Response to Reviewer Comments (MS # 2023-08208)

Our point-by-point response to all comments from the editor and three reviewers is provided below. Editor/reviewer comments are in black and our responses are in blue.

Editor

The authors did a thorough job responding to the editor and reviewer comments and revising their manuscript. There is consensus that the integration of these evolutionary and ecological datasets in an iconic system is of broad general interest. However, there are still concerns about the genomic analyses and strength of conclusions that can be made about the selection underlying disease resistance. One reviewer provides some clear suggestions for addressing these concerns that I agree with and encourage the authors to pursue.

For a detailed summary of how we addressed the remaining concerns about our genomic analyses and the strength of conclusions that can be made regarding selection underlying disease resistance, see our responses to the Reviewer 1 comments below.

Reviewer 1

The authors have addressed some of the reviewers' and editor's comments satisfactorily, which has improved the manuscript. I especially appreciate the restructuring and clarifications made throughout the text, which make the text much more efficient at communicating its message. That being said I still believe the current version has important issues that need to be addressed before it can be published:

1. I agree with the editor that evidence for recent selective sweep on putatively adaptive alleles that can be functionally connected with Bd resistance is essential to connect the divergence and association scans to the disease-mediated selection scenario in Fig. 1 and the discussion (lines 912-928). In their current form, the genetic analyses do not provide strong evidence in this regard.

First of all, although their results are consistent with recent selection, these analyses alone do not provide strong evidence for this scenario. Association analyses such as GEMMA are not meant to find evidence for selection. All they do is identify genetic variants that are statistically associated with a phenotype (or some other grouping), regardless of whether that phenotype has undergone any type of selection. High Fst or π-diff by themselves are expected under positive/balancing selection, but can also be caused by several other (neutral) processes, especially in the face of population bottlenecks, as is the case with the recovered populations. They can also arise as artifacts due to population stratification, which is present in the data.

Second, a key aspect of this system in particular is that there is good evidence for recent adaptation of MYL frog populations to Bd (i.e. sharp decline followed by recovery). In view of this, it is expected that the adaptive alleles that allow for Bd resistance became prevalent recently and quickly in recovering populations. Therefore, the authors should provide evidence that this is

the case for their candidate Bd adaptation loci. Of course the gold standard for this would be time series data, which are unfortunately unavailable. That being said, in most studies of adaptation time series data are not available, so this shouldn't be a limitation.

There are a wide variety of additional approaches to address whether selection has occurred at a specific region of the genome, as well as to estimate the strength and timing of selection, especially for the case of recent selective sweeps like the one that presumably occurred in Bd-resistant MYL frog populations. The authors should take advantage of these approaches to further assess the evidence for recent strong selection at their candidate loci.

We appreciate the detailed suggestions from Reviewer 1 regarding our genomics analyses. We have done our utmost to address the concerns, and we describe the changes we made to the manuscript in the fourth paragraph of our response to this comment (see below). As part of our response, we would also like to reiterate a key point. That is, the genomics analyses play a supporting and hypothesis-generating role in the manuscript. The truly groundbreaking work in this manuscript is the culmination of 15+ years of research demonstrating that reintroductions can bring an endangered species back from the brink of extinction despite the ongoing presence of the disease responsible for species endangerment.

Regarding our genomics analyses, as stated by the reviewer (see above), there is well-documented evidence of steep disease-caused declines in mountain yellow-legged (MYL) frogs followed by recovery of resistant individuals, consistent with recent selection for resistant genotypes. This intriguing pattern motivated us to conduct genomics analyses that could provide useful insights into this apparent disease-mediated selection (Figure 1). Sampling limitations (discussed in our response to Reviewer 1 - Comment 2 below) required a comparative pooled analysis (i.e., comparing the exomes of frogs from a limited number of naive and recovering frog populations), and we are well aware of the limitations inherent in this approach. Nonetheless, we sought to conduct the most robust analysis we could.

We agree with the reviewer (and editor) that our genomics analyses do not provide unequivocal evidence of recent selection nor do they allow us to strongly link selective sweeps at particular genetic loci to a specific episode of disease-mediated selection. Even with our full exome sequencing and the availability of a well-annotated genome, the pooled analysis approach precludes fully disentangling the effects of selection and demography in our dataset, nor can we confidently estimate the strength and timing of selection at specific candidate loci. That said, our analyses indicate that frogs from naive and recovering populatons show differences at several immune-function loci, consistent with selection following Bd expoure. In addition to providing valuable insights into possible disease-mediated selection, our genomics results generate important hypotheses about the mechanisms underlying these processes. Several of the candidate genes we identify were identified independently in different analyses, making them strong candidates for future study in this and other systems.

To clarify these points and ensure that we accurately describe the strengths, weaknesses, and uncertainties of our genomics results, we added numerous refinements and caveats to the genomics section of the manuscript and Supporting Information (SI). First, we added a new paragraph to the genomics section of the Results that clearly states the reasons for our pooled

analysis and the limitations inherent in this approach (lines 649-664). Second, throughout the manuscript we either removed outright any statement about results providing evidence of selection, or replaced such statements with wording that indicates that the results are consistent with selection but do not provide direct evidence of selection (e.g., lines 671, 713, 732, 742, 858, 966, 969, 973, 978, 982, 999, 1144). Finally, we changed the genomics section header in the Results, Materials and Methods, and SI from “Frog evolution in response to Bd” to “Frog genome evolution” to avoid any suggestion regarding the timing of selection (lines 589, 1344, SI:176).

2. I agree that small sample sizes can be a major limitation in selection scans, and understand why the authors decided to pool samples across populations and compare all resistant and all naive populations together. That being said, there are downsides to this approach, especially when there is population stratification, which may lead to incorrect inferences. Judging by the high degree of p-value inflation in the GEMMA results, despite this method's control for relatedness between samples (see Fig. S6B), suggests that the caveats of pooling populations have affected this dataset.

In view of this, I suggest the authors validate their results by analyzing pairs of closely related populations, especially at the reintroduction sites. This approach alone may be underpowered, but in combination with the pooled approach they can get as close as the "best of both worlds" as possible.

There are downsides to our pooled approach. It would be ideal to conduct pairwise comparisons among geographically-proximate populations, thereby minimizing the potentially confounding effects of population structure while gaining an opportunity to evaluate the shared and unique trajectories of adaptation in different sub-populations. However, we do not have the power for this approach. Genomic studies of endangered species are commonly limited in total sample size, but we have an added limitation, which is total existing populations. Our genomics work relied on comparisons between five recovering populations and four naive populations. Our samples from naive frogs are from the only naive populations remaining on the landscape, and we do not have pairs of closely related naive and recovering populations.

As the reviewer stated, the genomics data from Yosemite populations is of particular interest because these data are directly relevant to the reintroduced populations that are the primary focus of our study (all located in Yosemite). In Yosemite, we have three recovering populations and one naive population. Given this limitation, there is no amount of additional sampling that will increase the number of naive populations (at the time of sample collection there was only one naive population left and it too is now Bd-positive), or data analysis that will provide unambigous evidence of selection strength or timing. This limitation is why we used a broad-scale pooled study design. It is also worth noting that population structure and population status are not obviously confounded in this dataset (i.e., each genetic sub-group contains at least one naive and one persisting population). This does not eliminate, but it does reduce, the concerns about pooled analyses.

Limitations aside, we did try conducting additional analyses based on Reviewer 1’s suggestion, including using extended haplotype homozygosity (EHH) to detect signatures of selective

sweeps, as implemented in the program REHH. These analyses did not reveal new robust conclusions, and we elected not to bloat the revision with additional results. However, we did add a new figure to the SI (Figure S8) illustrating finer-scale results in Yosemite to give readers a closer-look at patterns of genetic diversity at outlier loci in the geographic area most relevant for the reintroduction study.

3. In the first round I thought that Fig 5 contained all candidate loci. I thank the authors for clarifying that only those identified by GEMMA are pictured. I strongly suggest adding additional panels to also depict the other outlier variants that show signatures of parallel evolution, especially those that can be tied to Bd resistance functionally (e.g those in the extended MHC, the complement factor genes, etc..). Information on how variation at outlier loci is distributed in the area where translocations occurred is key to connect the genome scan results with the rest of the paper.

We agree that, if possible, other outliers that are potentially involved in Bd resistance should be graphically represented. Figure 5 only contains GEMMA results because the π_diff and F_{ST} results cannot be displayed in the same way as GEMMA results. Specifically, the π_diff and F_{ST} results are sliding window analyses, which do not provide allele frequencies for specific SNPs. Therefore, instead of incorporating those results into Figure 5, we rely on Figure S8 and S10 to show additional candidate regions (including those containing MHC and complement factor genes).

I also have a couple of specific suggestions to improve the text:

L 653: The previous sections suggest an important role for intrinsic factors (such as resistance), not resistance specifically. I suggest rephrasing.

Corrected as suggested (lines 589-592).

L 732-733: I suggest replacing "principal component space" for "the first two principal components" to more accurately reflect this result.

Corrected as suggested (lines 680-682).

L 856: I suggest specifying that *some* of the recovering populations have high chances of persisting long term.

Corrected as suggested (lines 903-904).

L. 857 and elsewhere in the text: I wouldn't say there are "substantial" differences between naive and recovering populations. If anything the data show that they are more similar than expected in genome-wide patterns (e.g. similar heterozygocities and nucleotide diversities)

Corrected as suggested. (lines 28-30, 867-868, 904-907).

L 923-924: Pooling populations reduces the effect of population structure in some ways and

exacerbates it in others. I suggest rephrasing.

Corrected as suggested (lines 958-964).

L. 967: Maybe add a little context of what is meant by "the resistance phenotype" in salmon.

Corrected as suggested (lines 987-990).

L. 1340: Why were sites with AF<0.03 filtered out? I understand it may be advantageous to filter out allele frequencies that are For 53 diploids 0.03 corresponds to roughly 4 copies of the minor allele, so perhaps this threshold is a little high?

The wording of this comment appears to be incomplete. However, as the reviewer noted, the selection analyses included approximately 50 individuals, so the AF filter of 0.03 ensures that the allele was found in at least two individuals in the dataset (at least 3 copies). We argue that this is an appropriate threshold for the current dataset, and we did not make any changes in response to this comment.

Reviewer 2

Comments on Significance Statement:

1. Resistance needs to be defined.

We appreciate this suggestion. However, we offer that the general meaning of “disease resistance” is likely evident to most readers (i.e., “understandable to an undergraduate-educated scientist outside their field of specialty”). Given this and the limit of only 120 words for this section (our version is close to this word limit), we respectfully suggest that the significance of our study is better conveyed by highlighting the main results and their broader implications.

Comments:
I appreciate that the authors carefully considered our comments and recommendations. I believe that the manuscript contributes important data and employs integrative analyses to understand the impacts of the chytrid fungus in declining amphibian populations. However, I am not satisfied with the way that they have responded to the criticisms. The manuscript still has many issues that need to be addressed and it doesn't present a compelling story of the patterns and mechanisms involved in the success of these reintroductions.

2. From my previous comments (I was Reviewer 2), I pointed out to add more information about the frog system. I get it, the space is limited in this journal, but it is crucial to understand the efficacy of the reintroductions. For instance, it is known that populations at higher elevations take longer to reach adulthood. See this excerpt from AmphibiaWeb for Rana sierrae: "At lower elevations where the summers are longer, tadpoles are able to grow to metamorphosis in a single season (Storer 1925). At higher elevations where the growing season can be as short as three months, tadpoles must overwinter at least once and may take 2 or 4 years of growth before they are large enough to transform (Wright

and Wright 1949; Zweifel 1955)." My intention with bringing this up is to highlight that there is some really cool biology going on that was not even explained in the text, and it directly relates to survival, population viability, and the ability of these populations to withstand chytridiomycosis. The authors mentioned multi-year tadpole/juvenile stages as being taken accounted as part of the models (line 1179-1180), but this is not something to be 'taken into account' when there was variation in the probability of survival with elevation (Fig. 3B). Think about the consequences based on these findings, relocation strategies will need to move frogs to higher elevations, but that's also when they develop slower, thus, estimating the success of reintroductions will be more difficult and will take years in comparison with populations at lower elevations.

We thank the reviewer for elaborating on the previously-raised concern. The additional context is helpful. We heartily agree with the reviewer's comment that "there is some really cool biology going on", and acknowledge that we did not mention some of this biology in the text. In choosing what aspects of the frog's natural history to highlight in the manuscript, we had to make some hard decisions and we opted to focus on those aspects that are most relevant to the study results. Nonetheless, in response to the reviewer's comment, we made two significant changes to the revised manuscript. First, we added a paragraph to the Discussion that includes (in part) a description of the effect of elevation on MYL frog developmental rates and on frog survival and viability (lines 1070-1091). Second, we added additional tadpole and juvenile vital rates to the elasticity and sensitivity analysis shown in Figure S5 (p_L2: probabilty that year-2 tadpoles metamorph into juveniles; p_J1: probability that year-1 juveniles mature to adults; σ_L1: survival probability of year-1 tadpoles; σ_L2: survival probability of year-2 tadpoles; σ_L3: survival probability of year-3 tadpoles). Results from the expanded analysis support our assertion that these vital rates are likely to have only small effects on population growth rates and viability. In the following paragraphs, we provide additional details about our response.

First, we agree that variation in frog development and growth rate across the full elevational range occupied by MYL frogs is substantial (e.g., tadpole duration ranging from several months to a year or more) and could influence frog survival, population viability, and frog-Bd dynamics. However, differences in development and growth rates are substantially smaller across the narrower elevational range of lakes included in our frog reintroduction and viability studies (historical elevation range: 1370-3660 m, elevation range of reintroduction sites: 2417-3505 m), and this reduces the variation in frog development and growth rates. For example, in all but the most severe winters, tadpoles in all of our reintroduced populations overwinter once before metamorphosing.

Second, the elasticities and sensitivities of tadpole, juvenile, and adult vital rates in Figure S5 show that the two vital rates we focus on in our viability analyses, adult survival and year-1 juvenile survival/recruitment, generally had larger effects on population growth rate than the other tadpole and juvenile parameters. This result, in combination with our biological knowledge that juvenile survival and recruitment is variable between years and that adult survival varies among sites, justifies our choice to focus on variation in adult survival and year-1 juvenile survival/recruitment in our viability analysis.

Third, in our meta-analysis, any elevation-related differences in frog development and growth

rate are implicitly included in the elevation predictor variable in our statistical model. Results from this analysis clearly show that elevation has a strong positive effect on the survival of translocated frogs (and by extension, on population viability), contrary to the possible negative effect described by the reviewer. In summary, the several lines of evidence we provide here suggest that the effects of any elevation-driven variation in frog development and growth rates on survival and population viability across our study lakes is relatively small.

Abstract:

3. L21-22: "resistance against Bd infection, consistent with the evolution of resistant genotypes and/or acquired immunity". The authors have data on recaptured frogs (Fig. S2). If this pattern is consistent with acquired immunity, then we should see lower Bd loads at each time point for recaptured individuals. The authors mentioned (L519-522) "The absence of Bd load as an important predictor of frog survival is consistent with frogs in recovering populations having sufficient resistance to suppress Bd loads below harmful levels". That's not what these data are indicating, it shows that Bd loads are highly variable (Fig. S3) and don't decline. Again, going back to the theoretical definitions of resistance and tolerance, resistance should indicate a reduction in load, or maybe, it could also be reflected as recaptured frogs having more instances with no infections or lower infections, which is not supported by their results (i.e., models and Fig. S2). All of the data on this paper provides support for tolerance, not resistance.

Figure S2 shows the Bd loads of frogs before and after translocation. We included this figure to demonstrate that Bd loads did not change markedly following translocation. Based on this evidence, we concluded that “loads appear more strongly influenced by frog characteristics (e.g., resistance) than site characteristics.” (lines 486-488). However, the reviewer contends that “If this pattern is consistent with acquired immunity, then we should see lower Bd loads at each time point for recaptured individuals.” Contrary to the reviewer’s interpretation, there is no expectation that translocation or anything associated with it will boost acquired immunity and thereby reduce Bd loads following translocation. As we describe in the Introduction and in Figure 1, donor populations have recovered following previous Bd epizootics and associated steep population declines, and show typical enzootic frog-Bd dynamics (i.e, high Bd prevalence and low-to-moderate Bd loads: lines 294-301). These enzootic dynamics are consistent with those expected under the scenario in which frogs in these populations have either undergone selection for more resistant genotypes, and/or have acquired immunity following previous Bd exposure. Given the Bd infections experienced by frogs in donor populations throughout their entire tadpole-to-adult life cycle (including in the years before and after translocation), there is no mechanism by which translocation would affect acquired immunity. We assume the reviewer’s assertion that “we should see lower Bd loads at each time point for recaptured individuals” was based on a misunderstanding of when the selection for resistance and/or Bd exposure occurred. We did not make any changes in response to the reviewer’s comment.

Based on Figure S2, the reviewer also claims that our statement that frogs in recovering populations have sufficient resistance to suppress Bd loads below harmful levels (lines 475-478) is not true. In fact, the data in Figure S2 combined with the information in the figure caption strongly support the statement in question. Specifically, the median Bd load on frogs from before

and after translocation is 3.6 copies (on a $\log_{10}$ scale) and rarely exceeds 5 copies. In addition, as we stated in the caption, "Loads indicative of severe disease are > 5.8 ITS copies (on a log10 scale)." Collectively, the data clearly indicate that loads on frogs in recovering populations rarely exceed this disease threshold and support our conclusion that frogs in recovering populations have sufficient resistance to suppress Bd loads below harmful levels. To make the referenced disease threshold even more explicit, we added a horizontal line to Figure S2 to show this level. We also added text to the figure caption that states, "This threshold (5.8 ITS1 copies - on a log10 scale) is commonly exceeded during Bd epizootics, but is exceeded only rarely in recovering populations."

4. L23-24: "frogs collected from recovering populations and reintroduced to vacant habitats can reestablish populations despite the presence of Bd". This statement is partially true, and the authors should mention that the likelihood of establishment depends on other factors -as shown in their meta-analysis-namely environmental factors and elevation (Figure 3).

We added a sentence to the Abstract stating that the likelihood of establishment is influenced by site, population, and frog attributes (Lines 24-25). Because the original version of the Abstract was at the limit of 250 words, this addition required the removal of other text to remain within this word limit.

5. L339: Figure 5 was mentioned before the rest of the figures.

We referenced Figure 5 out of order to avoid placing the figure well before the associated *Results - Frog genome evolution* section. To avoid any confusion caused by refering to Figure 5 out of order, in the revised manuscript we replaced the reference to "Figure 5" with a reference to the *Frog genome evolution* section in the Results (lines 348-349).

6. How different are those 3 donor populations (70459, 70567, and 72996)?

The donor populations are similar to each other in that they are among the largest MYL frog populations in Yosemite. In the revision, we added text to lines 345-346 stating this.

7. L539-541: I would be more careful with statements like this. Frog tolerance is being maintained across generations, but frogs still die (P 1-year survivals vary from ~0.25-0.80, Fig. 3) - so there are some costs. These conditions do not support of population establishment and long-term population growth at lower elevations and when environmental conditions (winter severity) are extreme.

To provide some additional context, the statement in question is as follows: "...in 3 locations where longer CMR time series allowed us to assess the survival of new adults recruited to the population, naturally-recruited adults had equivalent or higher survival probabilities than the originally translocated adults (Figure S4). This suggests that frog resistance is maintained across generations." We stand by this statement for the following reasons. First, our suggestion that "frog resistance is maintained across generations" is reasonable given the results shown in Figure S4. Second, the reviewer's argument against our interpretations is factually incorrect. Specifically, although the probability of frog survival is < 1 (Figure 3), our analyses demonstrate that frog survival is unrelated to Bd load (lines 468-471). Therefore, in contrast to the reviewer's comment, nothing in Figure 3 supports the statement that there are costs associated with Bd infection. Instead, the figure makes the point that frog survival is affected by site, population,

and frog-specific attributes (but not Bd-load). Third, the fact that site, population, and frog-specific attributes influence survival does not contradict our original statement.

8. Genomic analyses

I still consider 53 as a low sample size considering that some of these populations have 1000s of individuals (Line 675). I would be more convinced if the authors could provide a largescale analysis of those 11 SNPs in at least 20-30 frogs per population.

We would also have liked to collect tissue samples from more frogs in each study population, but restrictions in our US Fish and Wildlife Service recovery permit prevented us from doing so. This and other similar limitations are inherent in studying endangered species, but the precarious status of these taxa and the potentially critical insights into recovery methods resulting from conservation-focused research argue strongly for making the best of this challenging situation.

9. Figure 1 and 6 should be sent to supplementary materials. These figures do not contribute significant information.

Some comments by reviewers on the first version of the manuscript indicated confusion about details of the study system and study design. In response, we developed Figure 1 to provide a conceptual model that outlines the study framework and design. This figure takes up less space than would be required if the model was described using text. Finding ourselves between the differing opinions of reviewers 2 and 3 about the value of Figure 1, we have opted to retain it.

Figure 6 is also important, and we have retained it in the manuscript as well. Figure 6 shows outliers from the π-diff and F_{ST} analyses (panels A and B), key results are not displayed anywhere else in the manuscript. Figure 6 also underscores the importance of specific outlier regions of the genome, including those containing immune function genes (panels C and D). To emphasize the meaning of the color-coded outliers, we modified the legend that is embedded in the figure to better highlight this element.

10. Figure 2 is misleading considering that Figure 3 shows a significant effect of elevation in the P(1-year survival). I suggest re-sorting these points by elevation. There's also no explanation for this effect and this is extremely important based on the natural history of the species.

We disagree with the reviewer on this point for several reasons. First, the goal of including this figure is to show the general structure of the data, in particular the large variation in frog survival across sites and much lower variation within sites. This information is important for readers' understanding of the data, and also serves to justify our hierarchical modeling approach. As such, it is difficult to understand how the reviewer could characterize the figure as "misleading". Second, our meta-analysis demonstrates that multiple factors influence frog survival. In addition to important effects of winter severity, elevation, donor population, and frog sex (Figure 3), all of the top models in this analysis also included the group-level ("random") effect of site_id and/or translocation_id, indicating that these variables also explained some of the variation in frog survival. The reviewer's claim that this figure is "misleading" because it displays the effect of one important variable (site) and not another important variable (elevation) does not add up, especially in light of the fact that Figure 3 shows the effects of all of the influential predictor variables identified in the meta-analysis. We did not make any changes in response to this comment.

A description of how we addressed the reviewer's comment regarding the effect of elevation on frog natural history (i.e., frog development) is provided in our response to Reviewer 2 - Comment 2 above.

Reviewer 3

I feel like the authors did a good job of addressing all the reviewers' comments, including my own. I was enthusiastic about this paper when I first reviewed it, and the authors addressed all my comments. I have only minor suggestions written directly on the uploaded manuscript and supporting information files.

We appreciate the reviewer's enthusiasm for our manuscript.

I like the reorganized and slightly refocused nature of the paper - the new Figure 1 is a nice addition.

Thank you for the positive feedback.

Opinion only: I still find the among-site survival modeling to test the influence of site, cohort, and individual level characteristics on post-translocation frog survival a little strange. I understand this can be impractical within the Bayesian CMR framework, but I probably would chosen to adopt a maximum likelihood framework, where this type of analysis is extremely common, rather than generating data (the response yi a binary outcome, representing whether individual i survived in the year following translocation) using the values from separate analyses for each site and then analyzing those predicted values...at least I think that is what they did. I'm not saying it's wrong, or that the authors need to change anything, it just seems like they are doing statistical gymnastics to stay within the Bayesian framework without clear benefits for biological inference. The posterior distributions for the yearly survival probabilities for translocated adults σAT and naturally recruited adults are calculated from the site-specific CMR models, so using posteriors in subsequent population viability modeling wouldn't be a justification for needing the Bayesian framework. That's my opinion, but I'm not a die-hard Bayesian. Again - just an opinion. The authors do not need to make any changes or even respond to this comment.

We appreciate this insightful and thought-provoking comment. We agree that our meta-analysis approach, conducted in a Bayesian framework, was somewhat more complex than an analysis conducted in a frequentist framework that combines our separate CMR and covariate analyses into a single model. However, the approach we used provides a consistent framework across all of the reintroduction and viability analyses, and that consistency has distinct advantages when interpreting the results. We suggest that either approach comes with its own advantages and disadvantages, and we are confident that our analyses are justifiable and the results are robust. As such, we did not make any changes in response to this comment.

<u>The following comment was added by Reviewer 3 directly to the manuscript, but we opted to address it here to allow the more detailed response that it deserves</u>: I wonder if there is any confounding with elevation since it seems like the donor and recipient populations are spatially

located (Fig S1). For example, is the reason that donor population 70459 yields higher 1-year survival is because you are translocating those individuals to only higher elevation recipient sites, relative to some of the recipient sites that received frogs from donor population 72996.

We cannot say that there is no confounding between the donor site and elevation of the associated recipient sites, but we believe that the potential for substantial confounding is low. Due to the high topographic relief of the portion of Yosemite in which the study sites in question are located, close proximity between donor and recipient populations is often not indicative of similar elevations between donor and recipient populations. For example, donor site 72996 is located at an elevation of 3176 m and contributed frogs to recipient sites with elevations ranging from the lowest site to two of the highest sites in the study (recipient site elevations: 2471, 2789, 2879, 2921, 3018, 3188, 3200, 3237, 3249 m.) Nonetheless, without conducting additional translocations from 70459 and 70567 to recipient sites that span a similar range of elevations as received frogs from 72996, we acknowledge that there may be no way to completely disentangle the effect of donor site from the effect of elevation. We did not make any changes in response to this comment.