

Migratory lineages rapidly evolve larger body sizes than non-migratory relatives in ray-finned fishes

Michael D. Burns and Devin D. Bloom

Article citation details

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Original submission: 21 June 2019
1st revised submission: 8 November 2019
2nd revised submission: 10 December 2019
Final acceptance: 11 December 2019

Note: Reports are unedited and appear as submitted by the referee. The review history appears in chronological order.

Review History

RSPB-2019-1471.R0 (Original submission)

Review form: Reviewer 1

Recommendation

Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Good

General interest: Is the paper of sufficient general interest?

Excellent

Quality of the paper: Is the overall quality of the paper suitable?

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

Yes

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

The paper infers the evolutionary history of migration in ray-finned fishes and its impact on their phenotypes. Specifically, the authors use a dataset of more than 4500 species extracted from a recently published actinopterygian megaphylogeny, and employ ancestral state inference to estimate the number of transitions between migratory and nonmigratory lifestyles. The authors then fit a number of trait evolution models to determine whether migratory species tend to evolve larger body sizes than nonmigratory species, as expected based on previous studies conducted on different clades or on different taxonomic scales, and whether the acquisition of a migratory lifestyle has been associated with increased rates of body size evolution. The authors identify a multipeak Ornstein-Uhlenbeck process with different stationary variances around the optima as the best-fit model, showing that migratory species tend to evolve larger body sizes, and at higher rates, compared to nonmigratory species. The analysis protocol established by the authors will hopefully facilitate testing similar hypotheses in other clades where relevant data are available.

Overall, the study is very clearly written, and thanks to its impressive scale, represents an important contribution to the study of migration on macroevolutionary scales. The methods are well-chosen for the task at hand, translating into convincing conclusions. The discussion nicely places the results in the broader context of recent research on migration syndromes, ranging from physiology to intraspecific variation, and points toward future applications of the same set of approaches to different clades and different traits.

As a result, I mostly have only minor edits to suggest, with an exception of one more substantial point concerning methodology (all listed in order of appearance):

Lines 53–54, 271–272: While evolution toward an optimum is a correct characterization of the Ornstein-Uhlenbeck (OU) family of models, and while one of these models (OUMV) was found to be the best-fit model by the authors, I would be cautious about couching the overall findings in this language. After all, all models – including the best-fit one – are necessarily oversimplifications of reality, and it is difficult to imagine that given their sheer

ecomorphological diversity, the 4500+ species analyzed by the authors all evolve toward just two body-size optima determined solely by their migratory status. To make it clear that these caveats have not been ignored, it would be helpful to discuss this particular result strictly in terms of theta values estimated under a multipeak OU model rather than in terms of biological reality.

Line 70: If I correctly understand the point being made here, it might be more straightforward to say that migration costs are “minimized” rather than “optimized”.

Subsection “Ecological Classification and Body Size”: The exact number of species analyzed should be given, as well as the numbers of migratory and nonmigratory species.

Lines 127–128: “personal” not “personnel”.

Paragraph spanning lines 181–186: I share the authors’ concern about the best-fit models possibly being overparameterized, i.e., incorrectly favored over simpler models. However, it seems to me that their simulation analyses do not address this problem. The authors ran 100 simulations under the OUMV model and under the parameter values estimated for this model from their empirical dataset, and analyzed the resulting simulated datasets using each of the seven models under consideration. They concluded that since the OUMV model was consistently recovered as best-supported in the simulation (Supplementary Figure 1), their dataset had enough power to recover the true model. However, since the true generating model (OUMV) was itself rather complex, such a simulation scenario could, at best, only demonstrate that the results were robust against the opposite and much less likely problem, i.e., simpler models being incorrectly favored over more complex ones.

One way to address this would be to extend the scope of the simulations and perform 100 runs under each of the seven models (with the parameter values of each model set to the estimates from the empirical data). Each set of simulations corresponding to a given model should then be analyzed using all seven models. If the simulations run under relatively simple Brownian motion models yielded consistently lower AIC scores for these models than for the more complex alternative models, this would be good evidence that the OUMV model is not too parameter-rich for the empirical data.

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Review form: Reviewer 2

Recommendation

Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Excellent

General interest: Is the paper of sufficient general interest?

Excellent

Quality of the paper: Is the overall quality of the paper suitable?

Marginal

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

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Yes

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Yes

Is it clear?

Yes

Is it adequate?

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No

Comments to the Author

RSPB-2019-1471 Review

General comments

This study uses a macroevolutionary framework to explore body size evolution for over 4500 migratory and non-migratory species of ray-finned fishes. The authors show that migratory species are evolving towards an optimal body size that is three times larger than non-migratory species and that migratory lineages evolve towards their optimal body size significantly more rapidly than non-migratory lineages, indicating body size is a key adaptation for migratory fishes. The study is well-written, addresses a very interesting theoretical question for evolutionary biology, and has compiled a substantial and diverse dataset for analysis. The study question is certainly relevant to, and appropriately targeted to the aims and scope of PRSB. Some aspects of the statistical methods used, however, need clarification in their description, but also presentation of Results. One of the major concerns with the study is the coarse classification of migratory behavior, and the default classification of more poorly studied species. Much of the dataset seems to rely on FishBase definition, and recognition of migratory behaviors, and some examples are provided (below) of where available data classifications are highly questionable. It may be that the inevitable mixed data quality of such a broad dataset has no overt effect on Results, but the authors need to provide some additional analytical control to satisfy these concerns at some level. For what is undoubtedly an interesting and provocative topic, the level of statistical rigor is also a little underwhelming (although the authors are to be commended for utilizing simulations of best-supported models, often overlooked in similar studies). Much of the current statistical basis of this study hinges upon analyses involving a priori designation of particular ecomorphs in state-dependent comparative modelling, an approach prone to elements of circularity. Given some of the potential biases inherent to the classification of migratory behaviors at the core of this study, it is highly recommended the authors consider incorporating additional analyses that potentially capture evolutionary shifts in body size optima in a continuously varying trait (without pre-defined classification of behavior). The l1ou R-package (Khabbazian et al. 2016)

could be used, for example, to document the purely data-driven shifts body length optima on the phylogenetic tree to suggest cases of positive body selection pressures toward larger optima in migratory species. Further explicit testing of convergence toward larger body sizes in migratory clades could also be included (just one example of several possible approaches or available packages). That particular package does offers several computational advantages for large trees (such as this study)

Specific comments.

Methods.

Line 118. One of the challenges with this topic (and compiling the dataset) is the definition of what constitutes migration in fishes. The study seems to emphasize 'long-distance' migration, with the authors defining a migratory species as any that 'cyclically and predictably moves long distances using active transport'. There is very possibly an inherent bias in underlying datasets toward larger, more conspicuous, better-studied commercially, recreationally or subsistence fishery based species (particularly those large enough to have movement biology quantified through tagging or telemetry based approaches). Large, mobile, marine and diadromous species will clearly be emphasized with this definition. Potadromous migration (which is clearly included in this definition and subsequent dataset) is much harder to capture and reliably quantify, and would again seem biased towards larger, better studied freshwater species. While the authors make this classification challenge (and their treatment) clear, the subsequent default classification of poorly known (and very possibly biased toward smaller-bodied, less conspicuous species in clades) as 'non-migratory' is potentially problematic.

As a brief example (and admittedly Australian-centric), the Australian freshwater percichthyid *Bostockia porosa* is defined as a migratory species in FishBase (and accordingly in this study) on the basis of moving out of river channels into tributaries for breeding. The small-bodied percichthyid 'pygmy perches' (*Nannoperca* spp.) are known to undertake migratory breeding behaviors like *B. porosa* (Allen et al., 2002), but are not defined as 'migratory' in this study (or Fishbase)? Several other Australian species included in the study appear to be classified as 'non-migratory' (*Ambassis agrammus*, *A. macleayi*, *Porochilus rendahlia*, some *Neoarius* spp. and most terapontids), but are well-documented as undertaking often substantial migrations (Allen et al., 2002; Pusey et al., 2004), at least of a scale to those seen in *B. porosa*.

For readers not familiar with rfishbase, it may also be worth documenting how it identifies and classifies migration. Is it a keyword search? As another example of a potential coarse classification challenge, several of the larger serranids in this study which form spawning aggregations are classified as migratory (*Epinephelus*), whereas smaller-bodied clades (*Paralabrax*) which exhibit similar spawning aggregation behaviors presumably involving migration are not?

How pervasive these artefacts are in Fishbase, and how to efficiently address this potential bias in such a large dataset is difficult to assess. The authors emphasize some smaller clades that exhibit the correlations between migration and larger body size (shads, herring etc.). It would be very informative to run the same analyses over several smaller, but still diverse clades (with migratory behaviors reliably classified) in the dataset to see if observed broader patterns are also reflected within these groups.

Line 121- While the phylogeny used in this study is still a substantial dataset (4500 species), the cited Rabosky phylogeny was considerably larger (>10,000 species). Is this exactly the same phylogeny, or has it been trimmed? If so, how exactly was the final phylogeny in this study derived and is it available?

Line 164. This definition of the two BM models needs clarification.

Line 173. The Methods define derivation of AIC weights for models, but no weights are presented in Results (Table 1 presents only AIC scores)?

Discussion.

It could be questioned as to whether interpretation of the main results in the Discussion is

possibly simplistic, at least at some levels? The authors frequently emphasize the benefits of larger body size in energetics and locomotory efficiency (which are very likely relevant to migratory birds and mammals). In many of the fish clades analyzed here, however, while larger adults may well undertake migrations, it is often the much smaller post-larval juveniles or elvers that undertake the most (relatively) substantial migrations in terms of distance or movement challenges (upstream migration into rivers). It would be worth exploring and elaborating on some of these differences associated with fish migration and at what life cycle stages in can occur.

Figure 1. Is this a single SIMMAP reconstruction of migratory evolution? It would be assumed there would be some variability emerging over 100 reconstructions (at particular nodes)?

Allen G.R., Midgley S.H. & Allen M (2002) Field guide to the freshwater fishes of Australia. Western Australian Museum, Perth, WA.

Pusey B.J., Kennard M. & Arthington, A. (2004) Freshwater Fishes of North-Eastern Australia. Melbourne: CSIRO Publishing.

Review form: Reviewer 3

Recommendation

Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Good

General interest: Is the paper of sufficient general interest?

Good

Quality of the paper: Is the overall quality of the paper suitable?

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

Yes

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Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

This study explores evolution towards larger body size in migratory fishes using an evolutionary model-fitting approach across a 4,500 species dataset. The authors find evidence for faster rates of body size evolution and evolution towards a larger optimal body size in migratory species.

Overall, I find the article interesting and novel. I was very impressed with how thorough the authors were with their OUwie analysis, even using simulations to ensure statistical power and interpreting their findings very conservatively. My only concern is the lack of log-transformation of the body size data. I took the liberty of downloading the dataset from the supplemental materials and found that the body size data are extremely non-normally distributed, as is quite common with size data. I worry about how the underlying distribution of the data may affect the findings, especially if the distribution differs between migratory and non-migratory species (as is indicated by figure 2). Primarily, this would potentially influence the phylogenetic ANOVA, as this statistical test assumes an underlying normal distribution. But, in the interest of consistency throughout the study and “good practice”, an argument could be made for running the entire model-fitting framework on the log-transformed body size data, though it isn’t necessarily required for this step of the analysis. That the authors find a rate twelve times more rapid in migratory lineages does imply that not log-transforming the data is potentially inflating these estimates.

Review form: Reviewer 4

Recommendation

Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Excellent

General interest: Is the paper of sufficient general interest?

Excellent

Quality of the paper: Is the overall quality of the paper suitable?

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

Yes

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Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

Thank you for giving me the opportunity to review this manuscript on the adaptive evolution of increased body size in migratory fishes. Overall, I think this paper has done a good job of outlining an interesting question, presenting multiple avenues for investigating that question, and completing a wide range of statistical analyses to answer the question. I believe the paper will be of broad interest to readers of the journal. However, all that being said, I do have some concerns, mostly with regards to the methodology, but also some with regards to the presentation of the results. Provided these concerns are addressed in a revision of the manuscript, I believe this paper would be very suitable for the journal.

Comments on Methodology (in no particular order):

My biggest concern is how the authors address the uncertainty of the phylogeny and the migratory ancestral states. In the ancestral state reconstructions section, the authors mention that they use stochastic character mapping across the 100 trees from Rabosky and Chang. It's unclear how many simmaps were created for each of those 100 trees, though. Using the 100 posterior trees is great for accounting for the phylogenetic uncertainty, but, as mentioned in the `make.simmap` documentation, many stochastic character maps should be made for each of those trees to account for uncertainty in the ancestral state estimations (I would say at least 50 for each posterior tree). If you have already done this, then the text should be updated to reflect exactly how many simmaps were made for each tree (and how many simmaps were made in total). If not, you will need perform such analyses before submitting a revision.

Furthermore, in the macroevolutionary model fitting section, you once again mention accounting for phylogenetic uncertainty but do not address the ASR uncertainty. These OUwie analyses should be run across the simmaps discussed above.

In terms of presentation of the results, the authors show a single ASR (Figure 1) and a single set of OUwie model fits (Table 1). However, even without the addition of the simmaps as discussed above, there should be at least 100 sets of results for the ASR and the OUwie results. It's unclear how the results in Figure 1 and Table 1 were selected from these distributions of results. Were they selected by random? Are they means or medians? The selection criteria should be discussed (at the very least, just say you picked one at random) in the text or captions.

Furthermore, the uncertainty that is revealed by the OUwie replicates (not simulations) should be presented, at least in supplemental figures/tables, as the authors have done with the insets in Figure 1 and Figure S2 for the OUwie simulations. This should include the uncertainty in model AIC and parameter values.

Another major concern I have is the authors' discussion of the σ^2 parameter. The authors mention (line 171) that this is the "rate parameter". However, I would instead consider this the "intensity of random drift" or something similar. The important distinction here is that the evolution associated with the σ^2 parameter is random, whereas the evolution associated with the alpha parameter is selective/directional. On line 111, the authors mention they are interested in the rate of body size as a proxy for the degree of selection. In this case, you are most definitely interested in the alpha parameter, which is a measure of the degree of selection, not the σ^2 parameter. In fact, I believe it would make much more sense for you to perform some model averaging on your parameter estimates (using AICc weights) to determine the estimated alpha (or transformed to phylogenetic half-life) parameters for the migratory and non-migratory fishes rather than to introduce a new method (compare.evol.rates) to calculate the rate of evolution. Ultimately, since your second best model is the OUMA model, you should find different alpha estimates for the different groups, even when you model-average.

Finally, body size is usually ln- or log10-transformed. It appears you performed the OUwie analyses on untransformed data. I wonder if and how your results would be different if you performed them on transformed data. At the very least, I would suggest transforming the body sizes in the figures.

Minor comments (in sequential order):

On lines 163-166, you mention two Brownian motion models, but don't discuss the differences between them.

On line 166, you mention OU for the first time without defining it.

On lines 197 and 198, you mention the simmaps show the ancestor was non-migratory. Is this true across all of the simmaps?

On line 200, I believe you meant Figure 1.

On lines 201 and 204, I would say "x times on average" instead of just "x times".

On line 206, you mention migratory species are generally larger than non-migratory species. Have you checked to see if this trend is driven by just a handful of families? Or is this pattern consistent across the entire tree?

On line 211, you mention that most migratory lineages have a larger size than their nearest non-migratory sister taxa. Is this statement based solely on visually assessing Figure 1? What is "most" in this case? Could you calculate this or even get some sort of statistic?

The model names in Table 1 should match those in the text (e.g. OU1, BM1, BMS).

Is there a reason you didn't use the AICc values returned by OUwie? Since all of your models have the same sample size, it probably doesn't matter, I'm just wondering.

Should the AIC numbers in Table 1 be an order of magnitude different from the AIC numbers in Figure S1?

You may want to log-transform the y-axes for alpha and σ^2 in Figure S2.

Decision letter (RSPB-2019-1471.R0)

06-Aug-2019

Dear Dr Burns:

I am writing to inform you that your manuscript RSPB-2019-1471 entitled "Migratory lineages rapidly evolve large body sizes in ray-finned fishes" has, in its current form, been rejected for publication in Proceedings B.

This action has been taken on the advice of referees, who have recommended that substantial revisions are necessary. With this in mind we would be happy to consider a resubmission, provided the comments of the referees are fully addressed. However please note that this is not a provisional acceptance.

The resubmission will be treated as a new manuscript. However, we will approach the same reviewers if they are available and it is deemed appropriate to do so by the Editor. Please note that resubmissions must be submitted within six months of the date of this email. In exceptional circumstances, extensions may be possible if agreed with the Editorial Office. Manuscripts submitted after this date will be automatically rejected.

Please find below the comments made by the referees, not including confidential reports to the Editor, which I hope you will find useful. If you do choose to resubmit your manuscript, please upload the following:

- 1) A 'response to referees' document including details of how you have responded to the comments, and the adjustments you have made.
- 2) A clean copy of the manuscript and one with 'tracked changes' indicating your 'response to referees' comments document.
- 3) Line numbers in your main document.

To upload a resubmitted manuscript, log into <http://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Resubmission." Please be sure to indicate in your cover letter that it is a resubmission, and supply the previous reference number.

Sincerely,
Professor Gary Carvalho
<mailto:proceedingsb@royalsociety.org>

Associate Editor
Board Member: 1
Comments to Author:

I have now received four detailed referee reports on this manuscript. (I aimed to obtain three reports only, but a late acceptance by one of the referees resulted in four recommendations – something admittedly unusual, but no doubt constructive). All referees have expertise in phylogenetic comparative analyses, and some are also ichthyologists. The paper addresses the relationship between body size and migration using a megatree of ray-finned fishes. The study question is interesting, and all reviewers see merit and significance. Some concerns raised, however, include ambiguity and coarse categorization of migratory behavior for poorly studied species (Ref. 2), lack of data-driven tests of body size convergence (without a priori designations, such as IIOu; Ref. 2), lack of log-transformation of body size data (Refs. 3,4), a rather superficial

accounting of phylogenetic uncertainty (Ref. 4; e.g., could use 50 SIMMAP replicates for each of the 100 trees analyzed; “there should be at least 100 sets of results for the ASR and the OUwie results”), and apparent misinterpretation of the σ^2 parameter. Several instances of possible miscoded species were also indicated by Ref. 2 (see ‘specific comments’).

Based on my own assessment of the paper, I also strongly encourage the authors to apply Felsenstein’s (2012) threshold model to test for evolutionary covariation between discrete (e.g., migratory behavior) and continuous (e.g., body size) traits.: “The model assumes that there is a quantitative character, called liability, that is unobserved and that determines the discrete character according to whether the liability exceeds a threshold value... In this way, one can make a comparative-methods analysis that combines both discrete and continuous characters.” See this blog post by Liam Revell for more details on the method: <http://blog.phytools.org/2012/08/bayesian-mcmc-for-threshold-model.html>. Finally, some discussion on cause vs. effect is also warranted here: did migratory fishes evolve large body sizes or did large species evolve migratory behavior? (a point raised at the ASIH meeting this summer, where the authors presented their work).

Sincerely,
Ricardo Betancur-R.
Associate Editor

Reviewer(s)' Comments to Author:

Referee: 1

Comments to the Author(s)

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Referee: 2

Comments to the Author(s)

RSPB-2019-1471 Review

General comments

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Specific comments.

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As a brief example (and admittedly Australian-centric), the Australian freshwater percichthyid *Bostockia porosa* is defined as a migratory species in FishBase (and accordingly in this study) on the basis of moving out of river channels into tributaries for breeding. The small-bodied percichthyid ‘pygmy perches’ (*Nannoperca* spp.) are known to undertake migratory breeding behaviors like *B. porosa* (Allen et al., 2002), but are not defined as ‘migratory’ in this study (or Fishbase)? Several other Australian species included in the study appear to be classified as ‘non-migratory’ (*Ambassis agrammus*, *A. macleayi*, *Porochilus rendahlii*, some *Neoarius* spp. and most terapontids), but are well-documented as undertaking often substantial migrations (Allen et al., 2002; Pusey et al., 2004), at least of a scale to those seen in *B. porosa*.

For readers not familiar with `rfishbase`, it may also be worth documenting how it identifies and classifies migration. Is it a keyword search? As another example of a potential coarse classification challenge, several of the larger serranids in this study which form spawning

aggregations are classified as migratory (*Epinephelus*), whereas smaller-bodied clades (*Paralabrax*) which exhibit similar spawning aggregation behaviors presumably involving migration are not?

How pervasive these artefacts are in Fishbase, and how to efficiently address this potential bias in such a large dataset is difficult to assess. The authors emphasize some smaller clades that exhibit the correlations between migration and larger body size (shads, herring etc.). It would be very informative to run the same analyses over several smaller, but still diverse clades (with migratory behaviors reliably classified) in the dataset to see if observed broader patterns are also reflected within these groups.

Line 121- While the phylogeny used in this study is still a substantial dataset (4500 species), the cited Rabosky phylogeny was considerably larger (>10,000 species). Is this exactly the same phylogeny, or has it been trimmed? If so, how exactly was the final phylogeny in this study derived and is it available?

Line 164. This definition of the two BM models needs clarification.

Line 173. The Methods define derivation of AIC weights for models, but no weights are presented in Results (Table 1 presents only AIC scores)?

Discussion.

It could be questioned as to whether interpretation of the main results in the Discussion is possibly simplistic, at least at some levels? The authors frequently emphasize the benefits of larger body size in energetics and locomotory efficiency (which are very likely relevant to migratory birds and mammals). In many of the fish clades analyzed here, however, while larger adults may well undertake migrations, it is often the much smaller post-larval juveniles or elvers that undertake the most (relatively) substantial migrations in terms of distance or movement challenges (upstream migration into rivers). It would be worth exploring and elaborating on some of these differences associated with fish migration and at what life cycle stages in can occur.

Figure 1. Is this a single SIMMAP reconstruction of migratory evolution? It would be assumed there would be some variability emerging over 100 reconstructions (at particular nodes)?

Allen G.R., Midgley S.H. & Allen M (2002) Field guide to the freshwater fishes of Australia. Western Australian Museum, Perth, WA.

Pusey B.J., Kennard M. & Arthington, A. (2004) Freshwater Fishes of North-Eastern Australia. Melbourne: CSIRO Publishing.

Referee: 3

Comments to the Author(s)

This study explores evolution towards larger body size in migratory fishes using an evolutionary model-fitting approach across a 4,500 species dataset. The authors find evidence for faster rates of body size evolution and evolution towards a larger optimal body size in migratory species.

Overall, I find the article interesting and novel. I was very impressed with how thorough the authors were with their OUwie analysis, even using simulations to ensure statistical power and interpreting their findings very conservatively. My only concern is the lack of log-transformation of the body size data. I took the liberty of downloading the dataset from the supplemental materials and found that the body size data are extremely non-normally distributed, as is quite common with size data. I worry about how the underlying distribution of the data may affect the findings, especially if the distribution differs between migratory and non-migratory species (as is indicated by figure 2). Primarily, this would potentially influence the phylogenetic ANOVA, as this statistical test assumes an underlying normal distribution. But, in the interest of consistency throughout the study and "good practice", an argument could be made for running the entire model-fitting framework on the log-transformed body size data, though it isn't necessarily required for this step of the analysis. That the authors find a rate twelve times more rapid in

migratory lineages does imply that not log-transforming the data is potentially inflating these estimates.

Referee: 4

Comments to the Author(s)

Thank you for giving me the opportunity to review this manuscript on the adaptive evolution of increased body size in migratory fishes. Overall, I think this paper has done a good job of outlining an interesting question, presenting multiple avenues for investigating that question, and completing a wide range of statistical analyses to answer the question. I believe the paper will be of broad interest to readers of the journal. However, all that being said, I do have some concerns, mostly with regards to the methodology, but also some with regards to the presentation of the results. Provided these concerns are addressed in a revision of the manuscript, I believe this paper would be very suitable for the journal.

Comments on Methodology (in no particular order):

My biggest concern is how the authors address the uncertainty of the phylogeny and the migratory ancestral states. In the ancestral state reconstructions section, the authors mention that they use stochastic character mapping across the 100 trees from Rabosky and Chang. It's unclear how many simmaps were created for each of those 100 trees, though. Using the 100 posterior trees is great for accounting for the phylogenetic uncertainty, but, as mentioned in the `make.simmap` documentation, many stochastic character maps should be made for each of those trees to account for uncertainty in the ancestral state estimations (I would say at least 50 for each posterior tree). If you have already done this, then the text should be updated to reflect exactly how many simmaps were made for each tree (and how many simmaps were made in total). If not, you will need perform such analyses before submitting a revision.

Furthermore, in the macroevolutionary model fitting section, you once again mention accounting for phylogenetic uncertainty but do not address the ASR uncertainty. These OUwie analyses should be run across the simmaps discussed above.

In terms of presentation of the results, the authors show a single ASR (Figure 1) and a single set of OUwie model fits (Table 1). However, even without the addition of the simmaps as discussed above, there should be at least 100 sets of results for the ASR and the OUwie results. It's unclear how the results in Figure 1 and Table 1 were selected from these distributions of results. Were they selected by random? Are they means or medians? The selection criteria should be discussed (at the very least, just say you picked one at random) in the text or captions.

Furthermore, the uncertainty that is revealed by the OUwie replicates (not simulations) should be presented, at least in supplemental figures/tables, as the authors have done with the insets in Figure 1 and Figure S2 for the OUwie simulations. This should include the uncertainty in model AIC and parameter values.

Another major concern I have is the authors' discussion of the σ^2 parameter. The authors mention (line 171) that this is the "rate parameter". However, I would instead consider this the "intensity of random drift" or something similar. The important distinction here is that the evolution associated with the σ^2 parameter is random, whereas the evolution associated with the α parameter is selective/directional. On line 111, the authors mention they are interested in the rate of body size as a proxy for the degree of selection. In this case, you are most definitely interested in the α parameter, which is a measure of the degree of selection, not the σ^2 parameter. In fact, I believe it would make much more sense for you to perform some model averaging on your parameter estimates (using AICc weights) to determine the estimated

alpha (or transformed to phylogenetic half-life) parameters for the migratory and non-migratory fishes rather than to introduce a new method (compare.evol.rates) to calculate the rate of evolution. Ultimately, since your second best model is the OUMA model, you should find different alpha estimates for the different groups, even when you model-average.

Finally, body size is usually ln- or log10-transformed. It appears you performed the OUwie analyses on untransformed data. I wonder if and how your results would be different if you performed them on transformed data. At the very least, I would suggest transforming the body sizes in the figures.

Minor comments (in sequential order):

On lines 163-166, you mention two Brownian motion models, but don't discuss the differences between them.

On line 166, you mention OU for the first time without defining it.

On lines 197 and 198, you mention the simmaps show the ancestor was non-migratory. Is this true across all of the simmaps?

On line 200, I believe you meant Figure 1.

On lines 201 and 204, I would say "x times on average" instead of just "x times".

On line 206, you mention migratory species are generally larger than non-migratory species. Have you checked to see if this trend is driven by just a handful of families? Or is this pattern consistent across the entire tree?

On line 211, you mention that most migratory lineages have a larger size than their nearest non-migratory sister taxa. Is this statement based solely on visually assessing Figure 1? What is "most" in this case? Could you calculate this or even get some sort of statistic?

The model names in Table 1 should match those in the text (e.g. OU1, BM1, BMS).

Is there a reason you didn't use the AICc values returned by OUwie? Since all of your models have the same sample size, it probably doesn't matter, I'm just wondering.

Should the AIC numbers in Table 1 be an order of magnitude different from the AIC numbers in Figure S1?

You may want to log-transform the y-axes for alpha and σ^2 in Figure S2.

Author's Response to Decision Letter for (RSPB-2019-1471.R0)

See Appendix A.

RSPB-2019-2615.R0

Review form: Reviewer 2

Recommendation

Major revision is needed (please make suggestions in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Excellent

General interest: Is the paper of sufficient general interest?

Excellent

Quality of the paper: Is the overall quality of the paper suitable?

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

Yes

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

Yes

Comments to the Author

This study is a revised submission that uses a macroevolutionary framework to explore body size evolution for over 4500 migratory and non-migratory species of ray-finned fishes. The authors show that migratory species are evolving towards a larger optimal body size than non-migratory species, and that migratory lineages evolve towards their optimal body size significantly more rapidly than non-migratory lineages, indicating body size is a key adaption for migratory fishes. The study addresses a very interesting theoretical question for evolutionary biology, and has compiled a substantial and diverse dataset for analysis.

While the authors have done a creditable job of addressing several earlier concerns, much of the Results of this paper still hinges on the evolutionary modelling component. A range of recent

methodological meta-analyses (Silvestro et al., 2015; Pennell et al., 2015; Cooper et al., 2016 etc.) have all identified very small amounts of error in datasets can have profound effects on the inferences derived from OU models, and that large trees are particularly susceptible to issues arising from unaccounted measurement error in the data (consistently biasing model selection towards erroneous rejection of simpler models in favour of more parameter-rich models such as the OU model). As was identified in even a cursory review of data with the initial submission, errors in discrete ecological classification, (as well as maximum body size data in poorly known species) are likely not uncommon in this dataset. These limitations mean that when evidence for the OU model is found, the results should be interpreted with caution, particularly where there is likely to be intraspecific variation or measurement error in the data. Obtaining accurate measurement error estimates (which can alleviate these concerns) is difficult with the current data (a single observation per species), so other avenues should be considered.

This is a fantastic dataset, but given some of the inherent limitations of the evolutionary modelling approaches, I would still recommend the authors consider incorporating additional analyses that potentially capture evolutionary shifts in body size optima in a continuously varying trait, without pre-defined classification of behavior. The points the authors make regarding explicit convergence analyses are warranted, but approaches such as l1ou R-package (Khabbazian et al. 2016) allow for simple identification of adaptive shifts (without convergence) in a continuous trait. Additional, purely data-driven evidence of increased body size in migratory clades compared to closely related non-migratory clades would tell a much more compelling story. While such approaches may also capture broader shifts in the body size adaptive landscape of fishes, if migratory behavior is driving significant shifts in body size, they should be apparent. These are not necessarily computationally demanding approaches, and this is also not an apparently overly long paper.

Review form: Reviewer 3

Recommendation

Accept with minor revision (please list in comments)

Scientific importance: Is the manuscript an original and important contribution to its field?

Good

General interest: Is the paper of sufficient general interest?

Good

Quality of the paper: Is the overall quality of the paper suitable?

Good

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

This study looks at the evolution of body size in migratory fishes on a macroevolutionary scale. The authors find evidence for a trend towards larger body size in migratory fishes, reasoning that larger size is more energetically efficient when moving long distances. I think this paper would make an excellent contribution to Proceedings B with some minor revisions detailed below.

It is a little unclear how the authors ran their phylogenetic analyses across the posterior distribution from the Rabosky tree, as maximum likelihood methods were used to produce this phylogeny. If the phylogeny used is the version of the Rabosky tree with imputed tips, it should be clarified in the paper that not all tips have underlying genetic information determining their positions.

Page 4, Line 87. Goby is spelled incorrectly

Line 175. ACR analysis should be defined prior to use of the acronym

Review form: Reviewer 4

Recommendation

Accept as is

Scientific importance: Is the manuscript an original and important contribution to its field?

Excellent

General interest: Is the paper of sufficient general interest?

Excellent

Quality of the paper: Is the overall quality of the paper suitable?

Excellent

Is the length of the paper justified?

Yes

Should the paper be seen by a specialist statistical reviewer?

No

Do you have any concerns about statistical analyses in this paper? If so, please specify them explicitly in your report.

No

It is a condition of publication that authors make their supporting data, code and materials available - either as supplementary material or hosted in an external repository. Please rate, if applicable, the supporting data on the following criteria.

Is it accessible?

Yes

Is it clear?

Yes

Is it adequate?

Yes

Do you have any ethical concerns with this paper?

No

Comments to the Author

The authors have substantially revised their manuscript based on the input from the editor and reviewers on a previous version of the manuscript. I'm happy with their changes and believe the manuscript is suitable for publication.

Decision letter (RSPB-2019-2615.R0)

02-Dec-2019

Dear Dr Burns:

Your manuscript has now been peer reviewed and the reviews have been assessed by an Associate Editor. The reviewers' comments (not including confidential comments to the Editor) and the comments from the Associate Editor are included at the end of this email for your reference. As you will see, the reviewers and the Editors have raised some concerns with your manuscript and we would like to invite you to revise your manuscript to address them.

We do not allow multiple rounds of revision so we urge you to make every effort to fully address all of the comments at this stage. If deemed necessary by the Associate Editor, your manuscript will be sent back to one or more of the original reviewers for assessment. If the original reviewers are not available we may invite new reviewers. Please note that we cannot guarantee eventual acceptance of your manuscript at this stage.

To submit your revision please log into <http://mc.manuscriptcentral.com/prsb> and enter your Author Centre, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions", click on "Create a Revision". Your manuscript number has been appended to denote a revision.

When submitting your revision please upload a file under "Response to Referees" in the "File

Upload" section. This should document, point by point, how you have responded to the reviewers' and Editors' comments, and the adjustments you have made to the manuscript. We require a copy of the manuscript with revisions made since the previous version marked as 'tracked changes' to be included in the 'response to referees' document.

Your main manuscript should be submitted as a text file (doc, txt, rtf or tex), not a PDF. Your figures should be submitted as separate files and not included within the main manuscript file.

When revising your manuscript you should also ensure that it adheres to our editorial policies (<https://royalsociety.org/journals/ethics-policies/>). You should pay particular attention to the following:

Research ethics:

If your study contains research on humans please ensure that you detail in the methods section whether you obtained ethical approval from your local research ethics committee and gained informed consent to participate from each of the participants.

Use of animals and field studies:

If your study uses animals please include details in the methods section of any approval and licences given to carry out the study and include full details of how animal welfare standards were ensured. Field studies should be conducted in accordance with local legislation; please include details of the appropriate permission and licences that you obtained to carry out the field work.

Data accessibility and data citation:

It is a condition of publication that you make available the data and research materials supporting the results in the article. Datasets should be deposited in an appropriate publicly available repository and details of the associated accession number, link or DOI to the datasets must be included in the Data Accessibility section of the article (<https://royalsociety.org/journals/ethics-policies/data-sharing-mining/>). Reference(s) to datasets should also be included in the reference list of the article with DOIs (where available).

In order to ensure effective and robust dissemination and appropriate credit to authors the dataset(s) used should also be fully cited and listed in the references.

If you wish to submit your data to Dryad (<http://datadryad.org/>) and have not already done so you can submit your data via this link

[http://datadryad.org/submit?journalID=RSPB&manu=\(Document not available\)](http://datadryad.org/submit?journalID=RSPB&manu=(Document not available)), which will take you to your unique entry in the Dryad repository.

If you have already submitted your data to dryad you can make any necessary revisions to your dataset by following the above link.

For more information please see our open data policy <http://royalsocietypublishing.org/data-sharing>.

Electronic supplementary material:

All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI. Please try to submit all supplementary material as a single file.

Online supplementary material will also carry the title and description provided during submission, so please ensure these are accurate and informative. Note that the Royal Society will not edit or typeset supplementary material and it will be hosted as provided. Please ensure that the supplementary material includes the paper details (authors, title, journal name, article DOI). Your article DOI will be 10.1098/rspb.[paper ID in form xxxx.xxxx e.g. 10.1098/rspb.2016.0049].

Please submit a copy of your revised paper within three weeks. If we do not hear from you within this time your manuscript will be rejected. If you are unable to meet this deadline please let us know as soon as possible, as we may be able to grant a short extension.

Thank you for submitting your manuscript to Proceedings B; we look forward to receiving your revision. If you have any questions at all, please do not hesitate to get in touch.

Best wishes,
 Professor Gary Carvalho
 mailto: proceedingsb@royalsociety.org

Associate Editor

Comments to Author:

I commend the authors for addressing the concerns raised by the four referees and myself during the first round of review as well as for running new analyses, which were clearly computationally demanding. All three referees who reviewed the revised manuscript agree that it offers a clear improvement over the first version. Referee 2, however, still feels that data-driven l1ou tests, without an a priori designation of migratory behavior, are needed here. I think authors should give l1ou a try. I agree with Ref. 2 that the method is designed to identify adaptive shifts that are not necessarily the result of convergence. I see three potentially contrasting outcomes here based on conceivable results and computational requirements:

- 1- l1ou analyses are not computationally prohibitive for a dataset of this size and the results are concordant with those obtained using a priori designations of migration behavior. This is a best-case scenario, which can be reported in the main text.
- 2- Analyses are not computationally demanding, but results prove difficult to interpret. Having run l1ou myself on other datasets, I see how this is a highly likely outcome here, but I don't think it necessarily invalidates other results. My feeling is that l1ou has power issues. If that happens to be the case, just downplay the importance of these analyses (e.g., report in supplement). The more empirical studies showing that a particular method fails to capture biological reality, the more likely are developers to be motivated to improve their package.
- 3- Analyses prove computationally demanding for this dataset. I do not expect authors to spend three additional months running l1ou. Just resubmit stating so.

Referee 2 also raise concerns about how minor behavior miss-classifications can have profound effects on the results. I agree with the authors that, given the scale of the dataset, some species will no doubt be misclassified. This is surely also the case for other large-scale studies addressing comparative questions (e.g., Rabosky et al.'s study on latitude, our own paper on marine and freshwater habitats). I think a short comment on this is warranted, also discussing the papers pointed out by the referee (Silvestro et al., 2015; Pennell et al., 2015).

Finally, Referee 3 makes a minor, though important point here. The authors should clarify that not all tips in Rabosky's tree are placed based on underlying genetic data. How many species are actually placed using data? How many are imputed?

Ricardo Betancur

Associate Editor

Reviewer(s)' Comments to Author:

Referee: 3

Comments to the Author(s).

This study looks at the evolution of body size in migratory fishes on a macroevolutionary scale. The authors find evidence for a trend towards larger body size in migratory fishes, reasoning that larger size is more energetically efficient when moving long distances. I think this paper would make an excellent contribution to Proceedings B with some minor revisions detailed below.

It is a little unclear how the authors ran their phylogenetic analyses across the posterior distribution from the Rabosky tree, as maximum likelihood methods were used to produce this phylogeny. If the phylogeny used is the version of the Rabosky tree with imputed tips, it should be clarified in the paper that not all tips have underlying genetic information determining their positions.

Page 4, Line 87. Goby is spelled incorrectly

Line 175. ACR analysis should be defined prior to use of the acronym

Referee: 4

Comments to the Author(s).

The authors have substantially revised their manuscript based on the input from the editor and reviewers on a previous version of the manuscript. I'm happy with their changes and believe the manuscript is suitable for publication.

Referee: 2

Comments to the Author(s).

This study is a revised submission that uses a macroevolutionary framework to explore body size evolution for over 4500 migratory and non-migratory species of ray-finned fishes. The authors show that migratory species are evolving towards a larger optimal body size than non-migratory species, and that migratory lineages evolve towards their optimal body size significantly more rapidly than non-migratory lineages, indicating body size is a key adaptation for migratory fishes. The study addresses a very interesting theoretical question for evolutionary biology, and has compiled a substantial and diverse dataset for analysis.

While the authors have done a creditable job of addressing several earlier concerns, much of the Results of this paper still hinges on the evolutionary modelling component. A range of recent methodological meta-analyses (Silvestro et al., 2015; Pennell et al., 2015; Cooper et al., 2016 etc.) have all identified very small amounts of error in datasets can have profound effects on the inferences derived from OU models, and that large trees are particularly susceptible to issues arising from unaccounted measurement error in the data (consistently biasing model selection towards erroneous rejection of simpler models in favour of more parameter-rich models such as the OU model). As was identified in even a cursory review of data with the initial submission, errors in discrete ecological classification, (as well as maximum body size data in poorly known species) are likely not uncommon in this dataset. These limitations mean that when evidence for the OU model is found, the results should be interpreted with caution, particularly where there is likely to be intraspecific variation or measurement error in the data. Obtaining accurate

measurement error estimates (which can alleviate these concerns) is difficult with the current data (a single observation per species), so other avenues should be considered. This is a fantastic dataset, but given some of the inherent limitations of the evolutionary modelling approaches, I would still recommend the authors consider incorporating additional analyses that potentially capture evolutionary shifts in body size optima in a continuously varying trait, without pre-defined classification of behavior. The points the authors make regarding explicit convergence analyses are warranted, but approaches such as l1ou R-package (Khabbazian et al. 2016) allow for simple identification of adaptive shifts (without convergence) in a continuous trait. Additional, purely data-driven evidence of increased body size in migratory clades compared to closely related non-migratory clades would tell a much more compelling story. While such approaches may also capture broader shifts in the body size adaptive landscape of fishes, if migratory behavior is driving significant shifts in body size, they should be apparent. These are not necessarily computationally demanding approaches, and this is also not an apparently overly long paper.

Author's Response to Decision Letter for (RSPB-2019-2615.R0)

See Appendix B.

Decision letter (RSPB-2019-2615.R1)

11-Dec-2019

Dear Dr Burns

I am pleased to inform you that your manuscript entitled "Migratory lineages rapidly evolve larger body sizes than non-migratory relatives in ray-finned fishes" has been accepted for publication in Proceedings B.

You can expect to receive a proof of your article from our Production office in due course, please check your spam filter if you do not receive it. PLEASE NOTE: you will be given the exact page length of your paper which may be different from the estimation from Editorial and you may be asked to reduce your paper if it goes over the 10 page limit.

If you are likely to be away from e-mail contact please let us know. Due to rapid publication and an extremely tight schedule, if comments are not received, we may publish the paper as it stands.

If you have any queries regarding the production of your final article or the publication date please contact procb_proofs@royalsociety.org

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Electronic supplementary material:

All supplementary materials accompanying an accepted article will be treated as in their final form. They will be published alongside the paper on the journal website and posted on the online figshare repository. Files on figshare will be made available approximately one week before the accompanying article so that the supplementary material can be attributed a unique DOI.

Thank you for your fine contribution. On behalf of the Editors of the Proceedings B, we look forward to your continued contributions to the Journal.

Sincerely,

Professor Gary Carvalho
Editor, Proceedings B
mailto: proceedingsb@royalsociety.org

Associate Editor:
Board Member
Comments to Author:
(There are no comments.)

Appendix A

Associate Editor Comments

Based on my own assessment of the paper, I also strongly encourage the authors to apply Felsenstein's (2012) threshold model to test for evolutionary covariation between discrete (e.g., migratory behavior) and continuous (e.g., body size) traits.

We implemented Felsenstein's 2012 threshold model to test for evolutionary covariation between discrete (e.g., migratory behavior) and continuous (e.g., body size) traits using the threshBayes function in phytools. We ran threshBayes for 1.0×10^6 generations, sampling every 100 generations. See lines 163-169 for more details. Under the threshold model, we found strong evolutionary covariation between body size and migratory behavior ($r=.93$). We updated lines 230-232 with the results of the threshold model.

Finally, some discussion on cause vs. effect is also warranted here: did migratory fishes evolve large body sizes or did large species evolve migratory behavior?

We updated the discussion (Lines 300-305) to convey that multiple intraspecific studies have shown that phenotypic traits, such as body size, rapidly evolve in response to the evolution of migratory behavior in a population, or the loss of migration, rather than the predisposition of larger species to evolve a migratory behavior. These studies suggest that migratory fishes evolve larger body sizes, rather than larger species evolving a migratory behavior.

Referee: 1

Lines 53–54, 271–272: While evolution toward an optimum is a correct characterization of the Ornstein-Uhlenbeck (OU) family of models, and while one of these models (OUMV) was found to be the best-fit model by the authors, I would be cautious about couching the overall findings in this language. After all, all models – including the best-fit one – are necessarily oversimplifications of reality, and it is difficult to imagine that given their sheer ecomorphological diversity, the 4500+ species analyzed by the authors all evolve toward just two body-size optima determined solely by their migratory status. To make it clear that these caveats have not been ignored, it would be helpful to discuss this particular result strictly in terms of theta values estimated under a multipeak OU model rather than in terms of biological reality.

We agree that given the ecomorphological diversity across 4600 species analyzed that is incredibly unlikely that ray-finned fishes are evolving towards just two optima. We have updated the text to focus strictly in terms of theta values estimated under a multipeak OU model and the overall pattern rather than in terms of biological reality.

Line 70: If I correctly understand the point being made here, it might be more straightforward to say that migration costs are “minimized” rather than “optimized”.

Line 70: Changed “optimize” to “minimize”.

Subsection “Ecological Classification and Body Size”: The exact number of species analyzed should be given, as well as the numbers of migratory and non-migratory species.

The number of total species, number of migratory species and the number non-migratory species analyzed was added to Lines 135-137. There was a total of 4648 species in our dataset with, with 585 species classified as migratory and 4063 classified as non-migratory

Lines 127–128: “personal” not “personnel”.

Lines 127-128: Changed “personnel” to “personal”.

Paragraph spanning lines 181–186: I share the authors’ concern about the best-fit models possibly being overparameterized, i.e., incorrectly favored over simpler models. However, it seems to me that their simulation analyses do not address this problem. The authors ran 100 simulations under the OUMV model and under the parameter values estimated for this model from their empirical dataset, and analyzed the resulting simulated datasets using each of the seven models under consideration. They concluded that since the OUMV model was consistently recovered as best-supported in the simulation (Supplementary Figure 1), their dataset had enough power to recover the true model. However, since the true generating model (OUMV) was itself rather complex, such a simulation scenario could, at best, only demonstrate that the results were robust against the opposite and much less likely problem, i.e., simpler models being incorrectly favored over more complex ones.

One way to address this would be to extend the scope of the simulations and perform 100 runs under each of the seven models (with the parameter values of each model set to the estimates from the empirical data). Each set of simulations corresponding to a given model should then be analyzed using all seven models. If the simulations run under relatively simple Brownian motion models yielded consistently lower AIC scores for these models than for the more complex alternative models, this would be good evidence that the OUMV model is not too parameter-rich for the empirical data.

During revision, we ran six different simulation sets using the empirical values from each model. We then ran OUwie on each simulation set to determine whether we could recover the model that the data was simulated under. See Lines 198-204 for an updated description in the methods. We found that we were able to recover the simpler models (BM1 +BMS +OU1) when simulating data under these parameters indicating that the OUMV and OUMA models were not too parameter rich for our dataset. See lines 251-263 in the results as well as Supplementary Figures 4 and 5.

Line 254: This is more of a semantic quibble, but if the nonmigratory-to-migratory transition took place 70 times, it should not be described as only happening “rarely”.

Line 254-255: We agree this was a mischaracterization and have removed the part of the sentence stating that migration is rarely lost.

Referee: 2

It is highly recommended the authors consider incorporating additional analyses that potentially capture evolutionary shifts in body size optima in a continuously varying trait (without pre-defined classification of behavior). The *l1ou* R-package (Khabbazian et al. 2016) could be used, for example, to document the purely data-driven shifts body length optima on the phylogenetic tree to suggest cases of positive body selection pressures toward larger optima in migratory species. Further explicit testing of convergence toward larger body sizes in migratory clades could also be included (just one example of several possible approaches or available packages). That particular package does offers several computational advantages for large trees (such as this study)

We believe our analyses are the best available and most appropriate for the questions and data. Additionally, we bolstered our analyses by following PRSB Associate Editor Betancur's suggestion to include Felsenstein's 2012 threshold model to test for evolutionary covariation between discrete (e.g., migratory behavior) and continuous (e.g., body size) traits.

The reviewer suggested using *l1ou* to document purely data-driven shifts in adaptive optima to suggest cases of positive body selection toward larger optima in migratory species. However, an analysis like *l1ou* will recover shifts in the adaptive optima caused by a multitude of selective pressures, while fully recovering the adaptive landscape of body size evolution in ray-finned fishes. We want to clarify that the goal of this study was not to recover evolutionary processes driving body size evolution across ray-finned fishes, nor show that adaptation to a migratory behavior is responsible for the majority of body size shifts in ray-finned fishes. Rather, we are interested in whether the evolution of a migratory behavior results in a repeated pattern of body size evolution in which migratory lineages are evolving towards a larger optimal body size than their non-migratory relatives. We think this question is best answered by a method with *a priori* assignment of the lineages that are migratory.

Also, the reviewer suggested using *l1ou* to test for convergence in body size. However, testing for body size convergence in the framework of *l1ou* or *surface* is not the goal of this study, nor is it the pattern we are arguing is evident in ray-finned fishes based our data and analyses. We are not suggesting that migratory ray-finned fishes are converging on a single optimal body size (trait value) and we do not expect to see rampant body size convergence among migratory lineages (which is what *l1ou* tests for). Rather we show that ray-finned fishes are exhibiting a repeated pattern of body size evolution in which migratory lineages are evolving towards a larger optimal body size than their non-migratory relatives. However, the reviewer's comment brought to our attention that confusion arises from the manner in which we discuss the pattern of body size evolution in a multiple peak OU model framework. We have updated our discussion to focus on the shared and repeated pattern of body size evolution when adapting to a migratory behavior rather than implying that a single optimal body size value is shared across broad lineages of migratory fishes.

Line 118. One of the challenges with this topic (and compiling the dataset) is the definition of what constitutes migration in fishes. The study seems to emphasize 'long-distance' migration, with the authors defining a migratory species as any that 'cyclically and predictably moves long distances using active transport'. There is very possibly an inherent bias in underlying datasets toward larger, more conspicuous, better-studied commercially, recreationally or subsistence fishery based species (particularly those large enough to have movement biology quantified

through tagging or telemetry based approaches). Large, mobile, marine and diadromous species will clearly be emphasized with this definition. Potadromous migration (which is clearly included in this definition and subsequent dataset) is much harder to capture and reliably quantify, and would again seem biased towards larger, better studied freshwater species. While the authors make this classification challenge (and their treatment) clear, the subsequent default classification of poorly known (and very possibly biased toward smaller-bodied, less conspicuous species in clades) as 'non-migratory' is potentially problematic.

As a brief example (and admittedly Australian-centric), the Australian freshwater percichthyid *Bostockia porosa* is defined as a migratory species in FishBase (and accordingly in this study) on the basis of moving out of river channels into tributaries for breeding. The small-bodied percichthyid 'pygmy perches' (*Nannoperca* spp.) are known to undertake migratory breeding behaviors like *B. porosa* (Allen et al., 2002), but are not defined as 'migratory' in this study (or Fishbase)? Several other Australian species included in the study appear to be classified as 'non-migratory' (*Ambassis agrammus*, *A. macleayi*, *Porochilus rendahlia*, some *Neoarius* spp. and most terapontids), but are well-documented as undertaking often substantial migrations (Allen et al., 2002; Pusey et al., 2004), at least of a scale to those seen in *B. porosa*.

For readers not familiar with fishbase, it may also be worth documenting how it identifies and classifies migration. Is it a keyword search? As another example of a potential coarse classification challenge, several of the larger serranids in this study which form spawning aggregations are classified as migratory (*Epinephelus*), whereas smaller-bodied clades (*Paralabrax*) which exhibit similar spawning aggregation behaviors presumably involving migration are not?

How pervasive these artefacts are in Fishbase, and how to efficiently address this potential bias in such a large dataset is difficult to assess. The authors emphasize some smaller clades that exhibit the correlations between migration and larger body size (shads, herring etc.). It would be very informative to run the same analyses over several smaller, but still diverse clades (with migratory behaviors reliably classified) in the dataset to see if observed broader patterns are also reflected within these groups.

We appreciate the reviewer's concerns regarding the use of fishbase data and the challenges of classifying migration across thousands of species. As the reviewer pointed out, our classification is clearly stated and transparent. There are several important points to highlight:

While we used FishBase as a starting point, however, *we also conducted extensive literature reviews to generate a database for migratory fishes* (Lines 123-126). We feel this database, which is represented by Supplementary Table 1, brings additional value to this study. This constitutes the most well documented database for migratory fishes to-date, and exceeds the steps typically taken for studies of this scale.

The reviewer suggests that migratory classification may be biased towards larger species. However, we note that our dataset includes many, relatively small migratory species, often from remote and poorly studied fauna. For example, sicydiinae gobies occur on remote islands and rarely exceed 10-15cm, yet the migratory strategy of these fishes is well known. Furthermore, we have many examples of small-bodied potadromous species in our dataset including multiple species of Characiformes and Cypriniformes from remote tropical regions in South America and Africa. We argue that migratory species are inherently conspicuous, and even small species are likely to be known as migratory. Nonetheless, there is no doubt that a small number of species

will be misclassified. We argue that the magnitude of the pattern we have uncovered and scale our study makes it very unlikely that these few instances are biasing our results. We note, that we have corrected the classifications for the specific species mentioned in our revised analyses (see Supplementary Table 1). Finally, we have indeed analyzed several small, yet diverse clades. As cited, we recently published an example on Clupeiformes, a clade of over 400 species. One can make repeated pairwise comparisons across the phylogeny and see that this pattern is remarkably consistent across subclades. Re-running all comparative analyses on random subclades is beyond the scope of our study and computationally prohibitive (re-analyzing for this revision took over 3 months on two different computing clusters). Moreover, the fact that this pattern emerges across the phylogenetic scale of our study is strong evidence that it must be present in most subclades. Note, that we are not arguing that every single pairwise comparison will hold this pattern, but that it is a robust general pattern across fishes.

Line 121- While the phylogeny used in this study is still a substantial dataset (4500 species), the cited Rabosky phylogeny was considerably larger (>10,000 species). Is this exactly the same phylogeny, or has it been trimmed? If so, how exactly was the final phylogeny in this study derived and is it available?

We trimmed the larger Rabosky et al. 2018 phylogeny down to a tree containing only the species that we had data for using the `drop.tip` function in the R package `ape`. We updated the methods (Lines 138-139) to clarify how we reduced the phylogeny. We added the reduced phylogeny to Dryad.

Line 164. This definition of the two BM models needs clarification.

Lines 163-166[166-168]: The methods were updated to clarify that one Brownian motion model (BM1) was a single σ^2 model, while the other (BMS) was a Brownian motion model with different σ^2 parameters for migratory and non-migratory lineages.

Line 173. The Methods define derivation of AIC weights for models, but no weights are presented in Results (Table 1 presents only AIC scores)?

We updated Table 1 to include the AICw values.

It could be questioned as to whether interpretation of the main results in the Discussion is possibly simplistic, at least at some levels? The authors frequently emphasize the benefits of larger body size in energetics and locomotory efficiency (which are very likely relevant to migratory birds and mammals). In many of the fish clades analyzed here, however, while larger adults may well undertake migrations, it is often the much smaller post-larval juveniles or elvers that undertake the most (relatively) substantial migrations in terms of distance or movement challenges (upstream migration into rivers). It would be worth exploring and elaborating on some of these differences associated with fish migration and at what life cycle stages in can occur.

While we agree that there are certain lineages of migratory fishes that migrate as post-larval juveniles, we would not characterize these as representing many of the migratory lineages. These are primarily restricted to gobioids; most lineages undergo substantial migrations as adults (and in many cases on a fixed energy budget e.g. Salmoniformes). To illustrate this point with your example, juvenile eels (elvers) undertake migrations from the ocean into streams/rivers, however, adult eels migrate from freshwater rivers to spawn in the Sargasso Sea with tagged individuals travelling over 2,400 kilometers to spawn (Beguer-Pon et al. 2015). The 2400 km travelled by adult eels represents a substantial distance covered. Furthermore, the bioenergetics studies that show the benefits of larger body size in energetic and locomotor efficiency are based on studies of fishes, with the findings shown to be relevant for multiple diverse fish clades including diadromous, potadromous, and oceandromous lineages. For instance, Roff 1991 discusses their predictions using six orders of fishes including Gasterosteiformes, Clupeiformes, Petromyzontiformes, Gadiiformes, Osmeriformes, and Salmoniformes. Hein et al. 2012 shows that migratory fishes from 9 different orders of fishes fit their theoretical prediction of a larger body size increasing energy efficiency when migrating. These fish lineages include Carcharhiniformes, Laminiformes, Petromyzontiformes, Clupeiformes, Istiophioformes, Pleuronectiformes, Salmoniformes, Scombriformes, and Beloniformes. The lineages that these studies are based on include fishes from across the tree of life including Elasmobranchs and many basal and derived ray-finned fishes. Many of the migratory lineages in our study include species where a larger body size was documented to show an increase in energetic and locomotor efficiency when migrating. Therefore, while we agree that there is substantial variation in migration strategies in fishes (which we discuss in Lines 82-85) that warrants future research, we disagree that the benefits of larger body size for energetics and locomotor efficiency which have been clearly documented in many of the lineages in our study, is a “simplistic” interpretation. Moreover, we argue our results speak for themselves – there is a remarkably consistent pattern of migratory fishes being larger than non-migratory relatives, which demands explanation. It is beyond the scope of our study (and available space in a PRSB paper) to discuss the nuances of all migratory species. Instead, we focus on the principles that generalize across the phylogenetic scale of our study.

Figure 1. Is this a single SIMMAP reconstruction of migratory evolution? It would be assumed there would be some variability emerging over 100 reconstructions (at particular nodes)?

Figure 1 depicts the SIMMAP reconstruction across all reconstructions, but with the pies removed for ease of seeing the state. We have included a figure in the supplement (Supplementary Figure 1) with the major clades condensed and the node variability shown to give an idea of uncertainty at the deeper nodes.

Referee: 3

My only concern is the lack of log-transformation of the body size data. I took the liberty of downloading the dataset from the supplemental materials and found that the body size data are extremely non-normally distributed, as is quite common with size data. I worry about how the underlying distribution of the data may affect the findings, especially if the distribution differs

between migratory and non-migratory species (as is indicated by figure 2). Primarily, this would potentially influence the phylogenetic ANOVA, as this statistical test assumes an underlying normal distribution. But, in the interest of consistency throughout the study and “good practice”, an argument could be made for running the entire model-fitting framework on the log-transformed body size data, though it isn’t necessarily required for this step of the analysis. That the authors find a rate twelve times more rapid in migratory lineages does imply that not log-transforming the data is potentially inflating these estimates.

We thank the reviewer for pointing out the need to log transform the data. For the revision we log transformed the body size data (available in the Dryad supplement) and re-ran all of the analyses. We updated the methods section (Lines 136-137) to state that we log transformed the data and why we did it. We updated the Results section to reflect any new values generated using the log transformed body size data.

Referee: 4

My biggest concern is how the authors address the uncertainty of the phylogeny and the migratory ancestral states. In the ancestral state reconstructions section, the authors mention that they use stochastic character mapping across the 100 trees from Rabosky and Chang. It's unclear how many simmaps were created for each of those 100 trees, though. Using the 100 posterior trees is great for accounting for the phylogenetic uncertainty, but, as mentioned in the make.simmap documentation, many stochastic character maps should be made for each of those trees to account for uncertainty in the ancestral state estimations (I would say at least 50 for each posterior tree). If you have already done this, then the text should be updated to reflect exactly how many simmaps were made for each tree (and how many simmaps were made in total). If not, you will need perform such analyses before submitting a revision.

Furthermore, in the macroevolutionary model fitting section, you once again mention accounting for phylogenetic uncertainty but do not address the ASR uncertainty. These OUwie analyses should be run across the simmaps discussed above.

We performed 100 ancestral state estimations for each tree in the posterior for a total of 10000 total reconstructions (this was mentioned in the Lines 162-163, but not in the section on ancestral state reconstructions). We agree this should be clearer in the methods and have updated the section accordingly (see Line 141). We also followed this methodology when we re-ran OUwie with the new log transformed body size data.

In terms of presentation of the results, the authors show a single ASR (Figure 1) and a single set of OUwie model fits (Table 1). However, even without the addition of the simmaps as discussed above, there should be at least 100 sets of results for the ASR and the OUwie results. It's unclear how the results in Figure 1 and Table 1 were selected from these distributions of results. Were they selected by random? Are they means or medians? The selection criteria should be discussed (at the very least, just say you picked one at random) in the text or captions.

We mention that model fits and parameters values were averaged across all iterations for each model in Lines 176-177. We have updated the Table 1 header to clarify that the values are the average model fits and parameter values across all iterations.

Figure 1 depicts the SIMMAP reconstruction across all reconstructions, but with the pies removed for ease of visualizing states. We have included a figure in the supplement with the major clades condensed and the node variability shown to give an idea of uncertainty at the deeper nodes. We acknowledge there are tradeoffs in the presentation of such larger datasets, and feel that by including the simplest depiction in the main text and further details in the supplementary materials that we can offer clear figures and also offer additional materials to readers interested in greater details. We updated the Figure 1 legend to clarify that the reconstruction was a summary of all the ancestral state reconstructions.

Furthermore, the uncertainty that is revealed by the OUwie replicates (not simulations) should be presented, at least in supplemental figures/tables, as the authors have done with the insets in Figure 1 and Figure S2 for the OUwie simulations. This should include the uncertainty in model AIC and parameter values.

We added the distribution of AIC scores and parameters values for the two best fit models to the supplementary materials (see Supplementary Figures 2 and 3).

Another major concern I have is the authors' discussion of the σ^2 parameter. The authors mention (line 171) that this is the "rate parameter". However, I would instead consider this the "intensity of random drift" or something similar. The important distinction here is that the evolution associated with the σ^2 parameter is random, whereas the evolution associated with the alpha parameter is selective/directional. On line 111, the authors mention they are interested in the rate of body size as a proxy for the degree of selection. In this case, you are most definitely interested in the alpha parameter, which is a measure of the degree of selection, not the σ^2 parameter. In fact, I believe it would make much more sense for you to perform some model averaging on your parameter estimates (using AICc weights) to determine the estimated alpha (or transformed to phylogenetic half-life) parameters for the migratory and non-migratory fishes rather than to introduce a new method (`compare.evol.rates`) to calculate the rate of evolution. Ultimately, since your second best model is the OUMA model, you should find different alpha estimates for the different groups, even when you model-average.

We have updated the text in the methods and results to reflect that the σ^2 parameter is the rate of stochastic variance around the theta parameter, while alpha represents the strength of selection towards the theta parameter (Lines 178-186 & Lines 233-245).

We removed the `compare.evol.rates` analysis from the manuscript. We determined the estimated alpha through model averaging using AICc weights with the value located in Table 1. We also show the entire distribution of parameter estimates in Supplementary Figure 4 to illustrate the uncertainty in parameter values.

Finally, body size is usually ln- or log10-transformed. It appears you performed the OUwie analyses on untransformed data. I wonder if and how your results would be different if you

performed them on transformed data. At the very least, I would suggest transforming the body sizes in the figures.

[See response to reviewer 3.](#)

Minor comments (in sequential order):

On lines 163-166, you mention two Brownian motion models, but don't discuss the differences between them.

[Lines 163-166\[166-168\]: We updated the methods to clarify that one Brownian motion model \(BM1\) was a single rate model, while the other model \(BMS\) was a Brownian motion model with different rate parameters for migratory and non-migratory lineages.](#)

On line 166, you mention OU for the first time without defining it.

[Line 166: Defined OU as Ornstein–Uhlenbeck.](#)

On lines 197 and 198, you mention the simmaps show the ancestor was non-migratory. Is this true across all of the simmaps?

[We edited this section to state that the ancestor was non-migratory in more than half of the simmap reconstructions. We added a figure \(Supplementary Figure 1\) that shows the node variability to give an idea of uncertainty of the ancestral state of ray-finned fishes.](#)

On line 200, I believe you meant Figure 1.

[Line 200: Changed Figure 2 to Figure 1.](#)

On lines 201 and 204, I would say "x times on average" instead of just "x times".

[Changed to "times on average".](#)

On line 206, you mention migratory species are generally larger than non-migratory species. Have you checked to see if this trend is driven by just a handful of families? Or is this pattern consistent across the entire tree?

On line 211, you mention that most migratory lineages have a larger size than their nearest non-migratory sister taxa. Is this statement based solely on visually assessing Figure 1? What is "most" in this case? Could you calculate this or even get some sort of statistic?

[Yes, this assessment is based on visually assessing Figure 1, We have investigated a wide number of subclades and found the pattern is remarkably consistent. Indeed, it would be unlikely that our results could be driven by just a few clades across such a large dataset. We argue that we have conducted extensive statistics \(comparative methods detailed throughout the manuscript\) that confirm the visual pattern – migratory fishes are larger than non-migratory fishes. Here we try to describe the general pattern without presenting a random assortment of subclades in our limited space. However, the tree and data are available so interested readers are free to investigate their clade of interest.](#)

The model names in Table 1 should match those in the text (e.g. OU1, BM1, BMS).

We updated the text (Lines 166-183) to make the model names consistent with the names in Table 1.

Is there a reason you didn't use the AICc values returned by OUwie? Since all of your models have the same sample size, it probably doesn't matter, I'm just wondering.

The AIC and AICc scores were the same value because of the large sample size in our study, so we reported the AIC score. However, after re-analysis we decided to report the AICc scores to make the reporting of statistics in our study more comparable with the broader literature.

You may want to log-transform the y-axes for alpha and σ^2 in Figure S2.

We decided to not log transform the y-axes because after reanalysis with the log transformed body size data the alpha and σ^2 values came out much more normal than the axes in the original Figure S2.

Appendix B

Referee: 3

It is a little unclear how the authors ran their phylogenetic analyses across the posterior distribution from the Rabosky tree, as maximum likelihood methods were used to produce this phylogeny. If the phylogeny used is the version of the Rabosky tree with imputed tips, it should be clarified in the paper that not all tips have underlying genetic information determining their positions.

We clarified that we ran the analyses across the distribution of trees from Raboksy et al. 2018 tree to account for uncertainty in the placement of taxa without underlying genetic data. We further clarified (Lines 142-146) that not all species in the Raboksy et al. 2018 have underlying genetic information determining their positions in the phylogeny, but that the majority (4635 of 4648) of the tips in our tree were placed based on underlying genetic data.

Page 4, Line 87. Goby is spelled incorrectly

Fixed the spelling error.

Line 175. ACR analysis should be defined prior to use of the acronym

Changed ACR to ancestral character reconstruction.

Referee: 2

Comments to the Author(s).

While the authors have done a creditable job of addressing several earlier concerns, much of the Results of this paper still hinges on the evolutionary modelling component. A range of recent methodological meta-analyses (Silvestro et al., 2015; Pennell et al., 2015; Cooper et al., 2016 etc.) have all identified very small amounts of error in datasets can have profound effects on the inferences derived from OU models, and that large trees are particularly susceptible to issues arising from unaccounted measurement error in the data (consistently biasing model selection towards erroneous rejection of simpler models in favour of more parameter-rich models such as the OU model). As was identified in even a cursory review of data with the initial submission, errors in discrete ecological classification, (as well as maximum body size data in poorly known species) are likely not uncommon in this dataset. These limitations mean that when evidence for the OU model is found, the results should be interpreted with caution, particularly where there is likely to be intraspecific variation or measurement error in the data. Obtaining accurate measurement error estimates (which can alleviate these concerns) is difficult with the current data (a single observation per species), so other avenues should be considered.

This is a fantastic dataset, but given some of the inherent limitations of the evolutionary modelling approaches, I would still recommend the authors consider incorporating additional

analyses that potentially capture evolutionary shifts in body size optima in a continuously varying trait, without pre-defined classification of behavior. The points the authors make regarding explicit convergence analyses are warranted, but approaches such as l1ou R-package (Khabbazian et al. 2016) allow for simple identification of adaptive shifts (without convergence) in a continuous trait. Additional, purely data-driven evidence of increased body size in migratory clades compared to closely related non-migratory clades would tell a much more compelling story. While such approaches may also capture broader shifts in the body size adaptive landscape of fishes, if migratory behavior is driving significant shifts in body size, they should be apparent. These are not necessarily computationally demanding approaches, and this is also not an apparently overly long paper.

We have updated the methods (Lines 209-212) to state that models of continuous trait evolution may produce misleading results when the ecological state of taxa are misclassified and that in a study of this magnitude, misclassifications in the migratory status of species are possible, especially among more poorly studied taxa. To address these concerns, we have used the purely data driven method, l1ou, that was suggested.

We used the l1ou R package to reconstruct the adaptive shifts of body size for migratory and non-migratory lineages without any a priori assignment of ecological state (see Lines 209-217 in methods for description). We found a total of 100 adaptive shifts across the tree (Supplementary Figure 6). Most of the optima were recovered at deeper nodes in the phylogeny and adaptive shifts in body size occurred at the base of radiations that are dominated by migratory species. For example, adaptive shifts occurred at the branches subtending Acipensiformes, Osmeriformes, Salmoniformes, and families of Characiformes, Cypriniformes, Siluriformes, Gobiiformes and Galaxiformes, among others. However, radiations that evolved migration in only a few species dispersed across a larger clade did not exhibit any adaptive shifts at these transitions. The lack of shifts in these taxa are not surprising because the increased body sizes in migratory lineages can be relatively subtle compared to the overall variation observed between clades of ray-finned fishes. It is likely that l1ou lacks the power to detect subtle shifts in body size that occur within a few species dispersed in a larger clade. For instance, the few centimeters increase in body size that occurs in migratory species of the freshwater sculpin genus *Cottus*, is a magnitude lower than the difference in body size between the genus and the marine sculpins they are derived from. l1ou recovered a shift at the base of the genus *Cottus*, but not at the few migratory species within the clade. Overall, we believe that the results of the l1ou analysis further support our findings that shifts to a migratory behavior tend to result in adaptive shifts in body size, while also showing the limitations of purely data driven tests in recovering subtle, but biologically meaningful adaptive shifts over broad phylogenetic scales.