

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). 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.

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

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

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

For the definitive hosts we have,

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

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

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

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

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

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

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

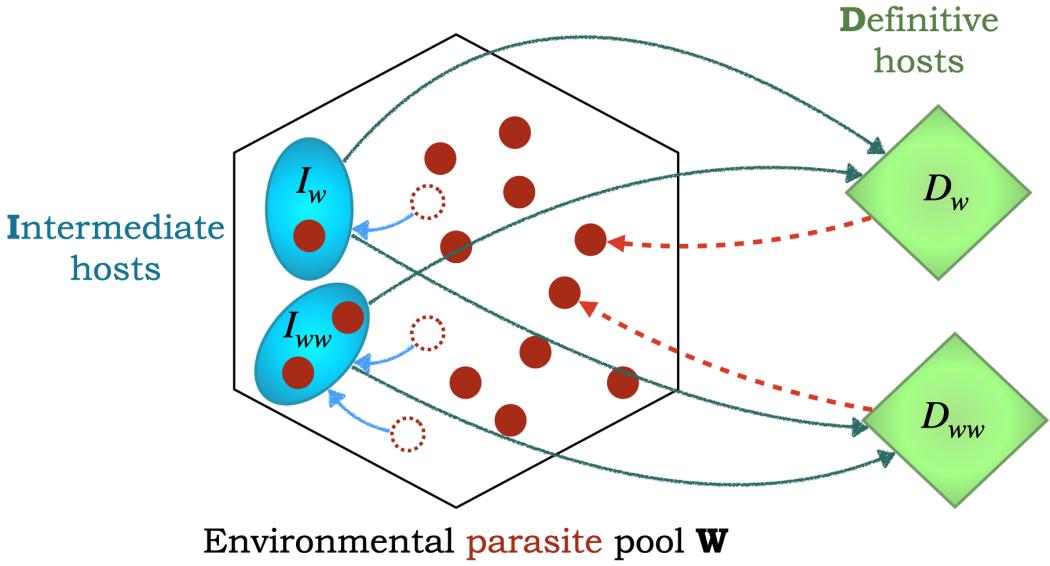


Figure 2: Schematic of the model. Blue ovals represent intermediate host compartment, green diamonds represent definitive host compartment, and the transparent hexagon represents the parasite pool compartment with red circles illustrating individual parasites.

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

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

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

¹³⁷ susceptible definitive host and eventually reproduces.

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

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

¹³⁸ where $D_{total} = D_s + D_w + D_{ww}$ is the total density of the definitive hosts, and ρ is the
¹³⁹ baseline capture rate of the predator on the prey. If an intermediate host is infected, it is
¹⁴⁰ captured by the definitive hosts with rate $\rho + \beta_w$ if it is singly infected and with rate $\rho + \beta_{ww}$
¹⁴¹ if it is doubly infected. Zero values for β_w and β_{ww} suggest no manipulation.

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

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

¹⁴² where c is the efficiency of converting prey into predator's offspring, and $I_{total} = I_s + I_w + I_{ww}$
¹⁴³ is the total density of the intermediate hosts. The birth rate of the predators depends on the
¹⁴⁴ capture rate, but it is not affected by host manipulation, as to our best knowledge, there is
¹⁴⁵ no supporting evidence to necessarily consider otherwise.

¹⁴⁶ The explicit form of I_s^* and D_s^* , capturing the predator-prey dynamics, depends on the
¹⁴⁷ precise form of all birth and predation functions B, R, P_s, P_w and P_{ww} . But, it does not
¹⁴⁸ depend on the manipulation ability or any other parameter of the parasite. Given that the
¹⁴⁹ birth rate of the predator and the predation rate are linear functions in prey and predator
¹⁵⁰ density, the form of the birth rate R of the prey has a significant effect on the susceptible
¹⁵¹ intermediate and definitive host dynamics. Hence we explore the exact structure of these
¹⁵² birth functions next.

153 **Linear birth function of intermediate hosts**

Here, we consider the system when the birth function R of the intermediate host is linear, specifically, $R(I_s, I_w, I_{ww}) = rI_{total}$. The equilibrium of intermediate and definitive hosts in the disease-free state are,

$$I_s^* = \frac{\mu}{c\rho}$$
$$D_s^* = \frac{r - d}{\rho}$$

154 This equilibrium is always unstable. In particular, it has a cyclic behaviour because, at this
155 equilibrium, the jacobian matrix of the system (1, 2, 3) always has two pure imaginary
156 eigenvalues (see SI2). This follows from the Lotka-Volterra system using linear functions for
157 prey birth and predation (Lotka, 1920). Since the disease-free dynamics is cyclic, it is difficult
158 to analyse the spread of a parasite (often evaluated when the disease-free state is stable).
159 Here, $R_0 > 1$ happens when γ , the transmission rate from the environment to intermediate
160 hosts, and the reproduction rates f_w, f_{ww} are significantly large (the specific mathematical
161 conditions can be found in SI2). However, even when this condition is satisfied, the parasite
162 may not be able to spread and persist in cyclic susceptible host dynamics (Figure 3). This
163 result agrees with the conclusion in (Ripa and Dieckmann, 2013), which suggests that it is
164 difficult for a mutant to invade a cyclic resident population. In our case, it is not the invasion
165 of a mutant in a resident population but the invasion of a parasite in a cyclic disease-free
166 host population; the argument, however, remains valid in both cases. This issue deserves a
167 more thorough investigation, which is out of the scope of this article. We, therefore, choose
168 a non-linear birth function of the intermediate hosts to obtain a stable disease circulation
169 state and focus on the effect of host manipulation on the ecological dynamics.

170 **Non-linear birth function of intermediate hosts**

We chose the logistic growth for the non-linear birth function, that is

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

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

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

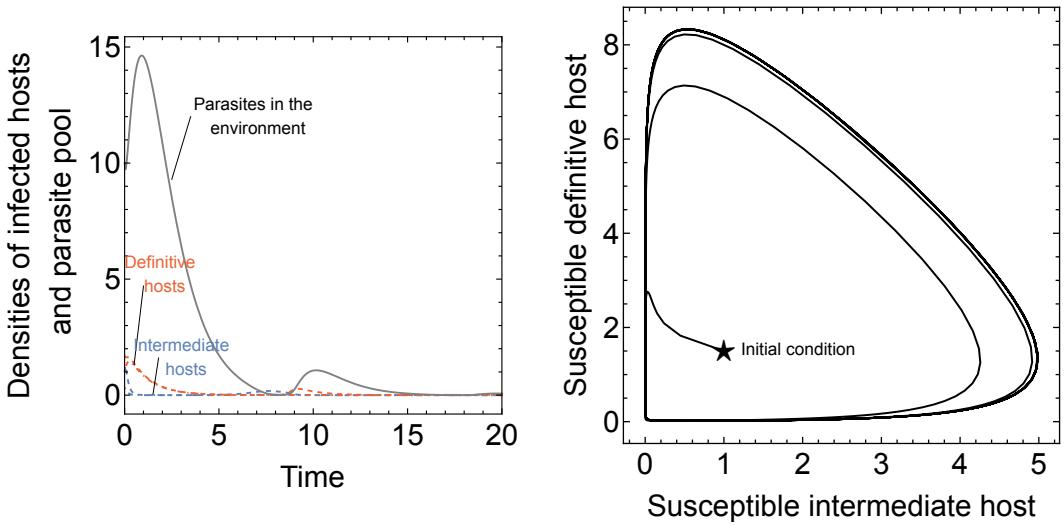


Figure 3: Disease-free equilibrium using linear birth function, where parasite goes extinct (left panel), and susceptible hosts demonstrate cyclic dynamics (right panel). Solid gray line indicate the density of free-living parasites, blue lines indicate infected intermediate hosts while red lines indicate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

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

$$(i) \quad r > d$$

$$(ii) \quad \frac{2c\rho \left(\sqrt{\frac{-d+\mu+r}{\mu}} - 1 \right)}{r} \leq k < \frac{c\rho(r-d)}{\mu r}$$

$$(iii) \quad \mu > \frac{4c^2\rho^2r - 4c^2d\rho^2}{4ck\rho r + k^2r^2}.$$

171 The above conditions suggest that (i) the intrinsic reproduction of intermediate hosts r needs
 172 to be greater than their natural mortality rate d . More importantly, (ii) the intraspecific
 173 competition coefficient has to be within a range allowing the population to survive. Finally,
 174 (iii) the definitive host's natural mortality rate must be sufficiently large. Satisfying such
 175 conditions, we obtain a stable disease-free equilibrium (Figure 4B).

176 When a parasite appears in the disease-free equilibrium, it spreads if its reproduction ratio

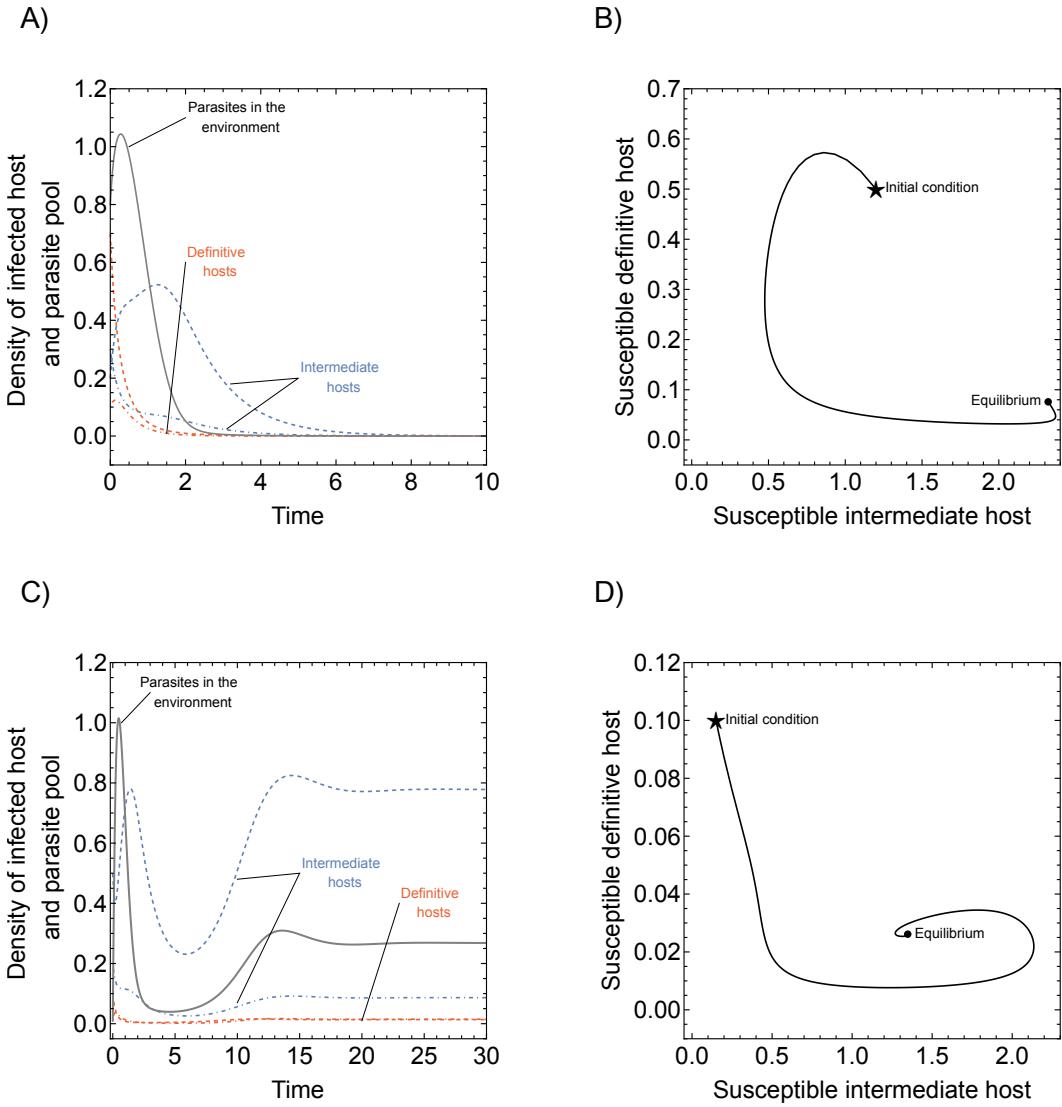


Figure 4: A, B) Disease free equilibrium where parasite cannot persist. C, D) Disease stable equilibrium. Annotations are the same in figure 3. Parameters for disease free equilibrium $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = \alpha_{ww} = 0$, $\beta_w = \beta_{ww} = 1.5$, $p =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

177 $R_0 > 1$. Since the expression is complicated, we could not obtain analytical solutions for
 178 this inequality without assumptions. We assume the same parasite virulence, $\alpha_w = \alpha_{ww}$,
 179 $\sigma_w = \sigma_{ww}$, 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).

Our numerical results show that the parasite reproduction is substantial compared to other parameters (its value is 40 times greater than other parameters). This observation suggests that trophically transmitted parasites must release many offspring into the environment to persist. Interestingly, bistability occurs if the reproduction rate of the parasite in double infections is greater than in the single infection state (Figure 5A, B).

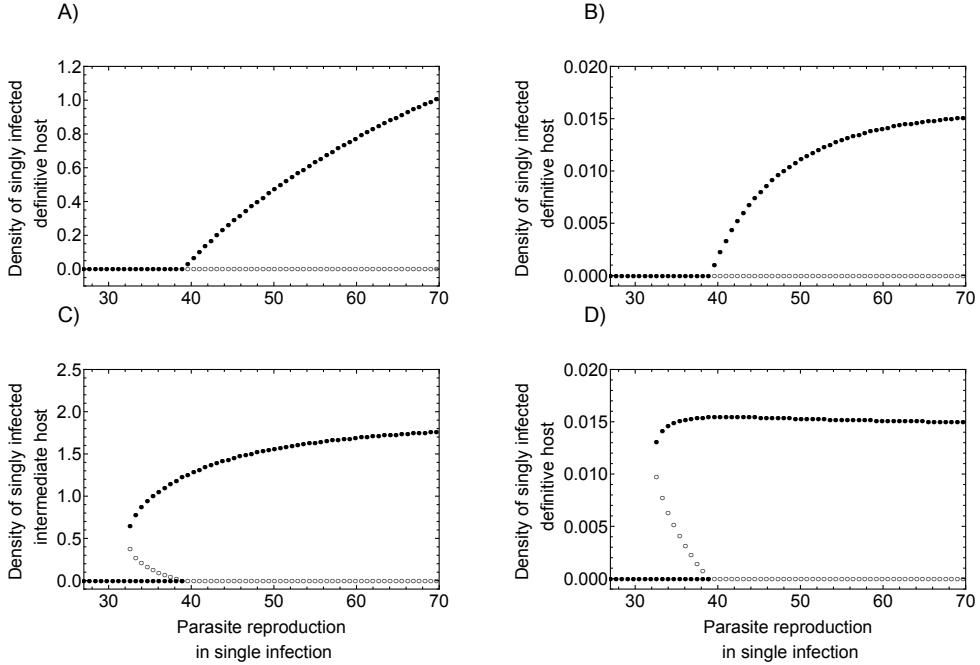


Figure 5: Effect of parasite reproduction on the ecological dynamics. A, B) When reproduction of parasites are the same in singly and doubly infected hosts $f_{ww} = f_w$. C, D) When reproduction of parasites in singly infected hosts is four times greater than those in doubly infected hosts $f_{ww} =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

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

191 Host manipulation can be cooperative; two parasites increase the predation rate on intermediate hosts, or $\beta_{ww} > \beta_w$. However, it can also be uncooperative; that is, the predation rate
192 on doubly-infected intermediate hosts lower than that on singly-infected ones, or $\beta_{ww} < \beta_w$.
193 Cooperation in parasite manipulation increases the parasite's basic reproduction ratio (R_0)
194 (Figure 6). However, suppose the ability to manipulate the host in a single infection is not
195 strong enough. In that case, cooperation in host manipulation leads to a bistable state of the
196 system. Within the bistable region, the basic reproduction ratio is less than one, suggesting
197 that the parasite cannot spread when its manipulative values are within this area of weak
198 manipulation when coinfecting. Parasites that can persist in the population may have weak
199 manipulative activity in a single infection but become much more manipulative in coinfection.
200 Likewise, parasites can persist if uncooperative but can manipulate the intermediate hosts
201 effectively when alone.

203 Cooperation between parasites need not be limited to host manipulation. Parasites can co-
204 operate to have a higher reproduction rate in co-infections, i.e. $f_{ww} > f_w$. Likewise, they can
205 compete for resources, so reproduction in double infection is smaller than in single infection.
206 Without any assumption on the relationship between manipulative ability and reproduction,
207 we explore all possible combinations of cooperation and sabotage in both manipulation and
208 reproduction. Interestingly, higher cooperation in manipulation and reproduction enlarges the
209 area of bistability even though it also shrinks the extinction space (Figure 7). This suggests
210 that systems in which parasites have much higher manipulative ability and reproduction rate
211 when co-infected than when singly infected are more prone to instability than systems with
212 less cooperative parasites or systems with parasites that sabotage each other in co-infection.
213 In other words, having the best of both worlds, effective manipulation and reproduction, at
214 the individual level may not benefit the population as a whole.

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

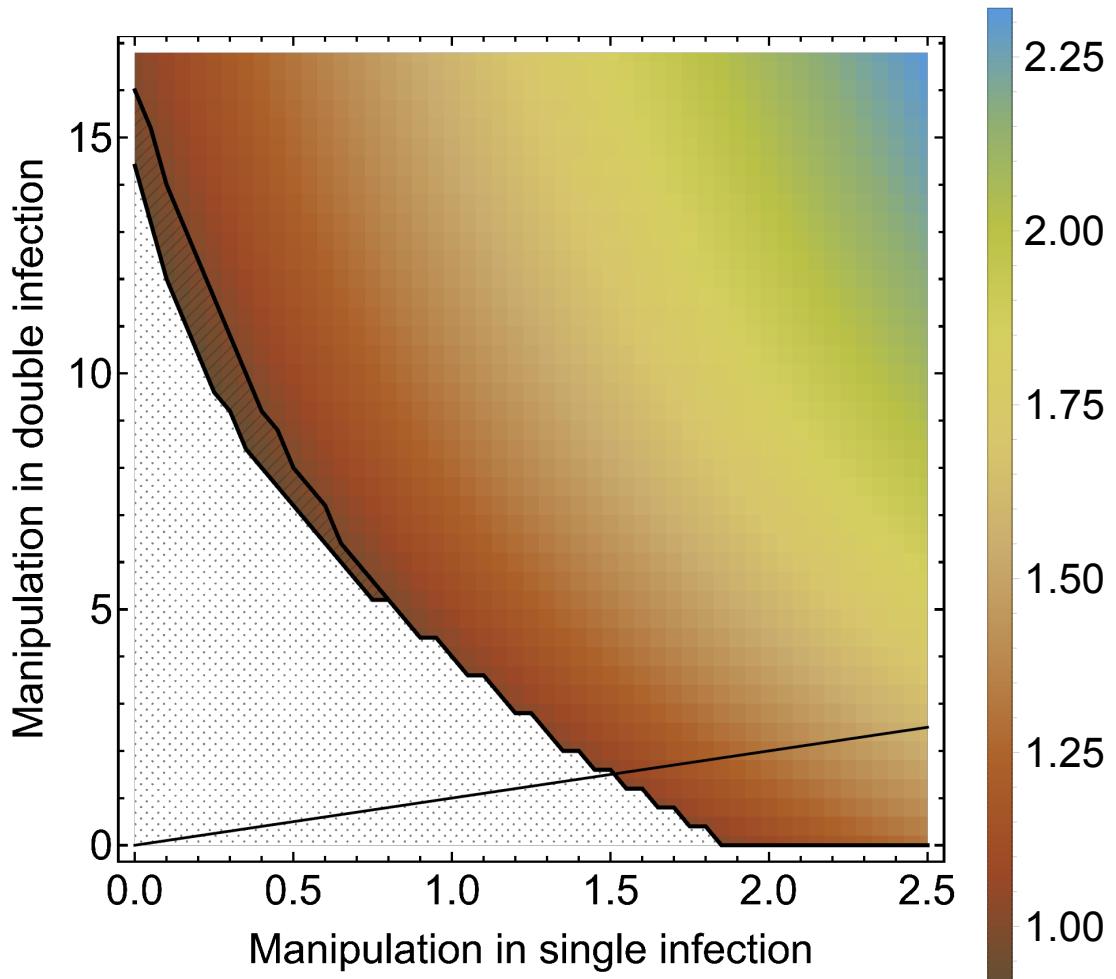


Figure 6: R_0 values increase with more efficient manipulation in both single and double infection. The parasite goes extinct if its manipulative ability is insufficient (dotted area). Bistability region occurs when cooperation in manipulation is intermediate (hatched area). As manipulation in single infection increases, the system only has one stable equilibrium. On the black line, manipulation is indifference between single infection and double infection ($\beta_w = \beta_{ww}$). $R_0 < 1$ in the hatched area indicates that the parasite cannot establish in a disease free prey-predator population.

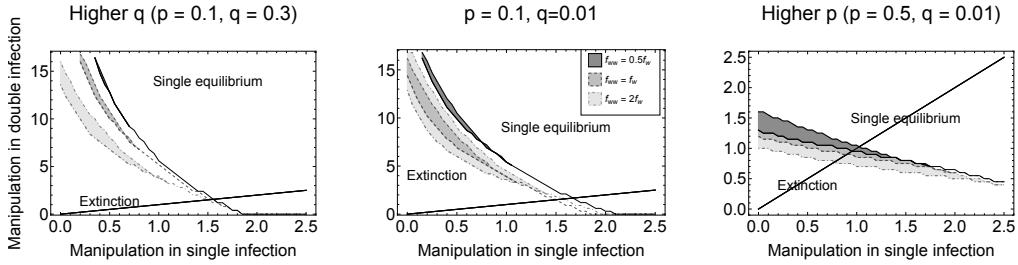


Figure 7: Changes of the bistability area (shaded areas) with respect to different reproduction rates in single and double infection (different boundary styles), and varying cotransmission probability. Manipulation is indifference between single infection and double infection on the black line. Common parameter: $\rho = 1.2$, $d = 0.9$, $r = 2.5$, $\gamma = 2.9$, $\alpha_w = 0$, $\alpha_{ww} = 0$, $p = 0.1$, $c = 1.4$, $\mu = 3.9$, $\sigma_w = 0$, $\sigma_{ww} = 0$, $q = 0.01$, $\delta = 0.9$, $k = 0.26$, $\epsilon = 0.5$. Parameter for the thick boundary $\epsilon = 0.5$, $f_w = 36$, the dashed boundary $\epsilon = 1$, $f_w = 36$, and the dot-dashed boundary $\epsilon = 2$, $f_w = 35$.

226 mediate hosts is low. In that case, general transmission from intermediate hosts is limited, which explains the wide extinction area.

228 Discussion & Conclusion

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

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

243 In the study by [Rogawa et al. \(2018\)](#), a non-manipulative parasite can invade a susceptible
 244 prey-predator population and causes the system to cycle. The system is stabilised when the

245 parasite becomes manipulative, and the stability increases with the manipulative ability. In
246 our model, non-manipulative parasites cannot persist in the system. The parasite does not
247 necessarily destabilise the predator-prey system, which may contradict the result of [Rogawa et al. \(2018\)](#). We suggest that the different results may be due to our introduction of a para-
248 site pool and multiple infections, unlike the model of [Rogawa et al. \(2018\)](#). In their system,
249 transmission from the definitive host to the intermediate host was assumed to result from
250 direct contact between the two hosts. Such immediate transmission could directly accelerate
251 the feedback loop between prey and predator. Hence, faster predator-prey dynamics occur,
252 which may lead to cyclic dynamics when parasites are introduced.

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

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

273 Finally, our study focuses on the ecological dynamics of the trophically transmitted para-
274 site. However, investigating the evolution of host manipulation is a natural extension beyond
275 the scope of a single manuscript, given the complexities that arise in the ecological dynamics
276 itself (we tried). The occurrence of bistability in our model suggests that the evolution of host
277 manipulation may drive the parasite population to extinction simply because of the scarcity
278 of the mutant and the Allee effect in the population dynamics. Moreover, the parasite can
279 enhance both values if there is no tradeoff between manipulation and reproduction. Never-
280 theless, our model shows that this strategy, which seems to make the best of both worlds,

281 can make the system even more unstable. Evolutionary dynamics here depend mainly on
282 the tradeoff between host manipulation and other traits of the parasites, such as reproduction,
283 virulence, and survivorship in the parasite pool, to list a few. This extension deserves
284 thorough analysis, and we will treat it as a separate matter.

285 **Acknowledgements**

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287 **Statement of Authorship**

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289 **Data and Code Availability**

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

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371 **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