Revision round #1
Decision for round #1: Revision needed
Minor revisions necessary

Reply (comments in italics, and our answers in bold characters)

To recommender *Ben Phillips*

Congratulations on an interesting piece of science. We now have three independent reviews, and they are unanimously positive about the work. Each reviewer has also included some useful thoughts about ways the manuscript might be improved. Some thoughtful responses to the reviewer's suggestions will improve the manuscript.

First of all, we would like to thank the recommender and referees for their positive and useful comments.

I see that you have already mentioned Chesson's framework. It might be worth touching back to that again in the Discussion.

Yes. The sentence in the Introduction (l. 85-87) introduces Chesson's *stabilizing* and *equalizing* mechanisms and we added a few sentences to come back to these concepts in the light of our results in the Discussion (l. 618-625 and 638-655). Basically, we observe an evolution of the resident species towards competition avoidance, and we propose to interpret this as a reinforcement of *stabilizing* mechanisms that strengthens the coexistence with the invasive species at the metapopulation scale (by enhancing their differentiation on the competitor-colonizer axis). We mention the parallel with other examples of character displacement (usually related to resource shifts), that also reinforce stabilizing mechanisms. We end with the open question of understanding when evolution is expected to reinforce stabilizing mechanisms (through avoidance of competition by the "inferior" species) versus equalizing ones (through increase in competitivity by the "inferior" species).
We also added a reference (Shea and Chesson, 2002), which introduce the concept of niche opportunity, and is relevant to our prediction of competitive asymmetry favoring the invader over the resident (l. 630).

I also agree that it might be worth thinking about the role of dispersal, at least in the discussion. Hutchinson's 'fugitive species' concept is probably also worth a look in the context of your results.

Yes, dispersal is a fundamental aspect of colonization ability. We have devoted a few lines (l. 513-536) to give more details on the natural history and dispersal of our two species, mostly based on our own previous published work (Dubart et al., 2019, 2022; Pantel et al., 2022). We indeed have lots of published, quantitative, evidence that *A. marmorata* (the resident species) is less competitive but more efficient at colonizing new sites and exploiting high-resource patches in the Guadeloupe environment. While the competitive hierarchy is visible in the laboratory, and therefore depends on traits also expressed in this environment, colonization is measured only in the field, and inferences on how life-history traits contribute to the higher colonization capacity of *A. marmorata* are necessarily more speculative. Based on long-term monitoring, dispersal is more efficient in *A. marmorata* as its colonization probability is not decreased in relatively isolated sites,

while that of *P. acuta* is (Dubart et al., 2019). However, we do not know exactly which traits are responsible for this difference (direct observation of dispersing individuals is notoriously difficult in freshwater invertebrates). We now summarize these arguments, citing field-based data, and acknowledging the importance (and, unfortunately, our little knowledge) of dispersal abilities in these snails in the Discussion (l. 513-536). We have also introduced (Appendix 2) the colonization and extinction rates estimated by Dubart et al. (2019) - for convenience, because we cite them at several places in the Discussion and readers might be curious to see the true numbers to get a better idea of how this metacommunity works, without having to unearth them from the literature.
Hutchinson's fugitive species concept is indeed an elegant summary of a phenotypic and ecological syndrome that does apply well to our resident species *A. marmorata*. It is now mentioned and shortly described (l. 508-513).

I also struggled a little with the figures. They are great in that they show a large amount of the structure of your data, but they are a lot to process. I wonder if it might be useful to remove the population labels and group the population points (jittered) for each treatment. Worth playing with, anyway.

We modified all three figures, also in accordance with one the referees' suggestions. We preferred to keep them as informative as possible on the population/replicate structure of our data but the effects are now made much more visible by the inclusion of model estimates and confidence intervals (shaded zones) that allow one to easily compare treatments and population types. This follows one of the reviewers' suggestions below. We also slightly jittered the data points as suggested to avoid superimposition when necessary. The legends were revised accordingly.

I look forward to reading the revised version.

To the reviewers
Review 1 (*David Reznick)*

The main idea advanced by this study is that resident species can resist competitive exclusion by an invasive species by evolving life history traits to exploit ephemeral low-competition situations after disturbances (i.e., avoiding competition), as opposed to a more common idea of evolving to be a better competitor. The study uses an interesting natural metacommunity context to cleverly make space for time substitutions to assay population phenotypes among replicated populations with suspected difference in their histories of selection and evolution in response to competitors and local conditions. It's a nice study.

The manuscript has a solid conceptual grounding, clear hypotheses and predictions, and is well-organized.
Having replicate populations within each 'treatment' type is necessary, and was a strength. The caveats and limitations (for example, of a contrived laboratory setting) are addressed well. The inferences being made are connected to the evidence presented, and are not overstatements or spin.

I wondered about the role of dispersal in the set of traits that might make A. marmorata better at exploiting ephemeral patches of resources. I didn't get a sense of how much disturbances (like floods) cause local extinction and how much of the community reorganization is due to *local recruitment from the few remaining individuals or if it depends on dispersal from elsewhere (other patches). If A. marmorata is less good at dispersing, then could some sort of priority effect shifts the advantage to P. acuta in field settings?*

See our answer to the Recommender, for a discussion on dispersal. The field data suggest true extinction and recolonization cycles. Perturbations are mostly related to desiccation, anoxia, occasional floods and can, as far as we know, lead to true local extinctions, the snails having to recolonize from other ponds. While it is never easy to tell with certainty that a population is extinct during a field survey, our (published) metapopulation models (Dubart et al., 2019) do account for imperfect detection (i.e., they estimate detection probabilities, using state-space models, and therefore do not assume absence when a species is not detected). So, the extinction rates estimated (now reported in Appendix 2) are as close as we can to true extinctions.
As mentioned above, based on these published data, *A. marmorata* (the resident) is more efficient at colonizing empty sites, exploiting high-resource patches (i.e., with lots of vegetation), and dispersing to isolated ponds than the invasive *P. acuta*. The competition effects observed in the field do indeed suggest some form of preemption by *P. acuta*, which decreases the probability that a site is colonized by *A. marmorata* (Dubart et al., 2019; see Appendix 2). Evolving towards higher colonization efficiency can partly compensate this effect and help *A. marmorata* to persist in the landscape after the arrival of *P. acuta*. We now mention this idea in Discussion (l. 567-571).

Some minor suggestions to improve clarity:
I would find it easier to read and interpret the results if I was reminded which species is the invader, and using the words in place of their acronym. I had to keep going back to remind myself what all the letters meant. Perhaps easier to just write the words out in the text?

Done each time we suspected an ambiguity.

Showing the population variation in Figures 2, 3, 4 is good, but the key information to assess the hypotheses are the dotted lines showing 'treatment' effects. To see the uncertainty' in how these means differ to each other, I think it would help to show 95% confidence intervals, such that the mean and 95%CI are those estimated from the GLMM's.

Done; see our answer to the recommender on how Figures were modified.

Finally, in the results section, I think it would help to have clear statements that say how a particular result provides support for or against the various hypotheses, instead of making the reader having to join the dots first.

We added a final paragraph in the Results section (l. 417-425) to summarize which ones of the predictions in Table 1 were fulfilled (or not), and for which trait. Hopefully, given that the result section is relatively short, this will help the reader to digest these results before the Discussion, while keeping a presentation of the statistical results and graphs as factual and free of interpretation as possible.

<u>Review 2</u>

Review of Preprint 2023.10.25.563987 – Rapid life-history evolution reinforces competitive asymmetry between invasive and resident species. Chapuis Elodie, Philippe Jarne and Patrice David

The authors present the results of laboratory experiments that characterize the interactions between an invasive species of snail (Physa acuta) and a resident species (Aplexa marmorata). The utilized P. acuta populations that had long since invaded new habitat (core populations) and those that were on the front of the invasion (front invasions), but emphasize that the front populations are derived from core populations so we should assume that all have adapted to the new environment. They compete them with A. marmorata from populations that have not yet been invaded or have only recently been invaded (N populations) with ones that have been occupied by P. acuta for an extended period of time (E populations). In earlier work they showed that the E. populations of A. marmorata were younger at maturity and had higher fecundity than N populations of A marmorata. There were more subtle differences in adult body size and fecundity between F and C populations of P. acuta – F populations tended to be larger and have lower fecundity. The new results in this paper show that P. acuta is competitively superior to A. marmorata and, paradoxically, A. marmorata from E populations are poorer competitors than those from N populations, meaning that their evolution in response to P. acuta invasion causes them to be poorer competitors. Simple ecological theory for co-existence predicts that the resident will go extinct, but they are continuing to co-exist with the competitor. The authors consider different explanations for this co-existence but miss a large body of ecological theory, originally proposed by Chesson (e.g. [1]), that propose general ecological conditions that can explain co-existence under such circumstances. While there is no need to go into these alternatives in detail, I think it is essential that they at least add a paragraph that refers to Chesson's models and considers how these alternatives might reconcile the observation of co-existence in spite of experimental data that suggest that co-existence is not possible.

This is now extensively discussed, as exposed in our response to the recommender.

Overall, I think this is a well-executed and well-presented experiment. I only ask that they adopt an interpretation that is less committed to their life history evolution explanation for the seemingly paradoxical results. It is natural to emphasize life history evolution since that is the one type of evolution that is well documented, but the ecological complexity of the actual invasion invites alternatives that need to be acknowledged.

We have enriched the Discussion (see response to recommender and the other referees), trying to delimitate as precisely as possible what our data do support and what is more speculative or based on other published data. Especially we made clear that (i) previous laboratory experiments document differences in life history traits between the two species, and evolution of these traits after invasion (Chapuis et al., 2017), (ii) previous field surveys indicate a that one species is a better colonist than the other (Dubart et al., 2019, 2022; Pantel et al., 2022), and (iii) this species is less competitive in the laboratory environment, and has evolved to be even less competitive (this study). But the associations between trait syndromes and colonization/extinction dynamics in the field, though they intuitively make sense, are not a proof that these traits are actually responsible for the differences in colonization/extinction. The link we make between the two, to suggest that evolution has improved *A. marmorata* colonization ability in addition to lower its tolerance to competition, remains hypothetical. As mentioned earlier, an aspect that remains poorly documented, while crucial to this hypothesis, is the dispersal ability of this species. We

hope we have now clarified our views, and how we basically combine field and laboratory data into an inference, not an established causation, by re-writing the relevant paragraphs of the Discussion (mainly l. 513-536 and 556-580).

Abstract*: Rather than say that Aplexa marmorata evolves "towards an apparently more colonization and less competition-oriented syndrome" I suggest that you state exactly how you know they evolved, which I assume is towards earlier maturity and higher fecundity.*

The reader should know the specifics after reading the abstract, but you never give them those specifics. Note that MacArthur and Wilson were not so specific in Island Biogeography about what the properties of a successful colonizer would be. Having a fast life history was one alternative, but being able to persist in the new environment was another. Likewise, later in the abstract when you refer to how A. marmorata's life history has evolved, you again just state that it has evolved a more colonizer lifestyle, leaving it to the reader to translate what this actually means. Please just give us the facts, not what you think the facts mean. The fact is apparently that they have evolved more rapid development. Your interpretation is that this means they have evolved a colonizer lifestyle.

Yes, see our clarification efforts in response to the previous point. Regarding the Abstract, we changed the sentence to "[*Aplexa marmorata*] has responded to invasion by rapid life-history evolution towards earlier maturity, higher fecundity and higher juvenile survival, traits that might favor rapid population growth in a noncompetitive context, but not necessarily in a competitive one."
In other words, we refocus on the facts and their predicted impact on competition, which is actually tested in the manuscript, rather than on the interpretation in terms of metapopulation dynamics and "colonizer" syndrome (which is interpretation). The next sentences relate how these tests are done.
Regarding the "colonizer" concept, we think that our now more detailed account of the metapopulation dynamics of these species, reporting previously published quantitative data in Appendix, clarifies what we mean by colonization in our system: re-establishing a population locally extinct in a pond by immigrants from other ponds at most a few kilometers away in the metapopulation, a regular process renewing a substantial proportion of local populations every year. This is not really comparable to the establishment of a new species in a distant oceanic island – the traits involved need not to be the same indeed, at such different time and space scales. This is why we preferred not to allude to island biogeography to avoid confusing the reader.

Introduction*: All that is said here makes sense and is well written. There is a good review of the relevant literature and a clear statement of how the resident species has evolved in response to the invader. Lines 130 through 146 give a good summary of the earlier research that establishes that the invasion of P. acuta imposes some selection on the resident A. marmorata, but also states a reciprocal impact. The earlier descriptions lead me to expect a smaller effect of the resident on the invader than the reverse.*

This is indeed what was observed in the field (Chapuis et al., 2017), expected in the current experiments, tested as point (i) in Table 1, and eventually observed in our results. As rightly pointed out below in another comment, we do observe demographic impacts of competition by *A. marmorata* on *P. acuta* (as well as the reverse), but because all the *P. acuta* we sampled have been exposed to *A. marmorata* the same way, we have no way to

measure any potential *evolution* of *P. acuta* since its arrival. So, the evolutionary consequences of being exposed to competition with the resident species, if there were any, are impossible to study in the invasive *P. acuta* (by lack of naïve control populations). For this reason, we do not discuss whether evolution was symmetrical or not in the two species, as we observe evolution in the resident only.

Table 1*: I do not understand what the second sentence (beginning with "changing the identity of the competitor…") of the prediction for hypothesis I means. It seems to repeat what is said in the first sentence. If so, delete it. If not, then it needs to be revised but I do not know what to recommend because I do not understand the intended message.*

We revised the writing of predictions in Table 1 to make this clearer and less redundant.

Methods*: We need to be told how you provisioned the snails in the experiment. What sort of food was used, how often were they fed and was food availability limiting? This gives us some clue about the extent to which there was resource competition. Do they eat each other's' eggs or larvae?*

We added this information (l. 269-273; snails are fed with ground boiled lettuce and in aquaria the quantity is regulated so that they usually consume all the lettuce before next feeding, and therefore are in competition). Freshwater snails have direct development, a small snail directly hatching from the egg. As far as we know, they are not feeding on conspecific capsules.

Figures 2 and 3*: Amend the captions to tell us that Figure 2 pertains to Model 1 and figure 3 to Model 2 of Table 4.*

We modified the caption of both figures to include this information.

Lines 524-529*: The absence of differences between front and core populations does not tell us that P. acuta does not evolve in response to A. marmorata. Earlier in the paper they state that front populations are derived from core populations, so the all have been exposed to A. marmorata, and the new habitat in general. From the evidence given here, it seems we cannot tell whether or not P. acuta has evolved in response to A. marmorata, but can certainly say that A. marmorata has evolved in response to invasion by P. acuta.*

Yes, absolutely, we tried to check everywhere for any ambiguity but this is precisely what we want to say (the lines are now 581-589 and start with "Invasive species may also evolve in response to interaction with residents, but our design is not able to detect it, as we do not have the "unevolved" states. Indeed, all the *P. acuta* populations, be they recent (front) or ancient (core), have been founded by ancestors exposed to *A. marmorata* during the spread of *P. acuta* in Guadeloupe.")

1. Chesson, P., Mechanisms of maintenance of species diversity. Annual Review of Ecology and Systematics, 2000. 31: p. 343-+.

<u>Review 3</u>

The paper is extremely well-written and conveys complex evolutionary ecological ideas in an easy-to-follow manner. Some of the treatment combinations have low replication that limits the interpretation of some of the results. Nevertheless, I found the thought processes explaining the findings and their placement within the various ecological theories valuable and exceptional. I only have minor comments.

Comments
Abstract
Line 30: I would spell out which species (A. marmorata) evolved a colonizer lifestyle. At first read I was a bit confused. It has become clear on 2nd/3rd read but better to include the species name here.
Introduction
Line 81: then be envisaged "on the resident species". Similarly, as in the abstract I had to reread a few sentences to clarify whether these predictions refer to the invader or the native species.
Line 103: between
Line 120: delete (iii) "is"
Line 121: These 3 main hypotheses (two typos)
Line 141: at 'the' invasion front
Line 142: confronted "by"
Line 145: the same set of populations as used by Chapuis et al. 2017? Please specify.
***Table 1**. (iii). The predictions here are a bit confusing. Maybe saying (or less) and (or lower) in the parentheses would help to clarify that the predictions can be in opposite directions.*
Line 215: individuals
Line 395: addressed.
Line 397: over the resident
Line 405: "in the latter" what? Trait or species? Please clarify
420: adults of P. acuta
428: environment

All these comments were taken into account, accepting the reviewer's suggestions and/or clarifying when required.