Peer Community In Ecology

While the quoll's away, the mice will play... and the seeds will pay

Denis Réale *based on peer reviews by 2 anonymous reviewers*

Chris J Jolly, Adam S Smart, John Moreen, Jonathan K Webb, Graeme R Gillespie and Ben L Phillips (2020) Trophic cascade driven by behavioural fine-tuning as naïve prey rapidly adjust to a novel predator. bioRxiv, ver. 1, peer-reviewed and recommended by Peer Community in Ecology. 10.1101/856997

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A predator can strongly influence the demography of its prey, which can have profound carryover effects on the trophic network; so-called density-mediated indirect interactions (DMII; Werner and Peacor 2003; Schmitz *et al.* 2004; Trussell *et al.* 2006). Furthermore, a novel predator can alter the phenotypes of its prey for traits that will change prey foraging efficiency. These trait-mediated indirect interactions may in turn have cascading effects on the demography and features of the basal resources consumed by the intermediate consumer (TMIII; Werner and Peacor 2003; Schmitz *et al.* 2004; Trussell *et al.* 2006), but very few studies have looked for these effects (Trusell *et al.* 2006). The study "Trophic cascade driven by behavioural fine-tuning as naïve prey rapidly adjust to a novel predator", by Jolly *et al.* (2020) is therefore a much-needed addition to knowledge in this field. The authors have profited from a rare introduction of Northern quolls (*Dasyurus hallucatus*) on an Australian island, to examine both the density-mediated and trait-mediated indirect interactions with grassland melomys (*Melomys burtoni*) and the vegetation of their woodland habitat. Jolly *et al.* (2020) compared melomys populations in four quoll-invaded and three quoll-free sites on the same island. Using capture-mark-recapture methods, they found a lower survival and decreased population size in quoll-invaded sites compared to quoll-free sites. Although they acknowledge that this decline could be attributable to either the direct effects of the predator or to a wildfire that occurred early in the experiment in the quoll-invaded sites, the authors argue that the wildfire alone cannot explain all of their results. Beyond demographic effects, Jolly *et al.* (2020) also examined risk taking, foraging behaviour, and predator avoidance in melomys. Quoll presence was first associated with a strong decrease in risk taking in melomys, but the difference disappeared over the three years of study, indicating a possible adjustment by the prey. In quoll-invaded sites, though, melomys continued to be more neophobic than in the quoll-free sites throughout the study. Furthermore, in a seed (*i.e.* wheat) removal experiment, Jolly *et al.* (2020) measured how melomys harvested seeds in the

presence or absence of predator scents. In both quoll-invaded and quoll-free sites, melomys density increased seed harvest efficiency. Melomys also removed less seeds in quoll-invaded sites than in quoll-free sites, supporting both the DMII and TMII hypotheses. However, in the quoll-invaded sites only, melomys foraged less on predator-scented seed patches than on unscented ones, trading foraging efficiency for an increased safety against predators, and this effect increased across the years. This last result indicates that predators can indirectly influence seed consumption through the trade-off between foraging and predator avoidance, strongly supporting the TMII hypothesis. Ideally, the authors would have run a nice before-after, impact-control design, but nature does not always allow for ideal experimental designs. Regardless, the results of such an "experiment in the wild" predation study are still valuable, as they are very rare (Trussell *et al.* 2006), and they provide crucial information on the direct and indirect interactions along a trophic cascade. Furthermore, the authors have effectively addressed any concerns about potential confounding factors, and thus have a convincing argument that their results represent predator-driven demographic and behavioural changes. One important question remains from an evolutionary ecology standpoint: do the responses of melomys to the presence of quolls represent phenotypically plastic changes or rapid evolutionary changes caused by novel selection pressures? Classically, TMII are assumed to be mostly caused by phenotypic plasticity (Werner and Peacor 2003), and this might be the case when the presence of the predator is historical. Phenotypic plasticity allows quick and reversible adjustments of the prey population to changes in the predator density. When the predator population declines, such rapid phenotypic changes can be reversed, reducing the cost associated with anti-predator behaviour (*e.g.*, lower foraging efficiency) in the absence of predators. In the case of a novel predator, however, short-term evolutionary responses by the prey may play role in the TMII, as they would allow a phenotypic shift in prey's traits along the trade-off between foraging efficiency and anti-predator response that will probably more advantageous over the longer term, if the predator does not disappear. The authors state that they could not rule out one or the other of these hypotheses. However, future work estimating the relative importance of phenotypic plasticity and evolutionary changes in the quoll-melomys system would be valuable. Phenotypic selection analysis, for example, by estimating the link between survival and the traits measured, might help test for a fitness advantage to altered behaviour in the presence of a predator. Common garden experiments, comparing the quoll-invaded and the quoll-free melomys populations, might also provide information on any potential evolutionary changes caused by predation. More work could also analyse the potential effects on the seed populations. Not only might the reduction in seed predation have consequences on the landscape in the future, as the authors mention, but it may also mean that the seeds themselves could be subject to novel selection pressures, which may affect their phenology, physiology or life history. Off course, the authors will have to switch from wheat to a more natural situation, and evaluate the effects of changes in the melomys population on the feature of the local vegetation and the ecosystem. Finally, the authors have not yet found that the observed changes in the traits have translated into a demographic rebound for melomys. Here again, I can see an interesting potential for further studies. Should we really expect an evolutionary rescue (Bell and Gonzalez 2009) in this system? Alternatively, should the changes in behaviour be accompanied by permanent changes in life history, such as a slower pace-of-life (Réale *et al.* 2010) that could possibly lead to lower melomys density? This paper provides nice in natura evidence for density- and trait-mediated indirect interactions hypotheses. I hope it will be the first of a long series of work on this interesting quoll-melomys system, and that the authors will be able to provide more information on the eco-evolutionary consequences of a novel predator on a trophic network.

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Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/856997
Version of the preprint: 3

Authors' reply, 10 July 2020

Editor's Comments

Dear authors, Both reviewers have appreciated your changes and considered that the paper has been improved a lot. One reviewer still has a few comments that should be dealt with easily. I encourage you to do these changes and resubmit a new draft of the paper before I recommend your preprint. Best wishes Denis Réale

Response: Thank you Prof. Réale. Your feedback has significantly improved the manuscript. We have made all of Reviewer 1's suggested changes.

Review's Comments Reviewer 1: Comments

In this manuscript the authors present new empirical data of a predator-introduction experiment in natural settings. Following the introduction of an ecologically (but not evolutionary) novel mammalian predator, they monitored mammalian prey population size and dynamics, behavioural responses towards novelty and seed predation in replicated populations for several years. They found changes in prey demography and behaviour that might have cascading effects on lower trophic levels due to direct effects (reduced population size of consumers) and indirect effects (reduced seed predation by the consumer under predation risk). Prey in

predator exposed populations had lower survival, faced deceasing population size, expressed lower levels of neophobia-related behaviours in novel-environment and novel-object tests, and removed less seeds from risky foraging patches. Overall, this is an interesting experimental case study for those interested in behaviour-mediated indirect ecological interactions and the study is highly suitable for an ecological journal. In this revised version of the manuscript the authors followed most of my (and the other's) suggestions. This version is largely improved, reads well, has clear figures, and includes all necessary detail. In my taste the discussion is still a bit lengthy in parts, but this is more for editorial decisions of the particular journal it will end up in. I only have a few very minor remarks (below).

General comments: All my general comments have been taken up and I do not have any additional major comments.

Specific comments: Comment: Introduction L100: In my view the predictions for the behavioural changes could be more nuanced. I do not think that in a predator-present population "foraging rates" in general decline but they might do in your specific experimental setting. Response: Good point. We have broadened the expectations of our predictions by not placing expectations on the direction in which they may change. Evidence suggests it can be unpredictable and may go either way. Now: "If behavioural adjustments are able to reduce the demographic effects of a novel predator, we predict rapid behavioural changes in quoll-exposed melomys populations, such as changes in personality composition, foraging behaviour and responses to predator-scent, may manifest through time."

Comment: Methods L129: "quoll-free sites" Response: Good catch. Pluralised from "site" to "sites".

Comment: L329: "number of seeds harvested as a linear function" Response: Added the missing "as" between "harvested" and "a".

Comment: L336: Please give R version used. Response: Added "R version 3.3.2"

Comment: Discussion L450-452: This sentence is hard to understand, consider rephrasing; What do you mean with "...that applies to the landscape becoming a fine-grained response..."? Reponse: Good point. Changed to "Thus, we see a reduction in seed take resulting in a fine-scaled aversive response varying on a spatial scale measured in the tens of metres."

Comment: The authors did a great job in discussing the potential impacts of confounds on their findings and make clear judgments. Response: Thanks you. We appreciate the feedback. And thank you for the detailed review, we agree that it has improved the manuscript.

Reviewer 2: Comments

Comment: I thank the authors for addressing all my comments. I am satisfied with the revisions made and have no other major comments to make. I congratulate the authors on a really nice manuscript.

Response: Thank you. We appreciate the feedback and the effort you went to in reviewing this manuscript. It was much improved by your suggestions.

Download author's reply

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Decision by Denis Réale, posted 06 July 2020

minor revision

Dear authors,

Both reviewers have appreciated your changes and considered that the paper has been improved a lot. One reviewer still has a few comments that should be dealt with easily. I encourage you to do these changes and resubmit a new draft of the paper before I recommend your preprint.

Best wishes

Denis Réale

Reviewed by anonymous reviewer 2, 29 June 2020

Review of the revised version of the manuscript Trophic cascade driven by behavioural fine-tuning as naïve prey rapidly adjust to a novel predator

In this manuscript the authors present new empirical data of a predator-introduction experiment in natural settings. Following the introduction of an ecologically (but not evolutionary) novel mammalian predator, they monitored mammalian prey population size and dynamics, behavioural responses towards novelty and seed predation in replicated populations for several years. They found changes in prey demography and behaviour that might have cascading effects on lower trophic levels due to direct effects (reduced population size of consumers) and indirect effects (reduced seed predation by the consumer under predation risk). Prey in predator exposed populations had lower survival, faced deceasing population size, expressed lower levels of neophobia-related behaviours in novel-environment and novel-object tests, and removed less seeds from risky foraging patches.

Overall, this is an interesting experimental case study for those interested in behaviour-mediated indirect ecological interactions and the study is highly suitable for an ecological journal. In this revised version of the manuscript the authors followed most of my (and the other's) suggestions. This version is largely improved, reads well, has clear figures, and includes all necessary detail. In my taste the discussion is still a bit lengthy in parts, but this is more for editorial decisions of the particular journal it will end up in. I only have a few very minor remarks (below).

General comments All my general comments have been taken up and I do not have any additional major comments.

Specific comments Introduction L100: In my view the predictions for the behavioural changes could be more nuanced. I do not think that in a predator-present population "foraging rates" in general decline but they might do in your specific experimental setting. Methods L129: "quoll-free sites" L329: "number of seeds harvested as a linear function" L336: Please give R version used. Discussion L450-452: This sentence is hard to understand, consider rephrasing; What do you mean with "...that applies to the landscape becoming a fine-grained response..."? The authors did a great job in discussing the potential impacts of confounds on their findings and make clear judgments.

Reviewed by anonymous reviewer 1, 28 May 2020

I thank the authors for addressing all my comments. I am satisfied with the revisions made and have no other major comments to make. I congratulate the authors on a really nice manuscript.

Evaluation round #1

DOI or URL of the preprint: **10.1101/856997**
Version of the preprint: 1

Authors' reply, 27 April 2020

Editor's Comments

Dear authors, Comment: Two reviewers have now read and commented on your preprint. They both found the paper interesting, but also made important constructive comments to help you improve it. These changes must be made before I could recommend your article. Having read the manuscript myself, I share the reviewers' general positive feelings about the study, and have made a few comments on it myself.

Response: Thank you for your consideration and comments. We have made the changes suggested by both you and two anonymous reviewers. Where we have not made suggested changes, we have provided justification for our decision.

Comment: Please clarify your research objectives at the end of the introduction. For now, you only mention that you want to "… test for the effects of quolls as a novel predator on an island ecosystem and observe how native prey adjust to mitigate the impacts of their arrival", but it would be important to be more specific: you are looking at survival rates, population size, exploration behavior (or boldness if this is what you prefer), and seed predation by Melomys. Ideally you may present hypotheses, but only at the condition that these hypotheses have been built up prior to conducting the study (I guess that you have set up these experiments and surveys based on some hypotheses).

Response: Good point. Additional lines in the final paragraph of the introduction have been added that clearly state our aims and predictions.

Comment: As you mention briefly in your discussion, your data will allow you to test whether the changes observed through time are caused by natural selection or plastic response to predators (i.e. individuals change their behavior as a response to predation risk). However, you seem to put more emphasis on the second hypothesis. For example, you say in the discussion that Melomys "adjust to mitigate the impacts of Quolls arrival", our you interpret your results as population adjustment to predation through reemergence "of appropriate antipredator response" (485-494). I find these interpretations speculative. I would rather suggest you to discuss your results by presenting these two hypotheses as alternative without towards one of the other.

Response: We suspect that you mean that our "data will (NOT) allow (us) to test whether the changes observed through time are caused by natural selection or plastic response to predators"? We certainly agree, but we suspect the problem you have identified is a semantics issue, rather than an attempt by us impose an opinion of the process of change. We genuinely suspect both processes are potentially acting to enact the changes we observe in the prey populations, and clearly state this in lines 471–472 (original ms). We chose the word "adjust", instead of "learn", "plastic", "evolve", "select" etc because we thought this word may be less tightly associated with one process or another. Populations can adjust to the arrival of a novel predator via natural selection, through plastic behavioural responses of individuals or both, but we do not want to make a judgement about what is acting here because we do not possess appropriate data. We reassessed our use of terminology throughout the manuscript to ensure that we cannot be misinterpreted as suggesting the population is changing due to any one mechanism. Additionally, we have made an effort to avoid speculating on the processes of the changes we have observed.

Comment: I do not think that after a 3-year study you can conclude that Melomys has mounted totally effective defences against the quolls. Population size has gone under 20 individuals on your predated grids, and may still be maintained by immigration from other parts of the island with less predation (i.e. Monsoon vine thicket). The chance of extinction does not seem negligible.

Response: This is a good point. We have now clarified that we do not know whether these populations are saved by this process of adjustment to the novel predator, and that extinction is still a possibility.

Comment: I am surprised that you did not consider a model where recapture rate differed between quoll-free and quoll-invaded sites (L 247-252 and Table 2). All your open-field tests and seed-removal results would suggest that melomys may have a lower probability entering traps than in the predator-invaded sites than in the predator-free sites. Unless p(g) in Table 2 means that you included a case where recapture probability differed between the two types of sites, for now I do not see evidence that you have consider that scenario, which may change a lot the results. Please clarify what p(g) means in the main text and the Table legends or add some models that will include different recapture probabilities.

Response: Thanks Prof. Réale, you raise a good point. We did in fact consider models where recapture probabilities differed between the two sites p (g). None of these models were well supported. We have now run some additional models, where recapture probabilities could differ with group and time, and again, they were not supported. We have clarified what p (g) means in the main text and legend, and have added some additional models to Table 1, as suggested. We also have an earlier paper that shows capture probability is unaffected by behavioural traits, because neophobia plays out over minutes, whereas we have a high density

of traps that are out for many hours (Jolly et al 2019). We now cite this paper also.

Comment: I am not sure that behavior can respond faster to predation pressures than other types of trait. See the recent study by Barrett et al. 2018 (Science 363: 499-504), where they show that fur color in Peromyscus maniculatus could change within a few years as in your system.

Response: We are not sure where specifically in our ms this comment is referring to? We agree that evolutionary changes to population behaviour via natural selection are not necessarily more rapid than change along any other evolutionary axis. However, behavioural change can and does occur in an individual's lifetime, often within hours as opposed to changes across generations. Behaviour is labile to the point that it can allow animals to mount an immediate response (via learning) to a pressure (i.e. quolls immediately learn to avoid toads after they have a non-fatal encounter with one). Morphology, physiology or life history changes typically require an evolutionary response, which wouldn't appear immediately.

Reviewer 1: Comments

Review of the manuscript Comment: In this manuscript the authors present new empirical data of a predator-introduction experiment in natural settings. Following the introduction of an ecologically (but not evolutionary) novel mammalian predator, they monitored mammalian prey population size and dynamics, behavioural responses towards novelty and seed predation in replicated populations for several years. They found changes in prey demography and behaviour that might have cascading effects on lower trophic levels due to direct effects (reduced population size of consumers) and indirect effects (reduced seed predation by the consumer under predation risk). Prey in predator exposed populations had lower survival, faced deceasing population size, expressed lower levels of neophobia-related behaviours in novel-environment and novel-object tests, and removed less seeds from risky foraging patches. Overall, this is an interesting experimental case study for those interested in behaviour-mediated indirect ecological interactions and the study is highly suitable for an ecological journal. However, the study has a few limitations. First, there is virtually no data of prey demography and behaviour from before the introduction of the predator. Second, predator-free (control) and predator-exposed (treatment) populations differ in several other confounding aspects. There are systematic differences in population sizes between control and treatment populations (see Fig. 2); some of the changes in treatment populations could (theoretically) also be due to the population decreasing towards sustainable levels. More importantly, only the treatment populations were affected by a wildfire shortly after the predator introduction. Certainly, this was beyond the authors control, but as they admit and discuss, it is a systematic bias impairing some of the results. The authors make a good job in addressing the last point and in arguing why the wildfire effects cannot solely explain their results; they might add similar considerations for the other systematic biases as well. Overall, the manuscript is well presented and results are illustrated in clear figures. Still, the manuscript would gain much from a thorough revision. Below I made some general suggestions and specific remarks.

Response: We kindly thank this reviewer for their detailed review. We have made all suggested changes and have provided justification where we thought the suggested changes were inappropriate. We have added an additional paragraph to the discussion to address the potential that a spatial confound and population cycling could explain the population changes we observed. The manuscript has been improved substantially by addressing your comments.

General comments

Comment: (1) Introduction: Overall the introduction introduces most relevant concepts but would gain much from clearer and more concise style. Please avoid jargon and global "we"-formulations. Clearly define concepts, such as landscape of fear. Instead of giving the main results away (L82-85), I would suggest to formulate a general aim of the study. Clear hypotheses and predictions are missing. Please provide a priori hypotheses and predictions in the introduction to avoid the impression that all analyses have been decided upon post-hoc data collection.

Response: We have now addressed all the above comments. See below for associated, specific changes we have made in line with these suggestions.

Comment: (2) Methods: There are a few issues with the methodology (behavioural tests, seed removal test), that need more explanation in the methods. Moreover, you might want to discuss their potential effects on the results in the discussion (see details below). I am not an expert on capture-mark-recapture statistics but also in the description of the stats, some more details are needed.

Response: Thank you for your comments. Please see below where we have addressed them directly.

Comment: (3) Discussion: Since there are systematic biases between control and treatment populations, which each reader has in mind throughout the manuscript, I would suggest to discuss them right away at the beginning. This would mean moving L502-537 up front and present it as the 2nd paragraph of the discussion. Please shorten this part to the most necessary arguments and add some discussion of other confounds (see above). This will make the discussion stronger because readers will focus on your results and conclusions that are robust against the problems. Furthermore, the discussion is very lengthy in parts and needs a thorough overhaul. The structure of the discussion is not always obvious and it seems to go back and forth. Please make an attempt to streamline the discussion, maybe add subheadings that guide the reader, remove all repetitions and reduce speculation.

Response: Thank you, we agree that this greatly improves the discussion. We have moved the discussion of confounds to paragraph 3 in the discussion, as it seems better placed here.

Specific comments

Title

Comment: In my view the title is too bold and not unambiguously supported by the data.

Response: We disagree with this comment. In this study, we provide data and analysis to support each aspect of the title. We observe a trophic cascade (novel predator affects prey altering seed predation), which is driven by behavioural changes mounted by prey in predator-exposed, but not predator-free populations.

Abstract

Comment: Could be shorter and more concisely written. Add sample size and number of replicates to abstract. Use scientific species names in abstract as well.

Response: It is not clear to us where to add sample sizes other than to the number of populations measured, but we have now added these details. Additional details are available in the methods. Since sample sizes to individual animal level change between sites by session, it seems neither appropriate nor intuitive to include these details in the abstract. We have added species names.

Comment: L26: Here and in the introduction, please be specific to what kind of plasticity you refer to (see e.g., Piersma & Drent 2003 TREE).

Response: We have now specified that the plasticity we are referring to is phenotypic plasticity. We do not, however, believe it is appropriate to be any more specific than that.

Comment: L29: Please be specific, you see change in mean behavioural phenotype at the population level. “Behavioural responses” to me sounds like you observed individuals to change their behaviour.

Response: Good point. We have now clarified this.

Comment: L43: Rephrase “as the nature of the threat become clearer”, sounds very anthropomorphic.

Response: Good point, this phrase has been removed and replaced with: “Presumably the significant and novel predation pressure induced by quolls drove melomys populations to fine-tune behavioural responses to be more predator-specific through time.”

Keywords

Comment: Please use keywords that are not part of the title.

Response: Good point, we have removed replicated keywords and added new ones.

Introduction

Comment: L52: Delete sentence “Although… a constant.” Not needed.

Response: Deleted.

Comment: L55: Reference missing?

Response: We have moved the mid-sentence references to the end of the sentence

Comment: L67: Consider rephrasing “We typically think…”. I do not think this is true.

Response: Global “we” removed and sentence revised.

Comment: L70-72: Can be deleted without losing information.

Response: We initially complied with this suggestion and removed the sentence, but after having done so we have decided we disagree with this comment. This sentence sets up the premise that behavioural, as well as demographic responses, may be mounted due to the arrival of a novel predator. Without this sentence, the next makes little sense.

Comment: L72: Please clearly define “landscape of fear”.

Response: Since neither reviewer liked this phrase it has been removed.

Comment: L85: You did show that prey avoided artificial seed patches with predator odor more in the predation treatment plots than in the control plots. In my view this result is not yet a “measurable change in downstream trophic interactions”. Please avoid over-interpretation.

Response: This sentence has now been removed.

Comment: L99: Avoid jargon. What is “toad-smart”.

Response: Removed.

Methods

Comment: L159: How do the captures distribute over the different sites?

Response: This information has been added to the methods.

Comment: L168: What happened with the individuals between the night of capture and the night of testing?

Response: Melmoys were retained between the night of capture and the night of testing within Elliot traps at the field station. Additional information has now been added to the ms. Much more detailed information and justification for methodology is provided in Jolly et al. 2019, but much of this information is beyond the scope of this study and would likely be relegated to supplementary material. Whether it should be included as supplementary material in this manuscript or left as a reference to previous, peer-reviewed manuscript is probably an editorial decision. We would be happy to provide this information in supplementary material if requested.

Comment: L175: What is a bait ball? Why did you provide food in the novel environment test? This is unusual and affects the behavioural measurements. and Since individuals will differ in energetic state and hunger, the bait will differ among individuals in its reward value. This difference in reward value will incur differences in motivation to enter into the novel environment. As a result, variation in motivation cannot be disentangled from variation in neophobia. Please discuss briefly, whether and how these aspects might have affected your results.

Response: Bait balls are described in L152, but we have now changed the terminology so it is clearer. The bait described is peanut butter, oats and honey rolled into a ball, which is the same bait used for trapping throughout our study. This is a very reasonable point which we didn’t make clear in the ms. We controlled for potential differences in motivation for resources by providing food ad libitum until 2 hours prior to the start of the experiment, when all individuals had their food removed. In a previously published study we included sex and size as factors when analysing this behavioural data and found neither affected behavioural phenotype (Jolly et al. 2019). We also avoided testing any animals that were clearly injured, unwell, underweight or pregnant (L196–197).

Comment: L183: Please provide more details on the novel object, size?

Response: Some details were provided in L181–182 and we have added additional detail here.

Comment: L187: These previous results are based on the same individuals? Please provide a repeatability value?

Response: Yes, these results are based on the same individuals, but only individuals from sessions 1 and 2. Repeatability scores from that study have now been added.

Comment: L195: Define “made contact”.

Response: We define “made contact” as touched. For clarity we have deleted “made contact” and changed it to “touched”.

Comment: L201: This means you divided all individuals only in two behavioural types, right?

Response: Yes, essentially melomys were classed as either “shy” or “bold”.

Comment: L212: Is wheat available in the natural habitats, or is this a novel food? If it is a novel food, your results on seed removal are also affected by neophobia/neophilia because individuals scoring high will have a higher probability to remove the seeds and this might interact with the predator odour cue (another novelty for the control individuals). Please provide more details and discuss these issues.

Response: Yes, wheat seeds are novel to melomys. We chose these because they posed a low weed risk and were easily available. Also, there were no native seeds that were easily accessible and abundantly available to us on the island. Natural seeds, however, are clearly available to melomys given they are granivores. Despite that, the novelty of seeds is common to all populations regardless of predator exposure, so it does not confound our results.

Comment: L215: If your predator-cue seeds smell strongly and are only placed 10 m apart, for a microsmatic animal the whole area will smell like predator. Did you do some pilot tests to ensure that the animals perceive a difference in predation risk between your treatment levels?

Response: While this is possible, and we did not test this directly in a pilot, we found there was no change in the seed harvesting behaviour of the quoll-free melomys populations, nor was there a difference in the number of control vs scented seeds they removed, and there was a distinct change in behaviour of quoll-invaded melomys populations towards predator-scented seed aversion. We should not observe a difference in the proportion of predator-scented vs control seeds in this population if melomys did not detect a difference in the risk associated with predator-scented seeds compared to unscented seeds. We, therefore, do not believe this confounds our results.

Comment: L245: I would suggest to move the hypotheses and predictions to the introduction.

Response: We have added predictions/hypotheses to our introduction. We do not think they are out of place here too, given we are specifically referring to hypothesis testing.

Comment: L246: Please use consistent terminology for your treatment levels; throughout the manuscript you switch between many.

Response: We now refer to treatment levels consistently as quoll-invaded (impact) or quoll-free (control) throughout the ms.

Comment: L252: Please add the criterion you used to evaluate models, e.g. a difference of 2 in AIC between models.

Response: We have now added this to the ms. “Models with AICc values < 2 were considered to be well supported by the data (Burnham & Anderson 1998). We used Akaike’s Weights, which are proportional to the normalized, relative likelihood of each model, and to determine which of these models was most plausible (Buckland et al. 1997).”

Comment: L256: Please explain in more detail how population size, your dependent variable, is “estimated in this process”.

Response: This is explained in the description of the analysis below this point.

Comment: L262: Define O and ds.

Response: All terms have now been defined in text.

Comment: L274: Why did you use this approach? What is the advantage of adding “zero-only” individuals to the data set?

Response: This is a standard approach to this particular problem. It converts the modelling framework into a Binomial rather than a Poisson process; the former being the more robust.

Comment: L279 and following: please define every term used.

Response: We have now defined Omega as the “proportion of the padded population that is real”.

Comment: L290 and following: If you divided the response into proportions, then each session is your

sample size? Please be specific here. Why did you not use binomial models with each individual being a sample?

Response: Apologies. That was poorly described. We did analyse it as a binomial response, but we graphically display this data as a proportion across sites in the figure. We have revised this section to clarify. In this case, it matters not to the likelihood whether we treat each individual as a sample or aggregate to population. We chose the former description.

Comment: L301: Total number of seeds at one site?

Response: Agreed. This wasn't clear but we have now clarified this sentence.

Comment: State significance level.

Response: This comment seems out of place and does not refer to anywhere specific.

Results

Comment: L: 314: Delete "When …. between sessions."

Response: Deleting this does not improve interpretation. In fact, it makes the results less clear. We believe this sentence fragment should not be deleted.

Comment: L341: Please move all interpretation of results to the discussion section, e.g. "suggesting…"

Response: Sentence removed.

Comment: L358: Please provide post-hoc tests for significant interactions and also indicate the results of the post-hoc tests in figure 3. (see also comment above, why not run binomial models to test the probability of emerging instead of population-level measures (proportion).

Response: We did in fact run binomial models to test the probability emerging, but for ease of interpretation we displayed them graphically as population proportions. We have now clarified that in our explanation of the statistical methods. Pairwise post hoc analyses can only be done on comparisons between factor levels, but in this analysis session (time) is treated as a continuous variable, so we cannot compare for pairwise effects. We have clarified this in the description of the statistical approach in the methods.

Comment: L363: Delete "on neophobic behaviour…. test" – the rest of the sentence states the result.

Response: Deleted.

Comment: L380 and following: Please report only results here and leave the interpretation for the discussion.

Response: These lines have been deleted from the results.

Discussion Comment: See also comments above. The structure of the discussion is not always obvious and it seems to go back and forth. Please make an attempt to streamline the discussion, maybe add subheadings that guide the reader, remove all repetitions and reduce speculation.

Response: The discussion has now been thoroughly revised to reflect the comments of both reviewers and the editor.

Comment: Please remove references to figures from the discussion. This is part of the results.

Response: Deleted.

Comment: L414: The few years encompassed by the study appear too few to allow natural selection to shape behavioural variation in the population. Phenotypic plasticity appears more likely as a mechanism. Are there any individuals that you tested twice across years or trapping sessions? Are there any indications of lower survival probability of bolder types? What do we know from other systems about the speed of microevolution?

Response: We disagree. If behavioural traits are innate and heritable (we know they can be), natural selection on adaptive behavioural traits can act immediately on a population. In this system, if all the bolder animals are killed by predators and behavioural traits are fixed within individuals, there is an immediate selection-driven shift in population-level phenotype. This will be transmitted to the next generation according to how heritable the trait is. Given both phenotypic plasticity and natural selection are both possible, nor are they mutually exclusive, we don't think it is appropriate to speculate which is more likely given our data. Although there are a handful of individuals tested between sessions, there are too few for formal analysis. Our approach is to be very clear that we cannot test between these possibilities, and we feel this is the most objective approach.

Comment: L420: These conclusions appear far-fetched. Individuals avoided taking seeds heavily smelling

like predator when other seeds were available nearby. This is an interesting result and a good attempt to quantify potential cascading effects of predation risk. However, it is not yet a proof of such effects. I would suggest tuning these conclusions down a bit.

Response: We agree that we are slightly short of perfect proof of cascade (we have demonstrated lowered predation on grass seed, but not the grass's numerical response to that predation), and have tuned that aspect of the paragraph. Much of the paragraph has been retained, however: it is our best interpretation of our results, we then go on in further paragraphs to examine how this interpretation might be incorrect. This seems a reasonable approach.

Comment: L426: You might want to cite here as well Sih et al. (2010, Integrated Comparative Biology 50:6).

Response: Added.

Comment: L438: In the manuscript you constantly change between neophobia, boldness and the variable names. This is confusing and misleading. Please clearly define/interpret your behavioural measures once and use the same terms throughout. Please see Carter et al. 2012 (Biol Rev) for guidance and fallacies.

Response: Thank you, that was a poorly written sentence and was quite misleading. We now refer to "boldness" as a willingness to emerge and "neophobia" as a willingness to emerge and interact with the novel object consistently throughout the ms.

Comment: L439-L443: Here and in the following there is a lot of speculation, please remove this and stay with the facts.

Response: We agree that the first sentence highlight was too speculative and has been removed. The following sentence was speculation on behaviour as dynamic trait in general, and speculation here is not unwarranted.

Comment: L441: What is the "behavioural state of a population"?

Response: We are referring to the behavioural composition of the population. We have clarified this.

Comment: L455: Could you test and compare behavioural-type specific survival across sessions? This would be a strong indicator of selective disappearance.

Response: Indeed. And the reason this isn't possible is explained in the paragraph starting L452.

Comment: L452-472: Please shorten, there is a lot of speculation here.

Response: We have largely left this paragraph unchanged. There is very little speculation here, and this is a genuine attempt to make it clear to the reader that we are not claiming one particular mechanism (selection vs plasticity).

Comment: L495: Please delete whole paragraph; these applied aspects are not directly related to your study/results and break the flow of the discussion.

Response: Deleted.

Comment: L502-537: Shorten this paragraph to half and move it further up (see above).

Response: This paragraph has been moved further up, but we disagree that much information can be removed from this paragraph. It provides a thorough justification for why, in this system, the fire is unlikely to explain the results we observe.

Comment: L538-566: Many aspects in this part have been discussed before.

Response: Although many aspects of this section have been discussed earlier in the discussion, we believe these final paragraphs act to conclude the manuscript and provide a rounded, global explanation for the results we observe.

References

Comment: The reference list needs serious proof-reading (species names in italic, missing page numbers, missing journal names, etc.).

Response: References have been proof-read and are now error free.

Tables and Figures

Comment: All figures, please use higher contrast for black-and-white printing.

Response: Good point and one that we did consider. We have tested this by printing these figures in black-and-white and they are quite distinct.

Comment: Fig.1: You might consider showing all sites separately.

Response: Given there's not much variation between sites (relatively small 95% CI), we believe that the results are more easily interpretable as means with their associated error.

Comment: Fig.2: Very nice figure, please explain symbols.

Response: Symbols simply denote different sites. This has been added to the figure legend.

Comment: Table 1: Show less digits after comma.

Response: Presumably, you are referring to Table 2? We have reduced all digits to 2 decimal places. Reviewer 2

Comment: This is an interesting paper that examines the impact of a novel predator (northern quoll) on an island population of melomys (a type of rodent). They found that melomys populations that are exposed to quolls suffered decreased survival and population declines. They also found that melomys from predator-exposed populations initially adopted shyer behavioural tendencies, but that this behavioural response became more refined over time and was eventually replaced by a more threat-specific, scent aversion antipredator behaviour. This result suggests that melomys were becoming better at dealing with quoll predation. Finally, they also found evidence that the seed intake rates of melomys in predator-exposed sites substantially decreased, potentially suggesting that the quoll introduction could be causing trophic cascades at multiple biological levels. This is a nice study. The authors had the rare opportunity to examine the impacts of a re-colonization program of a predator (i.e. quoll) on island populations and communities. This study represents a lot of hard work, which I commend the authors for. The results are interesting, albeit not that surprising, but the study offers a nice natural example of how novel predators can shape prey behaviour and the structure of ecological communities. Overall, I thought the experimental design was sound, but there are areas throughout the manuscript that need refinement and further justification, particularly in terms of the interpretation of the results.

Response: Thank you kindly for your supportive and thorough review. The manuscript has been improved substantially from addressing your comments. We have made all suggested changes and have provided justification where we thought the suggested changes were inappropriate.

Introduction

Comment: Line 57 – 61. I'm not a big fan of breaking up a sentence with a colon. It makes this sentence sound clunky and disjointed. Consider revising

Response: Replaced with a full stop.

Comment: Line 67 – 69: Same comment as above.

Response: This is the appropriate use of a colon. "The colon is used to separate two independent clauses when the second explains or illustrates the first." Since this is a matter of personal taste, we have decided to leave this sentence as it was.

Comment: Line 72: I would consider not using the term 'landscape of fear'. Some people don't like it, and to avoid people getting upset, I think you could just say "prey species living with predators".

Response: We were not aware of a dislike for this term. Since Reviewer 1 also requested it be changed, we have removed the phrase entirely. Now "Prey species living alongside predators".

Comment: Line 74: replace "will' with 'can'

Response: Good point. Changed.

Comment: Line 81 – 82: I would replace 'advance' with improve and then revise the last part of this sentence. How exactly does this improve our understanding of how communities are structured? Do you mean before or after invasions?

Response: "Advance" replaced with "improve". The second part of this sentence has been clarified.

Comment: Line 82 – 85: I would remove this last sentence. I think this is better said in the last paragraph. You also repeat more or less the same thing on lines 95 – 98 and on lines 103 – 105.

Response: Good point, we had not noticed the repetition. Lines 82–85 have been deleted. Lines 95–98 were moved to replace lines 103–105.

Comment: Line 95 – 96: 'We take advantage of….'

Response: "Exploit" was changed to "take advantage of" and this sentence has been moved to final introduction paragraph.

Comment: Line 95 -98: You say something similar to these 3 times in your introduction. I think it should be integrated more seamlessly, and when you say it the first time you should then tell us why this is such a good opportunity.

Response: Good point. We have now addressed this repetition.

Comment: Line 99: What does 'toad-smart' mean? I know what you mean, but others probably don't. I would probably just remove it.

Response: Good point. As requested by both reviewers, this jargon has been removed.

Comment: I think you should provide a clear set of hypotheses.

Response: As requested, hypotheses have now been built into the final paragraph of the introduction.

Methods

Comment: Methods I think overall, I found the structure of the methods a little hard to follow. I had to go back and forth at times to work out.

Response: We have now reviewed the methods and attempted to clarify any points of confusion.

Comment: Line 112 – 115: This is forth time that you mention the re-introduction as being a unique opportunity. I would remove this, but I would actually mention these specifics in the introduction the first time you talk about the re-introduction offering an opportunity to measure novel predator-prey dynamics.

Response: Repetition now addressed. However, we disagree that these specific details of the study species biology belong in the introduction.

Comment: Line 115 – 120: I found this part confusing. You mention three plots, but you drop two of the plots because quolls didn't use them. How big are these plots exactly? Why mention the plot you didn't actually use in this study? Also, how do they differ (or not) to the sites that you mention later on lines

Response: We include these lines because they provide context about why we missed an opportunity to do a more robust BACI designed experiment. This has now been clarified in the following lines. Thus, we only have data from a single "invaded" site before the introduction of quolls

Comment: 129 – 130? I might be worth considering incorporating a map outlining your plots into the manuscript.

Response: Good point, we have now added a map but have also retained the distance matrix to provide some information about relative distances between sites. The distance matrix can be removed or relegated to supplementary material if it appears redundant alongside a map.

Comment: Line 131 – 133: I think this site information should be presented before you talk about the sampling dates

Response: This paragraph has been rearranged to reflect the reviewer's suggestions.

Comment: Line 137 – 137: I would remove this sentence given that your next sentence helps to confirm this

Response: This sentence has been removed.

Comment: Table 1: I found this table not very informative. A map that outlines your plots and how far apart they are would be much more informative. You could also have different colour codes for sites with predator's vs those without predators. Just a suggestion.

Response: See comment above. We have added a map, but have retained the distance matrix.

Comment: Line 151 – 152: How did you know that you trapped the majority of the population? What were the population numbers for each site before quoll's were introduced? – you could incorporate this latter information into the map above

Response: We tested this directly in the Jolly et al 2019.

Comment: Any chance of behavioural-dependent trapping success? (see Dingemanse & Biro 2009, TREE)

Response: We tested this directly in Jolly et al 2019. There is no effect of personality (boldness or neophobia) on the willingness of melomys in these populations to enter and being trapped by Elliot traps.

Comment: Line 186 – 187: I think you should provide the repeatability estimates here (i.e. give the reader some indication of how repeatable these behaviours are without them having to go and look at the other paper).

Response: Good point. As this was requested by both reviewers, we have now provided these details in the ms.

Comment: Bummer about the wildfire!

Response: Tell us about it……. Very big bummer!

Comment: 198 – 202: I suppose I fail to see the link between neophobia of a plastic plate and predator avoidance. Why do the neophobia assay in the first place? Maybe some justification for these tests here would help.

Response: Neophobia is predicted to be linked to antipredator behaviour. When a specific antipredator response is lacking, a more general response, such as generalised neophobia, may affect survival after the arrival of a novel predator. Neophobia is regularly tested using a novel object that would have never been encountered during an individual's lifetime or during the evolutionary history of the species. We are less interested in specific antipredator behaviours with this tests, and more interested whether generalised neophobia in a population could be selected for following the arrival of a novel predator. Since we provide exploration of this in the discussion, we're not convinced it appropriate to repeat it here.

Comment: Line 274: What is site.session?

Response: site.session denotes site per session. What we are estimating is the population size at each site, during each session, which we denoted as "site.session". We agree that this wasn't clear before, but we have now clarified this.

Comment: It was hard for me to comment on some of the statistics because I am unfamiliar with some of the modelling used, however it seemed weird to me that you switched between a Bayesian framework and a frequentist framework for some of the stats. I am curious as to why the authors did this and why not just be consistent?

Response: This was done simply because we had the expertise to do a closed population model via a Bayesian framework, but not for an open population. Both statistical methods are valid, and the results will converge.

Results

Comment: Line 320 – What does the 'S' value represent?

Response: S represents "Survival" between sessions/years. We agree that this wasn't clear and have clarified it.

Comment: Link 321 – 323: Is there a statistical way of disentangling the effect of the wildfire and predation on these survival numbers?

Response: As far as we're aware, unfortunately, there is not. Because the arrival of predators and the wildlife event is confounded at most sites, there is no statistical way of disentangling them. We wish there was! We do, however, provided a thorough justification for why we think predation is the more parsimonious explanation for our results.

Comment: Figure 1: It might be useful to include what the initial population sizes were for comparative purposes. From this figure, it looks like both populations declined through years, and that the degree of decline isn't that much larger than for the quoll present population. This also makes me think that this decrease through time might also just be a result of your trapping success decreasing through time (as melomys habituate to the traps themselves)?

Response: This figure denotes survival (change in recapture rates of known individuals). It seems inappropriate to include population sizes here. Additionally, if we had the initial population sizes for these populations (before quolls) we would have included them. This would have considerable improved our ability to assign

causation of demographic changes to predation. Unfortunately, we only have an initial population size estimate for one invaded site, which we do present in Figure 2. We can, however, rule out habituation as affecting trapping success since we tested for this directly in Jolly et al 2019. Melomys do not habituate with traps within trapping sessions (Jolly et al 2019). Additionally, since we have such low survival between trapping sessions (Figure 1) the majority of trapped melomys during any session have never been trapped in a previous session, so have had no ability to habituate to traps.

Comment: Line 342: Perhaps be careful with using such emotive language given the potential confounding effects of the wildfire.

Response: "Dramatically" removed from this sentence.

Comment: Figure 2: This is really nice figure! It is also the first time in the manuscript that we get information about the initial population sizes – I think this information should be given to the reader much earlier. Also, what do the different shapes in this figure represent? This is not in your figure caption.

Response: Thank you. The different symbols denote different trap sites. We have now included that information in the figure legend.

Comment: Figure 3: It is weird that the standard errors extend to negative when negative proportions are impossible. I would suggest presenting this data as boxplots and have a clearer demarcation between the sampling periods in the plot. If you stick with the barplots, then you should really provide confidence intervals rather than standard errors. This is particularly true, given that you use credible intervals earlier.

Response: Boxplots are inappropriate here given the means are derived from only 3 impact and 3 control sites, respectively. We have now provided a figure using only positive error bars and provided 95% Cis instead of standard errors. We have replaced SE with 95% CI in Figure 1 as well.

Comment: Line 374 – 377: How did you disentangle lower density of melomys due to predator presence and less foraging intensity due to predator presence?

Response: Expanded on in Discussion, but if it was simply an effect of reduced abundance of melomys driving the difference in seed predation, then we wouldn't have gotten an effect of quoll presence independently of melomys density in this analysis.

Comment: Line 378 – 381: I would address these interactions first before addressing the main effects. You should tell the reader what these interactions (or lack thereof) actually mean.

Response: This interaction is unrelated to the main effects identified above. The result reported are addressing two different datasets and two different figures. We have made that clearer by splitting this paragraph into two and rearranging them a bit.

Comment: Figure 5: Again, I would use confidence intervals over standard errors.

Response: Good point. Figure altered.

Discussion

Comment: Line 396 – I think it's unusual to refer the reader to figures and tables throughout the discussion, and it usually only done in the results unless the figure/table relates to something specifically in the discussion. Maybe this is just a preference thing, but I would consider removing all of these.

Response: References to figures have been removed from the discussion.

Comment: Line 396 – replace 'immediate' with 'apparent'

Response: Good point. Removed.

Comment: Line 397 – remove 'immediate'.

Response: Removed.

Comment: Line 399 – 400: It could also be that predation presence reduces foraging intensity in melomys, particularly in open sites that are more vulnerable to predators. This makes me wonder about your seed plots and their location relative to shelter. Do your seed plots represent typically used foraging patches (i.e. do melomy's forage for seeds in the open)? Can melomys forage for these seeds within the safety of vegetation or near vegetation? If so, then perhaps in predator present sites, melomys were avoiding your seed plots because they were in open areas and thus considered them dangerous, rather than being an aversion response to the

predator scent. Maybe you should add some justification for your seed plot design earlier in the methods to clear some of this up.

Response: Our results indicate that when predators are present, melomys consume fewer seeds generally. But they also become increasingly avoidant of predator-scented seeds relative to unscented seeds through time. We believe this makes the possibility of what you are suggesting unlikely. At each site there are a large number of seed plots (81) and they are in a variety of locations relative to cover. This distance to cover is totally random and is not biased by whether a seed is predator-scented or not. If they were simply avoiding seeds that are further from cover than others, we wouldn't expect to see such an obvious difference driven by seed scent type. Details have been added to methods section.

Comment: Line 403 – 404: Did you measure melomys shyness before the introduction of predators? If not, then how do you know that these population differences in boldness didn't already exist? This could actually occur given the geographical differences between your predator-free and predator-present sites (north vs south), and thus could just be a result of differences in local environmental conditions.

Response: We did (Figure attached). However, we only have pre-invasion data for one population. "Boldness" in this population changed following the arrival of quolls. Unfortunately, our sample size for this is only one. Additionally, due to population declines following the arrival of quolls, the number of individual we could run behavioural assays on following the arrival of quolls at this site was too few to run formal analyses on this data and be confident of the results. Therefore we made the call to remove this data entirely from our analyses, despite it providing compelling evidence in favour of our interpretation. Given the other behavioural changes we observed in predator-invaded populations, we believe we have sufficient justification to interpret the changes we observed in the data we include as likely being the result of predation affecting population behaviour.

Unpublished Figure. Proportion of grassland melomys (Melomys burtoni) emerging from hiding during open field tests from site 1 in 2017 (n = 27), 2018 (n = 5) and 2019 (n = 4). Orange dotted line denotes the arrival of quolls between behavioural assays collected in 2017 and 2018.

Comment: Line 417 – 422: Okay but this only from your artificial seed plots. Melomys from predator-present sites could be getting their seeds from somewhere else considered less dangerous. I would just be a little more circumspect when extrapolating your results to the entire landscape.

Response: This is a reasonable point, and we have toned the language down, but if that was the case, we should not see a reduction in seed take generally, regardless of seed scent, at predator-invaded sites but not predator-free sites.

Comment: Line 455 – 456 – These descriptive statistics are in the results, so we don't need to see them here

Response: Removed.

Comment: Line 455 – 458 - How do you know this not a trapping effect. For example, if individuals are becoming more neophobic as a result of predator presence, then perhaps they are also becoming more neophobic towards entering a trap (see Dingemanse and Biro 2009, TREE; Michelangeli et al. 2016, Behav Ecol).

Response: Michelangeli et al 2016 describes a situation where skinks are equality likely to be trapped regardless of behavioural type (personality). So this seems a strange example here? But the papers you reference actually inspired us to investigate this exact question with some of the data presented in this study, which we published prior to this paper (Jolly et al 2019). No matter which co-variate we included, we found no effect of personality on trappability in melomys (Jolly et al 2019). Thus we can be fairly confident that neophobia was not responsible for differences in detection being misinterpreted as differences in population size/survival in this study.

Comment: Lines 467 – 471. See above comment. So, it sounds like you did have variation in trapping success (i.e. you were not trapping the same individuals over time), thus potentially you the population decreases you observed are somewhat explain by decreased trapping success. Is there some way that you can disentangle this?

Response: Yes, and we should have done this earlier, but we were confident it wasn't responsible for our

results given our earlier study. We have now added additional analysis (Table 2) that more robustly demonstrate that detection probability cannot account for the differences we observe in population estimates and survival. See above.

Comment: Line 548. Again I would avoid emotive language.

Response: Good point. Emotive language has been removed.

Comment: Line 564. Again, I would be more circumspect. Your results suggest that this could be occurring, but you don't explicitly show this. For example, you don't actually show that there was now a higher density of vegetation which melomys feed on post quoll introduction.

Response: Good point. This is speculative, and has been toned down.

Download tracked changes file

Decision by Denis Réale, posted 02 January 2020

Major changes

Dear authors,

Two reviewers have now read and commented on your preprint. They both found the paper interesting, but also made important constructive comments to help you improve it. These changes must be made before I could recommend your article. Having read the manuscript myself, I share the reviewers' general positive feelings about the study, and have made a few comments on it myself.

Please clarify your research objectives at the end of the introduction. For now, you only mention that you want to "... test for the effects of quolls as a novel predator on an island ecosystem and observe how native prey adjust to mitigate the impacts of their arrival", but it would be important to be more specific: you are looking at survival rates, population size, exploration behavior (or boldness if this is what you prefer), and seed predation by Melomys. Ideally you may present hypotheses, but only at the condition that these hypotheses have been built up prior to conducting the study (I guess that you have set up these experiments and surveys based on some hypotheses).

As you mention briefly in your discussion, your data will allow you to test whether the changes observed through time are caused by natural selection or plastic response to predators (i.e. individuals change their behavior as a response to predation risk). However, you seem to put more emphasis on the second hypothesis. For example, you say in the discussion that Melomys "adjust to mitigate the impacts of Quolls arrival", our you interpret your results as population adjustment to predation through reemergence "of appropriate antipredator response" (485-494). I find these interpretations speculative. I would rather suggest you to discuss your results by presenting these two hypotheses as alternative without towards one of the other.

I do not think that after a 3-year study you can conclude that Melomys has mounted totally effective defenses against the quolls. Population size has gone under 20 individuals on your predated grids, and may still be maintained by immigration from other parts of the island with less predation (i.e. Monsoon vine thicket). The chance of extinction does not seem negligible.

I am surprise that you did not consider a model where recapture rate differed between quoll-free and quoll-invaded sites (L 247-252 and Table 2). All your open-field tests and seed-removal results would suggest that melomys may have a lower probability entering traps than in the predator-invaded sites than in the predator-free sites. Unless p(g) in Table 2 means that you included a case where recapture probability differed between the two types of sites, for now I do not see evidence that you have consider that scenario, which may change a lot the results. Please clarify what p(g) means in the main text and the Table legends or add some models that will include different recapture probabilities. I am not sure that behavior can respond faster to predation pressures than other types of trait. See the recent study by Barrett et al. 2018 (Science 363: 499-504), where they show that fur color in Peromyscus maniculatus could change within a few years as in your system.

Best wishes Denis Réale

Reviewed by anonymous reviewer 2, 18 December 2019

Review of the manuscript

Trophic cascade driven by behavioural fine-tuning as naïve prey rapidly adjust to a novel predator

To the editor

In this manuscript the authors present new empirical data of a predator-introduction experiment in natural settings. Following the introduction of an ecologically (but not evolutionary) novel mammalian predator, they monitored mammalian prey population size and dynamics, behavioural responses towards novelty and seed predation in replicated populations for several years. They found changes in prey demography and behaviour that might have cascading effects on lower trophic levels due to direct effects (reduced population size of consumers) and indirect effects (reduced seed predation by the consumer under predation risk). Prey in predator exposed populations had lower survival, faced deceasing population size, expressed lower levels of neophobia-related behaviours in novel-environment and novel-object tests, and removed less seeds from risky foraging patches.

Overall, this is an interesting experimental case study for those interested in behaviour-mediated indirect ecological interactions and the study is highly suitable for an ecological journal. However, the study has a few limitations. First, there is virtually no data of prey demography and behaviour from before the introduction of the predator. Second, predator-free (control) and predator-exposed (treatment) populations differ in several other confounding aspects. There are systematic differences in population sizes between control and treatment populations (see Fig. 2); some of the changes in treatment populations could (theoretically) also be due to the population decreasing towards sustainable levels. More importantly, only the treatment populations were affected by a wild fire shortly after the predator introduction. Certainly, this was beyond the authors control, but as they admit and discuss, it is a systematic bias impairing some of the results. The authors make a good job in addressing the last point and in arguing why the wild fire effects cannot solely explain their results; they might add similar considerations for the other systematic biases as well. Overall, the manuscript is well presented and results are illustrated in clear figures. Still, the manuscript would gain much from a thorough revision. Below I made some general suggestions and specific remarks.

General comments

(1) Introduction: Overall the introduction introduces most relevant concepts but would gain much from clearer and more concise style. Please avoid jargon and global “we”-formulations. Clearly define concepts, such as landscape of fear. Instead of giving the main results away (L82-85), I would suggest to formulate a general aim of the study. Clear hypotheses and predictions are missing. Please provide a priori hypotheses and predictions in the introduction to avoid the impression that all analyses have been decided upon post-hoc data collection.

(2) Methods: There are a few issues with the methodology (behavioural tests, seed removal test), that need more explanation in the methods. Moreover, you might want to discuss their potential effects on the results in the discussion (see details below). I am not an expert on capture-mark-recapture statistics but also in the description of the stats, some more details are needed.

(3) Discussion: Since there are systematic biases between control and treatment populations, which each reader has in mind throughout the manuscript, I would suggest to discuss them right away at the beginning. This would mean moving L502-537 up front and present it as the 2nd paragraph of the discussion. Please shorten this part to the most necessary arguments and add some discussion of other confounds (see above). This will make the discussion stronger because readers will focus on your results and conclusions that are robust against the problems. Furthermore, the discussion is very lengthy in parts and needs a thorough overhaul. The structure of the discussion is not always obvious and it seems to go back and forth. Please make an attempt to streamline the discussion, maybe add subheadings that guide the reader, remove all repetitions and reduce speculation.

Specific comments

Title

In my view the title is too bold and not unambiguously supported by the data.

Abstract

Could be shorter and more concisely written. Add sample size and number of replicates to abstract. Use scientific species names in abstract as well.

L26: Here and in the introduction, please be specific to what kind of plasticity you refer to (see e.g., Piersma & Drent 2003 TREE).

L29: Please be specific, you see change in mean behavioural phenotype at the population level. "Behavioural responses" to me sounds like you observed individuals to change their behaviour.

L43: Rephrase "as the nature of the threat become clearer", sounds very anthropomorphic.

Keywords

Please use keywords that are not part of the title.

Introduction

L52: Delete sentence "Although… a constant." Not needed.

L55: Reference missing?

L67: Consider rephrasing "We typically think…". I do not think this is true.

L70-72: Can be deleted without losing information.

L72: Please clearly define "landscape of fear".

L85: You did show that prey avoided artificial seed patches with predator odor more in the predation treatment plots than in the control plots. In my view this result is not yet a "measurable change in downstream trophic interactions". Please avoid over-interpretation.

L99: Avoid jargon. What is "toad-smart".

Methods

L159: How do the captures distribute over the different sites?

L168: What happened with the individuals between the night of capture and the night of testing?

L175: What is a bait ball? Why did you provide food in the novel environment test? This is unusual and affects the behavioural measurements. and Since individuals will differ in energetic state and hunger, the bait will differ among individuals in its reward value. This difference in reward value will incur differences in motivation to enter into the novel environment. As a result, variation in motivation cannot be disentangled from variation in neophobia. Please discuss briefly, whether and how these aspects might have affected your results.

L183: Please provide more details on the novel object, size?

L187: These previous results are based on the same individuals? Please provide a repeatability value?

L195: Define "made contact".

L201: This means you divided all individuals only in two behavioural types, right?

L212: Is wheat available in the natural habitats, or is this a novel food? If it is a novel food, your results on seed removal are also affected by neophobia/neophilia because individuals scoring high will have a higher probability to remove the seeds and this might interact with the predator odour cue (another novelty for the control individuals). Please provide more details and discuss these issues.

L215: If your predator-cue seeds smell strongly and are only placed 10 m apart, for a microsmatic animal the whole area will smell like predator. Did you do some pilot tests to ensure that the animals perceive a difference in predation risk between your treatment levels?

L245: I would suggest to move the hypotheses and predictions to the introduction.

L246: Please use consistent terminology for your treatment levels; throughout the manuscript you switch between many.

L252: Please add the criterion you used to evaluate models, e.g. a difference of 2 in AIC between models.

L256: Please explain in more detail how population size, your dependent variable, is "estimated in this process".

L262: Define O and ds.

L274: Why did you use this approach? What is the advantage of adding "zero-only" individuals to the data set?

L279 and following: please define every term used.

L290 and following: If you divided the response into proportions, then each session is your sample size? Please

be specific here. Why did you not use binomial models with each individual being a sample?
L301: Total number of seeds at one site?
State significance level.

Results

L: 314: Delete "When …. between sessions."
L341: Please move all interpretation of results to the discussion section, e.g. "suggesting…"
L358: Please provide post-hoc tests for significant interactions and also indicate the results of the post-hoc tests in figure 3. (see also comment above, why not run binomial models to test the probability of emerging instead of population-level measures (proportion).
L363: Delete "on neophobic behaviour…. test" – the rest of the sentence states the result.
L380 and following: Please report only results here and leave the interpretation for the discussion.

Discussion

See also comments above. The structure of the discussion is not always obvious and it seems to go back and forth. Please make an attempt to streamline the discussion, maybe add subheadings that guide the reader, remove all repetitions and reduce speculation.
Please remove references to figures from the discussion. This is part of the results.
L414: The few years encompassed by the study appear to few to allow natural selection to shape behavioural variation in the population. Phenotypic plasticity appears more likely as a mechanism. Are there any individuals that you tested twice across years or trapping sessions? Are there any indications of lower survival probability of bolder types? What do we know from other systems about the speed of microevolution?
L420: These conclusions appear far-fetched. Individuals avoided taking seeds heavily smelling like predator when other seeds were available nearby. This is an interesting result and a good attempt to quantify potential cascading effects of predation risk. However, it is not yet a proof of such effects. I would suggest tuning these conclusions down a bit.
L426: You might want to cite here as well Sih et al. (2010, Integrated Comparative Biology 50:6).
L438: In the manuscript you constantly change between neophobia, boldness and the variable names. This is confusing and misleading. Please clearly define/interpret your behavioural measures once and use the same terms throughout. Please see Carter et al. 2012 (Biol Rev) for guidance and fallacies.
L439-L443: Here and in the following there is a lot of speculation, please remove this and stay with the facts.
L441: What is the "behavioural state of a population"?
L455: Could you test and compare behavioural-type specific survival across sessions? This would be a strong indicator of selective disappearance.
L452-472: Please shorten, there is a lot of speculation here.
L495: Please delete whole paragraph; these applied aspects are not directly related to your study/results and break the flow of the discussion. L502-537: Shorten this paragraph to half and move it further up (see above).
L538-566: Many aspects in this part have been discussed before.

References

The reference list needs serious proof-reading (species names in italic, missing page numbers, missing journal names, etc.).

Tables and Figures

All figures, please use higher contrast for black-and-white printing.
Fig.1: You might consider showing all sites separately.
Fig.2: Very nice figure, please explain symbols.
Table 1: Show less digits after comma.

Reviewed by anonymous reviewer 1, 18 December 2019

This is an interesting paper that examines the impact of a novel predator (northern quoll) on an island population of melomys (a type of rodent). They found that melomy populations that are exposed to quolls

suffered decreased survival and population declines. They also found that melomys from predator-exposed populations initially adopted shyer behavioural tendencies, but that this behavioural response became more refined over time and was eventually replaced by a more threat-specific, scent aversion antipredator behaviour. This result suggests that melomys were becoming better at dealing with quoll predation. Finally, they also found evidence that the seed intake rates of melomys in predator-exposed sites substantially decreased, potentially suggesting that the quoll introduction could be causing trophic cascades at multiple biological levels.

This is a nice study. The authors had the rare opportunity to examine the impacts of a re-colonization program of a predator (i.e. quoll) on island populations and communities. This study represents a lot of hard work, which I commend the authors for. The results are interesting, albeit not that surprising, but the study offers a nice natural example of how novel predators can shape prey behaviour and the structure of ecological communities. Overall, I thought the experimental design was sound, but there are areas throughout the manuscript that need refinement and further justification, particularly in terms of the interpretation of the results.

Introduction

Line 57 – 61. I'm not a big fan of breaking up a sentence with a colon. It makes this sentence sound clunky and disjointed. Consider revising

Line 67 – 69: Same comment as above.

Line 72: I would consider not using the term 'landscape of fear'. Some people don't like it, and to avoid people getting upset, I think you could just say "prey species living with predators".

Line 74: replace "will' with 'can'

Line 81 – 82: I would replace 'advance' with improve and then revise the last part of this sentence. How exactly does this improve our understanding of how communities are structured? Do you mean before or after invasions?

Line 82 – 85: I would remove this last sentence. I think this is better said in the last paragraph. You also repeat more or less the same thing on lines 95 – 98 and on lines 103 – 105.

Line 95 – 96: 'We take advantage of….'

Line 95 -98: You say something similar to these 3 times in your introduction. I think it should be integrated more seamlessly, and when you say it the first time you should then tell us why this is such a good opportunity.

Line 99: What does 'toad-smart' mean? I know what you mean, but others probably don't. I would probably just remove it.

I think you should provide a clear set of hypotheses.

Methods I think overall, I found the structure of the methods a little hard to follow. I had to go back and forth at times to work out

Line 112 – 115: This is forth time that you mention the re-introduction as being a unique opportunity. I would remove this, but I would actually mention these specifics in the introduction the first time you talk about the re-introduction offering an opportunity to measure novel predator-prey dynamics.

Line 115 – 120: I found this part confusing. You mention three plots, but you drop two of the plots because quolls didn't use them. How big are these plots exactly? Why mention the plot you didn't actually use in this study? Also, how do they differ (or not) to the sites that you mention later on lines 129 – 130? I might be worth considering incorporating a map outlining your plots into the manuscript.

Line 131 – 133: I think this site information should be presented before you talk about the sampling dates

Line 137 – 137: I would remove this sentence given that your next sentence helps to confirm this

Table 1: I found this table not very informative. A map that outlines your plots and how far apart they are would be much more informative. You could also have different colour codes for sites with predator's vs those without predators. Just a suggestion.

Line 151 – 152: How did you know that you trapped the majority of the population? What were the population numbers for each site before quoll's were introduced? – you could incorporate this latter information into the

map above

Any chance of behavioural-dependent trapping success? (see Dingemanse & Biro 2009, TREE)

Line 186 – 187: I think you should provide the repeatability estimates here (i.e. give the reader some indication of how repeatable these behaviours are without them having to go and look at the other paper).

Bummer about the wildfire!

198 – 202: I suppose I fail to see the link between neophobia of a plastic plate and predator avoidance. Why do the neophobia assay in the first place? Maybe some justification for these tests here would help.

Line 274: What is site.session?

It was hard for me to comment on some of the statistics because I am unfamiliar with some of the modelling used, however it seemed weird to me that you switched between a Bayesian framework and a frequentist framework for some of the stats. I am curious as to why the authors did this and why not just be consistent?

Results

Line 320 – What does the 'S' value represent?

Link 321 – 323: Is there a statistical way of disentangling the effect of the wildfire and predation on these survival numbers?

Figure 1: It might be useful to include what the initial population sizes were for comparative purposes. From this figure, it looks like both populations declined through years, and that the degree of decline isn' t that much larger than for the quoll present population. This also makes me think that this decrease through time might also just be a result of your trapping success decreasing through time (as melomys habituate to the traps themselves)?

Line 342: Perhaps be careful with using such emotive language given the potential confounding effects of the wildfire.

Figure 2: This is really nice figure! It is also the first time in the manuscript that we get information about the initial population sizes – I think this information should be given to the reader much earlier. Also, what do the different shapes in this figure represent? This is not in your figure caption.

Figure 3: It is weird that the standard errors extend to negative when negative proportions are impossible. I would suggest presenting this data as boxplots and have a clearer demarcation between the sampling periods in the plot. If you stick with the barplots, then you should really provide confidence intervals rather than standard errors. This is particularly true, given that you use credible intervals earlier.

Line 374 – 377: How did you disentangle lower density of melomys due to predator presence and less foraging intensity due to predator presence?

Line 378 – 381: I would address these interactions first before addressing the main effects. You should tell the reader what these interactions (or lack thereof) actually mean.

Figure 5: Again, I would use confidence intervals over standard errors

Discussion

Line 396 – I think it's unusual to refer the reader to figures and tables throughout the discussion, and it usually only done in the results unless the figure/table relates to something specifically in the discussion. Maybe this is just a preference thing, but I would consider removing all of these.

Line 396 – replace 'immediate' with 'apparent'

Line 397 – remove 'immediate'.

Line 399 – 400: It could also be that predation presence reduces foraging intensity in melomys, particularly in open sites that are more vulnerable to predators. This makes me wonder about your seed plots and their location relative to shelter. Do your seed plots represent typically used foraging patches (i.e. do melomy's forage for seeds in the open)? Can melomys forage for these seeds within the safety of vegetation or near vegetation? If so, then perhaps in predator present sites, melomys were avoiding your seed plots because they were in open areas and thus considered them dangerous, rather than being an aversion response to the predator scent. Maybe you should add some justification for your seed plot design earlier in the methods to clear some of this up.

Line 403 – 404: Did you measure melomy's shyness before the introduction of predators? If not, then how do you know that these population differences in boldness didn't already exist? This could actually occur given the geographical differences between your predator-free and predator-present sites (north vs south), and thus could just be a result of differences in local environmental conditions.

Line 417 – 422: Okay but this only from your artificial seed plots. Melomy's from predator-present sites could be getting their seeds from somewhere else considered less dangerous. I would just be a little more circumspect when extrapolating your results to the entire landscape.

Line 455 – 456 – These descriptive statistics are in the results, so we don't need to see them here

Line 455 – 458 - How do you know this not a trapping effect. For example, if individuals are becoming more neophobic as a result of predator presence, then perhaps they are also becoming more neophobic towards entering a trap (see Dingemanse and Biro 2009, TREE; Michelangeli et al. 2016, Behav Ecol).

Lines 467 – 471. See above comment. So, it sounds like you did have variation in trapping success (i.e. you were not trapping the same individuals over time), thus potentially you the population decreases you observed are somewhat explain by decreased trapping success. Is there some way that you can disentangle this?

Line 548. Again I would avoid emotive language.

Line 564. Again, I would be more circumspect. Your results suggest that this could be occurring, but you don't explicitly show this. For example, you don't actually show that there was now a higher density of vegetation which melomys feed on post quoll introduction.