppose the ability to manipulate the host in a single infection is not strong
236 enough. In that case, cooperation in host manipulation leads to a bistable state of the sys-
237 tem. Within the bistable region, the basic reproduction ratio can be less than one, suggesting
238 that the parasite cannot spread when its manipulative values are within this area of weak
239 manipulation when coinfecte.

240 Cooperation between parasites need not be limited to host manipulation. Parasites can
241 cooperate to have a higher reproduction rate in co-infections, i.e. $f_{ww} > f_w$. Likewise,
242 they can compete for resources, so reproduction in double infection is smaller than in sin-
243 gle infection. Without any assumption on the relationship between manipulative ability and
244 reproduction, we explore all possible combinations of cooperation and sabotage in both ma-
245 nipulation and reproduction. Intuitively, if parasites are uncooperative in both reproduction
246 and manipulation, they can not persist. In contrast, if they are highly cooperative in both
247 aspects, that is, reproduction and manipulation in double infections are much higher than in
248 single infection, there is one guaranteed single equilibrium for parasite existence. For interme-
249 diate cooperation of both reproduction and manipulation, there exist the area of bistability,
250 whose size is sensitive to the reproduction value in single infection. In particular, a higher
251 value of reproduction on single infection lead to smaller bistability area. If the parasites only
252 sabotage host manipulation, the system is highly prone to bistability, and only has a single
253 equilibrium when cooperation in reproduction is extremely high (i.e. reproduction in double
254 infections is much higher than reproduction in single infection). Interestingly, noncooperative
255 in reproduction always lead to single equilibrium of the system (Figure 7). Bistability area
256 indicates that a disturbance of the system may likely lead to extinction of parasite. This
257 suggests that, if the reproduction in single infection is sufficiently small, systems in which
258 parasites cooperate in both reproduction and manipulation may be more vulnerable to ex-
259 tinction than systems with less cooperative parasites. In other words, having the best of
260 both worlds, effective manipulation and reproduction, at the individual level may not benefit
261 the population as a whole.

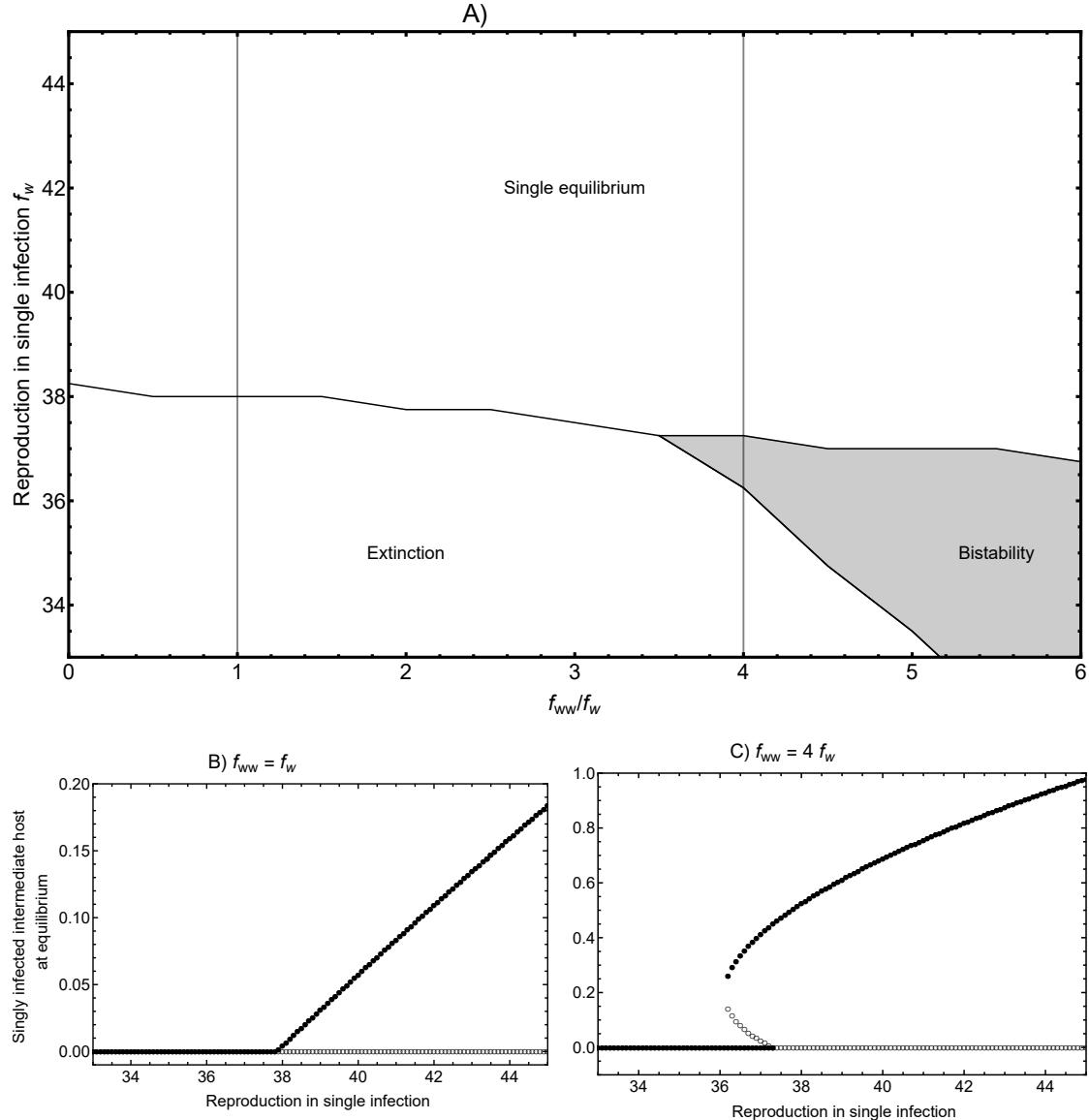


Figure 5: Effect of parasite reproduction on the ecological dynamics. A) Increasing reproduction in doubly infection leads to bistability, B, C) Density of singly infected host at equilibrium when reproduction of parasites are the same in singly and doubly infected hosts $f_{ww} = f_w$, and when reproduction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

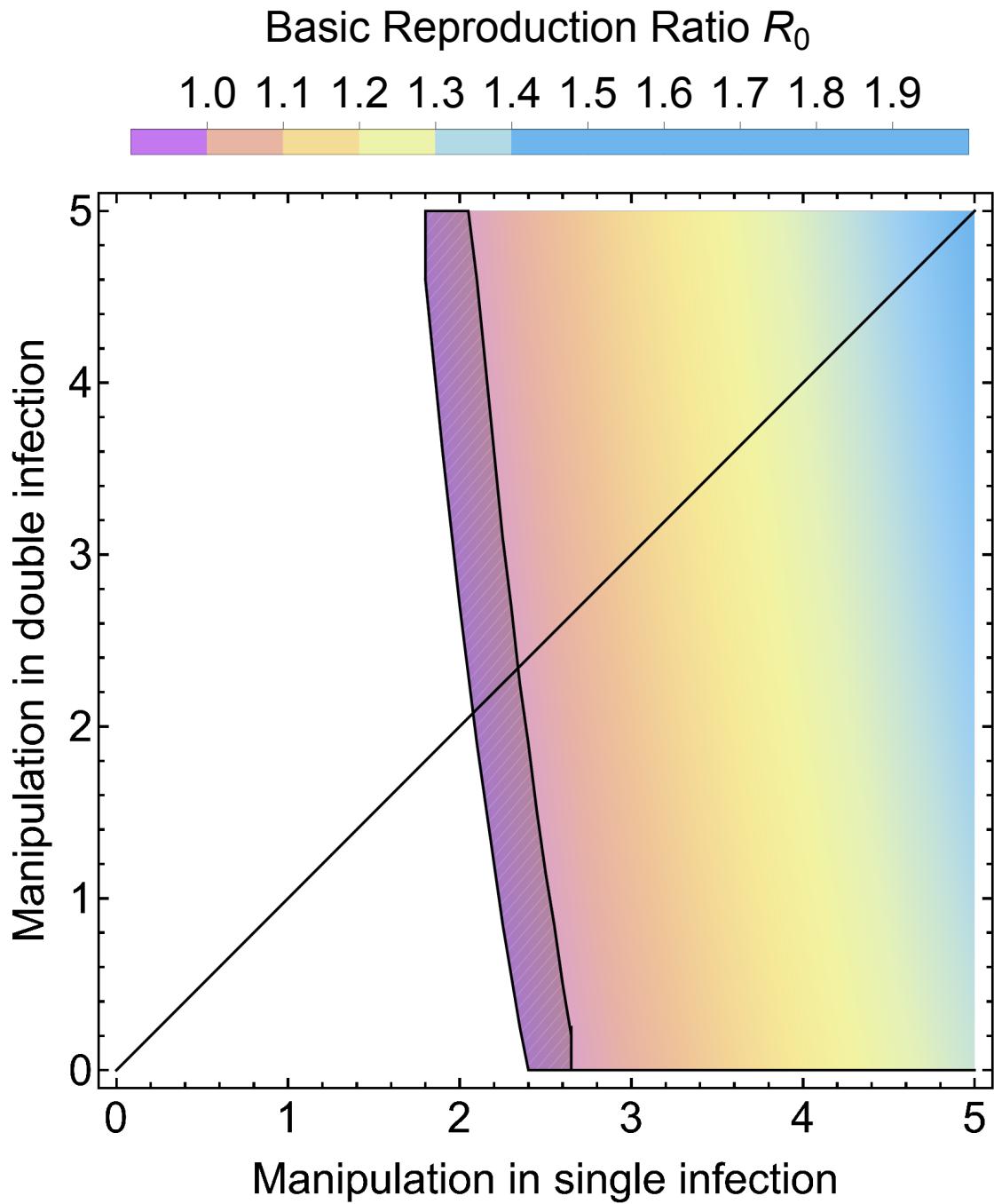


Figure 6: R_0 values increase with more efficient manipulation in both single and double infection. The parasite goes extinct if its manipulative ability is insufficient (white area). Bistability region occurs when cooperation in manipulation is intermediate (hatched area). As manipulation in single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

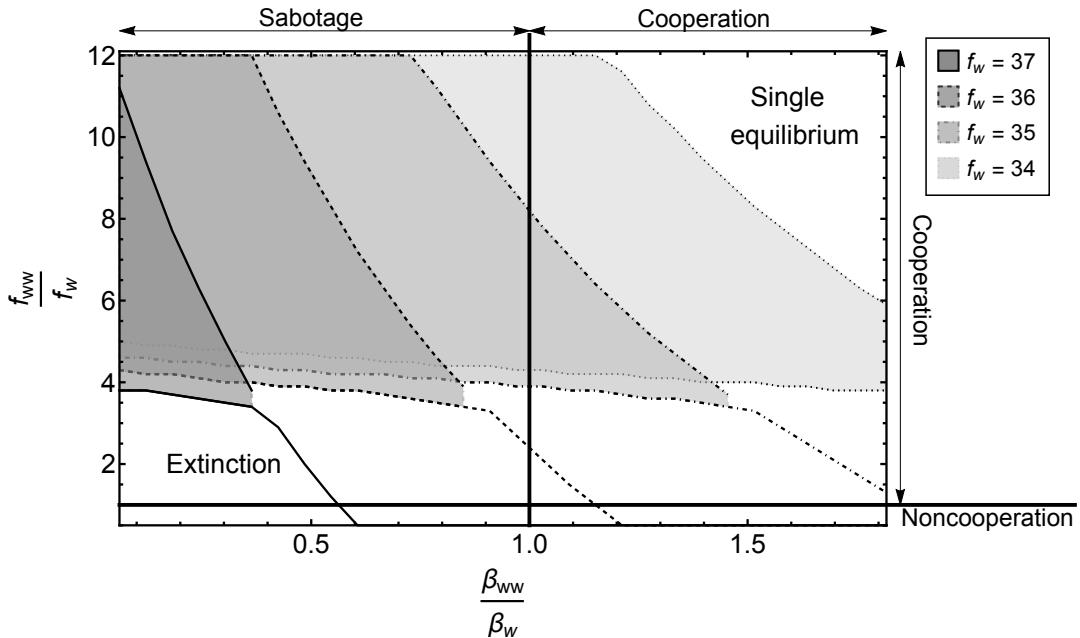


Figure 7: Changes of the bistability area (shaded areas) with respect to different reproduction rates in single infection (different boundary styles). Manipulation and reproduction is indifference between single infection and double infection on the vertical and horizontal lines respectively. Common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

Increasing the co-transmission probability p from the parasite pool to intermediate hosts reduces the extinction area. When p is high, doubly infected intermediate hosts are more abundant. Cooperation in host manipulation then need not be too high to bring the population out of the bi-stability state. However, it also means that the singly infected intermediate hosts are few and parasites in a single infection must make more manipulative effort to successfully transmit (Figure 7B). On the other hand, increasing the co-transmission probability q from intermediate hosts to definitive hosts broadens the extinction area. When q is high, successful transmission to definitive hosts relies on the predation of susceptible definitive hosts on doubly infected intermediate hosts. Cooperation in manipulation, therefore, needs to be sufficiently high to avoid bi-stability. Sequential transmission is also rarer because the probability of a single infection $1 - q$ is low. Suppose the number of doubly infected intermediate hosts is low. In that case, general transmission from intermediate hosts to definitive hosts is limited, which explains the wide extinction area.

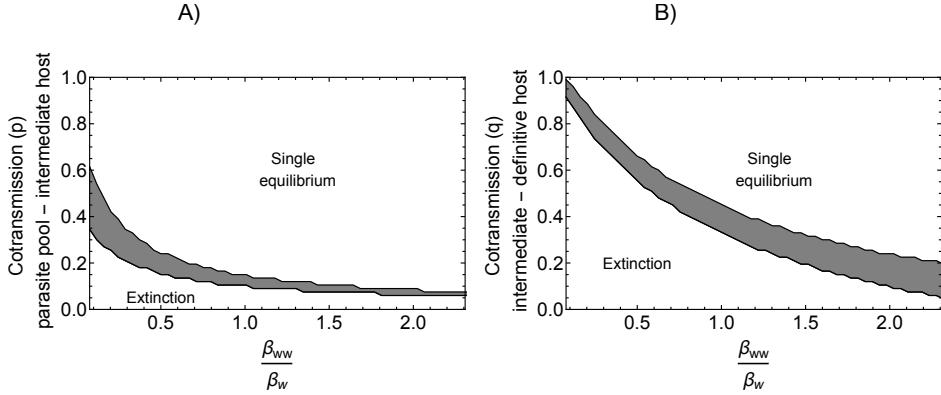


Figure 8: Common parameter: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.05$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.05$, $\delta = 0.9$, $k = 0.26$, $\epsilon = 0.5$, $\beta_w = 1.5$.

275 Discussion & Conclusion

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

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

290 In the study by [Rogawa et al. \(2018\)](#), a non-manipulative parasite can invade a susceptible
 291 prey-predator population and causes the system to cycle. The system is stabilised when the
 292 parasite becomes manipulative, and the stability increases with the manipulative ability. In
 293 our model, non-manipulative parasites cannot persist in the system. The parasite does not
 294 necessarily destabilise the predator-prey system, which may contradict the result of [Rogawa
 295 et al. \(2018\)](#). We suggest that the different results may be due to our introduction of a par-
 296 asite pool and multiple infections, unlike the model of [Rogawa et al. \(2018\)](#). In their system,

297 transmission from the definitive host to the intermediate host was assumed to result from
298 direct contact between the two hosts. Such immediate transmission could directly accelerate
299 the feedback loop between prey and predator. Hence, faster predator-prey dynamics occur,
300 which may lead to cyclic dynamics when parasites are introduced.

301 However, in our study, host manipulation can destabilise the predator-prey system under
302 particular circumstances and in a different way than the models of [Rogawa et al. \(2018\)](#). In
303 particular, the destabilisation of the system is possible due to the occurrence of bistability
304 when parasite reproduction in coinfection is boosted. In this bistability region, if the system is
305 disturbed (e.g. migration of the intermediate or definitive hosts or predation of intermediate
306 hosts by other predators), then the density of the infected hosts may crash, leading to parasite
307 extinction. The bistability region widens as the manipulation in double infection increases,
308 and manipulation in a single infection is insufficient. This extension is because the density of
309 the doubly infected hosts is always much smaller than the singly infected host density, limited
310 by sequential transmission and a small probability of co-transmission. Suppose manipulation
311 in a single infection is not sufficient. In that case, the transmission of the parasites depends
312 mainly on the double infection hosts, which is rare. So extinction is possible if manipulation
313 in double infection is not sufficiently high.

314 [Iritani and Sato \(2018\)](#) show that manipulative parasites can persist if they can alternate
315 manipulation between enhancing and suppressing predation rate. In our model, the parasite
316 cannot switch its manipulative strategy. However, we show that sabotage in manipulation
317 when parasites are coinfecting almost always leads to a single stable equilibrium scenario. This
318 result suggests that manipulation suppression, either by alternating manipulative strategy or
319 sabotaging, can be crucial in maintaining the parasite population.

320 Finally, our study focuses on the ecological dynamics of the trophically transmitted para-
321 site. However, investigating the evolution of host manipulation is a natural extension beyond
322 the scope of a single manuscript, given the complexities that arise in the ecological dynamics
323 itself (we tried). The occurrence of bistability in our model suggests that the evolution of host
324 manipulation may drive the parasite population to extinction simply because of the scarcity
325 of the mutant and the Allee effect in the population dynamics. Moreover, the parasite can
326 enhance both values if there is no tradeoff between manipulation and reproduction. Never-
327 theless, our model shows that this strategy, which seems to make the best of both worlds,
328 can make the system even more unstable. Evolutionary dynamics here depend mainly on
329 the tradeoff between host manipulation and other traits of the parasites, such as reproduc-
330 tion, virulence, and survivorship in the parasite pool, to list a few. This extension deserves
331 thorough analysis, and we will treat it as a separate matter.

332 **Acknowledgements**

333 Removed for review

334 **Statement of Authorship**

335 Removed for review

336 **Data and Code Availability**

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

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418 **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