

Comments to authors

This is a neat study showing adaptive manipulation of host behaviour by a parasite, by looking at changes following infection of the gammarid intermediate host *G. pulex* by the acanthocephalan helminth *P. laevis* (whose final host is a fish predator). More specifically the authors investigate how host behavioural changes vary according to the parasite's developmental stage (i.e., whether it is pre-infective or infective) and how they are affected by the presence of predator cues.

The main findings are:

- 1) early on in the infection and as long as the parasite *P. laevis* hasn't reached the (cystacanth) infective stage, *G. pulex* hosts hide in refuges to a greater extent than uninfected hosts - especially so in predator-scented water.
- 2) that a clear switch in behaviour occurs when the parasite is ready for transmission to the final host: infected gammarids start spending less time than uninfected ones in refuges, while increasing the proportion of time spent inactive (not swimming) – thus being more exposed to predation than uninfected gammarids.

Taken together, these results provide evidence for adaptive host manipulation in this study system – more than phenotypic changes in the host simply being byproducts of infection (although some of the increase in time spent inactive at the end of the infection period could in part be due to energy depletion in the host, too).

This is a valuable study that I enjoyed reading, but I do have a couple of comments and suggestions, see below.

General comments

My main general comment is that this paper does not introduce or discuss clearly enough the alternative explanation for phenotypic changes in infected hosts, namely that some of the changes following infection – including activity rates or predator exposure – might, at least in part, also be a byproduct of infection and not only adaptive manipulation by the parasite. This can easily be addressed by adding these aspects to the introduction, and then discussing your results in the light of this. It would make your paper stronger, as your results anyway build a convincing case for adaptive manipulation of host behaviour in this study system; acknowledging that some of the changes could also partly be byproducts would make your conclusion stronger, not weaker, as the two need not be mutually exclusive.

More specifically, you could in the introduction better explain what the difference is between PIPA (parasite-induced phenotypic alterations) and host manipulation – and how “true” manipulation can be recognized. Similarly in your discussion, your paper would be strengthened if you acknowledged the alternative explanation for phenotypic changes in the host. As it is

now, you only first refer to it L408 as “a non-adaptive pathogenic byproduct of the infection, as sometimes suggested”. The difference between by-product changes and adaptive manipulation deserves more attention earlier in your paper, precisely because your study provides evidence for host manipulation – so it is important to clarify to the broader readership what the alternative explanation could have been, and that some byproduct effects may also occur at the same time without invalidating the conclusion.

You might want to use these references:

Håkonsrud Jensen et al. (2023) Adaptive host responses to infection can resemble parasitic manipulation. *Ecology & Evolution*, in press, doi:10.1002/ece3.10318

Lefèvre et al. (2008) Exploiting host compensatory responses: the ‘must’ of manipulation? *Trends in Parasitology* 24, 435-439

Poulin & Maure (2015) Host manipulation by parasites: a look back before moving forward. *Trends in Parasitology* 31, 563-570.

Helluy (2013). Parasite-induced alterations of sensorimotor pathways in gammarids: collateral damage of neuroinflammation? *Journal of Experimental Biology* 216, 67.

Detailed comments

Introduction:

L46: tropical transmission -> trophical transmission

L47:by upstream host -> by the upstream host

L49: parasite’s life cycle -> the parasite’s life cycle

L48: what is the difference between PIPA and adaptive manipulation of hosts by parasites?

Please clarify and expand on this (see my general comment above).

L57: referred as -> referred to as

L79-81: here it sounds as if you are saying that the fact that a trait responds to selection makes it part of an extended phenotype. This can lead to confusion; please clarify, e.g. adding ‘rather than a trait of the host’, or something similar, at the end of the sentence

L82 rephrase -> Another well-studied parasite-host system etc.

L88-93: here you seem to interchangeably use behavioural changes and manipulation, but these are two different things; please clarify. Also, L91-93 are not needed, you could go straighter to the point by removing these two sentences

L97-102: these two points would work better if they were moved further up, e.g. before the sentence starting L96.

L105-108: a bit unclear, please try to rephrase.

L109-111 is the main prediction of your study, this should appear more clearly

L112: replace ‘forced’ with another word – that acanthellae induce – or cause – an increased used of refuges, for example

L114: increased use of refuges was found in both the presence and absence of predator cues: how does this fit with your hypothesis (see L97-102) of costs / investment into ‘protective manipulation’?

L122-124: move up to L118

Methods

L130-136: why did you only use males?

L146-148: how many hosts died prior to d20 post-infection?

L150-160: how often was the water changed between tests of individual gammarid behaviour? I may not know the study species well enough, but gammarids are rather social; could successive gammarids have left odor cues in the water that could have affected their behaviour? Do you know if their rank in the testing sequence has any effect on their use of refuges?

L163-164: a bit confusing here, I'd suggest moving this bit of information somewhere else, for example at the end of the paragraph

L166: at this point I wondered how the water was scented and how much, but it is only described further down; this can be avoided by moving L173-179 up in the methods description

L177: I do not understand how feeding trouts with live gammarids would reinforce their predator signal; can you explain this briefly? Do they take the scent of the prey they eat?

L180-188: move this up to the start of the 'behavioral measurements' paragraph

L192: control vs infected, or was it uninfected vs infected?

Results

L264-272: make a new paragraph for this, as this is a different line of results

L265: interpretation -> observation

L288 : rephrase, using neither / nor

L313: hypothesis -> observation / trend

Figure 5: in the labels, change 'non-signed' and 'signed water' to 'non-scented' and 'scented'

Discussion

L406-430 is the main conclusion of your study, the discussion should gradually lead to it: keeping the same structure to your discussion, you could turn it into a more explicit step-by-step explanation why your study gives evidence for manipulation, as opposed to only representing byproducts effects of infection.

Here are a few suggestions.

L332-341: at this point, this result alone could be either adaptive manipulation or byproduct – you could state this more explicitly

L347-353: what would you have expected to find instead, if there was no manipulation? More specifically, would you observe the same patterns of increased refuge use with time as a byproduct, for example if the parasites become increasingly costly and the host increasingly depleted with energy over time?

L353, 356: products -> chemicals, molecules, factors

L358: oscillate -> fluctuate

L350-360: could this be caused by energy depletion? What did they eat during the experiment?

L381-398: this is the step where adaptive manipulation becomes the main conclusion, because only manipulation allowed to explain the full range of your observations, and especially such a shift in host behaviour, synchronised with the parasite reaching infective stage – you could consider making this a distinct paragraph and presenting it as your final piece of evidence for adaptive host manipulation.