

Dear reviewer,

We thank you for taking your time to consider our manuscript. You will find below in blue our response to your comments.

This study extends the theoretical understanding of the effects of host manipulation on ecological dynamics, predator (definitive host)- prey (intermediate host)- manipulative parasites, by considering multiple infection. Previous theory has suggested that predation-enhancing manipulation can reduce prey density, increasing the risk of parasite extinction (Fenton & Rands 2006 Ecology). In this study, the authors suggested that multiple infection is common among manipulative parasites (supported in empirical studies) and incorporating the nature of multiple infection may lead to different outcomes of the predator (definitive host) - prey (intermediate host) - manipulative parasite dynamics. I have mixed feelings about the evaluation of this paper. While multiple parasitism is an important topic, the model seems complex and lacks sufficient justification for parameter settings. On the other hand, as the authors point out, it could serve as a foundation for future theoretical considerations for host-parasite dynamics given the limited empirical information on parasites.

I believe the lack of knowledge on parasite ecology is inevitable, so I don't mean to criticize the paper solely based on that. But, I appreciate the authors' efforts to discuss the validity of the model explanation more thoroughly, based on studies of the natural history of parasites. In addition, I wonder if the authors may point out, from a theoretical perspective, the parameters that parasitologist should focus on collecting. Otherwise, the paper would be more appropriate for the theoretical journals.

Below I have mainly commented where I did not fully understand the ecological meaning of the parameters and how they relate to parasitic ecology.

1. The reproduction rate of parasite and the relative value of that with the host's reproductive rate are crucial for understanding the model outcomes, but they were difficult to understand in the current explanation (I made comments when I did not fully understand the ecological meaning of the parameters and how they relate to parasitic ecology).

The reproduction rate of parasite ( $f_w$  and  $f_{ww}$ ) is the *per capita* reproduction rate of the parasite inside a definitive host (indicated in line L143). Since we consider multiple infections, it can be understood as the number of offspring released into the environment per infected definitive host.

The reproduction rate of the intermediate host ( $r$ ) is the *per capita* reproduction rate of the intermediate host regardless of whether they are infected or not (indicated in line L214). We show that numerically, the reproduction rate of the parasites is relatively large compared to the reproduction rate of the intermediate host (almost 20 times larger, L231-232). We are aware that there is no empirical evidence for this qualitative result. However, we think that this result is reasonable as parasites are often simpler organisms than hosts (e.g. nematodes compared to fishes) and, thus, normally produce more offspring than hosts. This might also be an interesting parameter to measure in experiments.

Finally, the reproduction rate of definitive hosts concerns the manipulation parameters  $\beta_w$  and  $\beta_{ww}$  (line L123-125). In fact, these parameters are not really the reproductive rate of the definitive host because this is a trophic system in which a definitive host makes contact with an infected intermediate host, consumes it and then reproduces.

The transmission parameter can be equivalent to the consumption rate of the definitive host on the intermediate host. However, the consumption rate does not have the same dimension as the *per-capita* reproduction rate (see Table 1); we therefore cannot compare these two types of parameters.

To reduce confusion and add clarity, we have now added the dimensionality of the variables and parameters in Table 1.

L36 Haderler and Freedman (1989), Fenton & Rands (2006) and Rogawa et al. (2018).

L43 Here or somewhere, the authors may explain they focus on multiple infection of one parasite species in this paper. Then, the authors may concisely discuss about how their findings can be (or cannot be) applied to multiple infection of different species.

Thanks for the suggestion. We have now added more explanation as suggested in L73, L96. In the Discussion we highlight how our findings can be applied to multiple infection of different species in L371-374.

Figure 1. Since the figure legend explain the life-cycle of the parasite, it would be better to have a diagram of the life-cycle here.

We have added the life-cycle of the parasite as suggested and state how this specific example is but an exemplar of our genetic theoretical model.

L65-71 I understand the limitations of the previous studies, but I would like to know what mechanisms might be responsible for the different results between the previous studies and this study.

Indeed, we discuss this point in the Discussions, where we compare our results with the results of Rogawa et al. 2018. The main difference between our results and the results of other studies is that we do not have cyclic population dynamics, and it might very well due to the introduction of the parasite pool in the environment. In nature, new parasites are released from the definitive hosts into the environment before transmitting to the intermediate hosts. In the studies that we cited (Haderler and Freedman 1989, Fenton and Rands 2006, and Rogawa et al. 2018), transmission from definitive hosts to intermediate hosts as a new cycle of the parasite is due to direct contact between infected definitive hosts and uninfected intermediate hosts. We suggest that this transmission due to such direct contact may speed up the dynamics of the system, resulting in cyclic dynamics under some set of parameters. A clarified explanation to this effect can now be found in lines 332-343

L108 and Figure 2 I am not entirely convinced by the term "co-transmission" from the environmental parasite pool to intermediate hosts, because it assumes that the parasites are free-living and infect the intermediate hosts individually. To me, sequential transmission would be more appropriate here.

In fact, we do mean that the parasites have a free-living state, which is very common in the literature, especially for trophically transmitted parasites (Chubb et al. 2006, Poinar et al. 2008). Note that the parasites cannot reproduce by themselves in the free-living environment. Therefore, in this case, parasites infect the intermediate hosts individually, resulting in a single infection if only one parasite infects an intermediate host. If two parasites infect an intermediate host at the same time, i.e. co-transmission, then we have

two parasites that immediately infect the intermediate hosts, i.e. double infections. Sequential transmission happens when an intermediate host infected with one parasite gets infected with another parasite from the parasite pool. However, we omit this scenario in our model for simplicity, and also for the ecological explanation that we made in L147-152.

L119 Are there any empirical studies related with this parameter setting? The authors may have some information from the following papers:

Lafferty, K.D. 1992 Foraging on prey that are modified by parasites. *The American Naturalist* 140, 854-867.

Shanebeck, K.M., Besson, A.A., Lagrue, C. & Green, S.J. 2022 The energetic costs of sub-lethal helminth parasites in mammals: a meta-analysis. *Biological Reviews* 97, 1886-1907.

To our best knowledge, there are not many empirical studies on trophically transmitted parasites that consider parameter setting that is relevant to our model. We thank the reviewer for the suggested literature and indeed the work of Lafferty 1992 is one of them. There is also the study by Seppala et al. 2004 that measured the susceptibility to predation between fishes infected by *Diplostomum spathaceum* (Trematodonta) and their uninfected counterparts. Note that these measurements do not explicitly reflect the manipulation parameter ( $\beta_w$  and  $\beta_{ww}$ ) that we use in our model because our model simplifies these complex systems, e.g. multiple infections are limited to two co-infecting parasites. To achieve the measurements tailored to our model, it requires experiments with strict controls on the number of infecting parasites (e.g. Hafer and Milinski 2015).

There is also the study of Gopko et al. 2015 that measured the time spent to catch infected versus uninfected fishes, which reflect the exposure of the fishes to predation. This would require modifications of the predation function in our model to encompass the exposing time.

These studies are now cited and discussed by us in the L380-382 section.

Seppälä, O., Karvonen, A., & Tellervo Valtonen, E. (2004). Parasite-induced change in host behaviour and susceptibility to predation in an eye fluke–fish interaction. *Animal Behaviour*, 68(2), 257–263.

Gopko, M., Mikheev, V.N. & Taskinen, J. Changes in host behaviour caused by immature larvae of the eye fluke: evidence supporting the predation suppression hypothesis. *Behav Ecol Sociobiol* 69, 1723–1730 (2015).

Figure 3 I'd like the text inside the panel B to be larger.

Thank you for the suggestion. We made the text larger and in some other corresponding figures where it might help readability.

L236 In general, parasites may compete (or not compete) within the hosts, reducing (or not reducing) their reproductive rate. So, what mechanisms should we expect when co-infection enhance the reproductive rate of parasites?

There may be several mechanisms for the enhancement of reproduction of the parasite in co-infection, one of which is that some parasites suppress the immune system of the host so that their siblings could establish and reproduce. This hypothesis fits our model set up

as we do not consider sexual reproduction and only take into account one strain of parasite so that co-infecting parasites share the same genetic background.

In fact, the reviewer brings up a very interesting point as to the best of our knowledge there is no literature investigating the reproduction of the parasite. Most literatures focus on the host's fitness, such as host mortality and fecundity. We believe that experimental inference of whether co-infection can enhance the reproduction of parasites could be tested in the laboratory.

L237-238 In the early stages of invasion, a few infected hosts invade a susceptible host population. So, what is the ecological situation, where sufficient parasites are introduced into the disease-free predator-prey populations? Since this assumption affects important model predictions, it requires sufficient justification.

The invasion criteria of trophically transmitted parasites follow exactly the principle of invasion criteria of pathogens in epidemiology. In ecological situation, it could be the case that infected intermediate hosts migrate or are introduced into areas where the definitive host population are not yet infected. For instance, animals such as rats or cockroaches that contain parasites are introduced in different areas via human mobility. Alternatively, it could also be the case that the free-living parasite pool is introduced into the disease-free predator-prey system.

L243-245 Please provide citations supporting both cooperative and uncooperative manipulation.

We added the citation as suggested.

L246 As long as my visual inspection in Figure 4, almost no effect of cooperation in parasite manipulation on  $R_0$ .

We thank the reviewer for pointing out this issue. Indeed, the increase of  $R_0$  with respect to cooperation ( $\beta_{ww}$ ) is relatively small compared to its increase with respect to manipulation in single infection ( $\beta_w$ ) such that it is not visible on the graph. We added in the supplementary the analytical result of  $d R_0 / d \beta_{ww}$  and  $d R_0 / d \beta_w$  to show that  $R_0$  in fact increases with respect to both  $\beta_w$  and  $\beta_{ww}$ .

L251 The mechanisms generating the bistable state would be ecologically important. Can the authors provide more mechanistic explanations on this by mentioning the dynamics of both intermediate and definitive hosts?

Within the bistable area, we observe the Allee effect. In particular, if there are sufficient parasite in the free-living pool as well as inside the intermediate and definitive hosts, the parasite will persist and spread. In contrast, if there is not enough parasite in the beginning, the parasite population will perish. We added more explanation in L256-260, and bistable graph for the population dynamics of all compartments in the Supplementary.

L286-287 As long as my visual inspection of Figure 6, this statement are only applicable to Figure 6B.

We thank the reviewer for pointing out this issue. We actually mean sufficient cooperation (now stated so L292).

As we increase the amount of relative manipulation (x-axis) we see that the region denoting single equilibrium keeps expanding. While we see the existence of only the single equilibrium already in Fig 6A, we would need to explore much higher manipulation ratios to see a similar feature in Figs 6B, C and D.

Therefore, if we extend the value of  $\beta_{ww}/\beta_w$  in Figure 6A, we would observe only a single equilibrium in this area. We modified the text accordingly in L292-295.

L367-371 I basically agree with the statement here, but would appreciate if the authors could suggest which ecological parameters empirical researchers should study in natural systems.

Our model is not tailored to any specific parasite species. Its purpose is to study the qualitative behaviour of population dynamics of both parasites and their hosts. While we are grateful that the reviewer sees value in our qualitative work, it does encourage us to provide some quantitative expectations. Further knowledge of some parameters definitely will contribute to the general understanding of trophically transmitted parasites. From discussion with experimental colleagues, we have identified three sets of parameters that empiricists could measure in laboratory or fieldwork:

- Conditions of the host depending on none, single vs multiple parasites ( $\alpha_i$ ,  $\sigma_i$ )
- Distribution of parasites in the ecological pool ( $W$ )
- Reproduction of the parasite in single and multiple infections ( $f_i$ )

We have added a discussion about these parameters in L383-389.

We have also now added the appropriate dimensionality for the parameters in Table 1. This will help relate to what is possible to be measured in ecologically explicit experiments. Even getting qualitative input from such experiments will be extremely useful in delineating the domains of behaviour that we see in the extent of manipulation. Quantitative fits with experimental data will need the development of bespoke models based on our general theoretical findings. This and a connection to evolutionary processes form parts of our future goals beyond the scope of the current presentation that focuses primarily on the qualitative ecological aspects.

We thank the reviewer for the suggestions that have greatly improved the readability of the manuscript and made it broader than a theoretical exploration.