Round #1

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by Gabriele Sorci, 17 Mar 2022 10:25
Manuscript: **https://doi.org/10.1101/2022.02.24.481784**

Both referees agreed that this is an intersting work. However, they also suggested that some revision is needed in order to improve the clarity of the manuscript. In particular, they thought that more details should be provided on the rationale underlying the definition and use of the reproductive indeces, the structure of the statistical models, the meaning of selection gradient in this context, etc. They also did a terrific job in spotting the typos and mispelling. I am looking forward to seeing the revised version of this nice piece of work.

Reviews

Reviewed by Thomas Haaland, 14 Mar 2022 18:35

General comments:

This is a really nice study that aims to quantify and explain a puzzling phenological pattern, and it has an elegant mix of empirical and theoretical work that I enjoy a lot. It is well written overall with a good structure, good motivation, and nice discussion. Most of my comments are quite detailed, as there is just a little bit of grammar/punctuation that isn't very tidy – I hope you don't mind me nitpicking on this. Otherwise I just miss a little bit more clarity on the derivation of the bet-hedging model, as well as clarifying the many various metrics and indeces that you measure (as you will see in my line-by-line comments below)… just make sure that everything is consistent, and actually necessary! It is a bit easy as a reader to get lost. However, once these small things are addressed, I have no hesitation to recommend this preprint for PCI Evol Biol. Thanks for an enjoyable read and a very cool study!

Specific comments:
Line 23: Should "…breeds from autumn to spring" be changed to "…breeds both in autumn and spring"?

Done

Line 51: You give the definition of bet-hedging in the following sentence, line 53-54, so I might move the ending of this sentence, "a strategy known as bet-hedging", to the end of that next sentence instead. The current sentence (line 50-51) is already quite long with several subclauses - and only gives an example of a bet-hedging strategy, not the definition.

We move this part of the sentence after the definition in a separate sentence, as advised. L56

Line 57: Change "genotypic" -> "genotype"

Done

Line 65: Change “dormancy seeds” -> “dormant seeds”

Done

Line 67: Could add a citation to Graham et al. 2014 (http://dx.doi.org/10.1098/rspb.2014.0706) here, perhaps the most convincing example of experimental evolution of bet hedging in my opinion.

Thanks a lot for sharing this great paper! We added this reference.

Line 79: Missing plural s: “partitioning brood” -> “partitioning broods”

Done

Line 89: Missing plural s: “insect” -> “insects”.

Done

Line 114: Remove word “than”: “it thus prefers seasonally flooded habitats to large permanent water bodies”.

Done

Line 121: Change “in altitude” -> “at higher altitudes”

Done

Line 123: Change word order: “Adults have thus to” -> “Adults thus have to”

Done

Line 125: Missing plural s: “tadpoles”

Done

Line 139-140: I’m not sure this parenthesis is adding anything. Could be removed.

Done

Line 161: Add starting parenthesis before 2001? Or remove the closing parenthesis?

Done

Line 167: Add missing s, remove comma and change word order: “In only 2% of the larval cohorts produced were small larvae observed…”

Done

Line 168: Perhaps remove “Note that”, since you haven’t actually shown any data yet (referred to a figure/table), so we (the reader) can’t note anything.

Done

Line 193-198: I'm having a hard time following what these different ratios that you're describing are. If hatch rate is #tadpoles/#eggs, and survival form egg to metamorph is #metamorphs/#eggs, and you claim that you calculate "survival during larval stage as the product of the two former ratios", then somehow survival becomes (#tadpoles * #metamorphs) / #eggs^2 ?? What exactly do you define as the larval stage? Does survival during sarval stage just be #metamorphs/#tadpoles?

This was a mistake and this was not clear enough. To simplify the text, we corrected it as follow (L201):

"Finally, we calculated the survival during larval stage as the ratio of the number of metamorphs over the number of small tadpoles. This index could only be estimated in about one third of the breeding events when hatching was successful (i.e. the number of small tadpoles was not null)."

Line 251: Here and elsewhere the formatting of references is a bit weird. I guess it should say "This model was primarily inspired by Cohen (1966, reviewed by Seger and Brockman 1987)." Initials haven't been used elsewhere, and place parentheses only around the year when author names are part of the text.

We removed the reference to Seger and Brockman which was already in the beginning of the paragraph and we corrected the reference to remove initials.

Line 254: Add comma after "environmental conditions".

Done

Line 264: Remove space between "below" and closing parenthesis.

Done

Line 280: I didn't understand how this selection gradient was arrived at. Could you add some derivation, or at least a bit of written explanation to guide the reader from the expressions on the previous page to this result?

We expanded the explanation as (L290):

"The selection gradient on c is the derivative of the function Log(W), which indicates whether selection favors an increase in c (if positive) or a decrease (if negative): [Formula]; If some value of c within the authorized interval [0,1] results in G[c]=0 then it is considered an evolutionary stable strategy (ESS) provided the second derivative is negative (i. e. G[c] is positive below the ESS and negative above).

We explored numerically the selection gradients in order to find potential ESS using Mathematica […]"

Line 320: The different measures of breeding success and survival rates continues to confuse me. Aside from the sheer number of these measures being calculated, some confusion about the terms used to describe the stages of the life cycle just makes this a lot to keep track of. There is: hatching success (% of breeding events producing at least one larva), hatch rate (#tadpoles/#eggs), breeding success (% of breeding events producing at least one metamorph), survival until metamorphosis (#metamorphs/#eggs), and larval survival (uncertain). It's fine that there are many, just make sure they are clearly defined and used consistenly throughout.

Thanks for this useful comment. We decided to rename some variables.

Regarding survival (rates and binary derived variables), we now have (L198 and 248):
Hatching rate (percentage of eggs that become tadpoles) and *hatching success* (binary variable : whether some tadpoles hatch or none at all for a given breeding event)
Survival rate from egg to metamorph (percentage of eggs that become metamorphs) and *metamorphosis success* (binary variable: whether some metamorphs are produced or none at all for a given breeding event)
Survival rate during larval stage: percentage of tadpoles that become metamorphs

We also changed the name of variables describing breeding. We now have (L190):
Presence of egg masses (binary: whether some eggs are laid or not when we visited a pond) and *number of egg masses* (integer, non-zero; applies only to cases where egg masses are present)

Finally, we decided to write all variable names in italics to help the readers.

Line 346: Add missing s, remove word "the": "Finally, the figure 5 summarize" -> "Finally, figure 5 summarizes".

Done

Line 348-349: This last sentence is too vague to be helpful. How exactly does it illustrate the quasi-exclusion between the two cohorts? Either add some more detail (what features of the figure or lack thereof can we look at to see this?), or remove the sentence. If keeping it, change illustrated -> illustrates.

We added some more details (L374).

"It illustrates the quasi-exclusion between the two cohorts: there were only 4 cases where metamorphs from the two seasonal cohorts emerged in spring in the same pond during the same year (compared to 47 cases where parsley frogs spawned during both seasons)."

Line 355: Shouldn't the reference to Figure 4 here be Figure 6?

Yes

Line 364: Remove extra opening parenthesis. Your reference manager probably has an option that lets you "add prefix" to a reference, so the "but see" can be inside the auto-generated reference.

Done

Line 370: Remove extra closing parenthesis.

Done

Line 383: Remove "compared to our study area" on line 383? You can add "our study area in" on line 384, making it (also add missing s in "makes"): "It is thus possible that increased competition for Pelodytes punctatus larvae in autumn and winter makes the autumn niche less favourable in northeastern Spain compare to our study area in southern France…"

Modified

Line 395: Remove extra opening and closing parenthesis. The "e.g." can be added as a prefix :)

Done

Line 399: Add comma after "(Baradun & Reyer 1997)".

Done

Line 407: Remove extra "*perezi*"? Or is it to specify subspecies, in which case the comma between the *perezi*s can be removed.

It was a mistake.

Line 410: hypothesized -> hypothesize.

Modified

Line 422: Change words: "predates" -> "depredate". And "predaceous" -> "predatory"

We changed "predates" to "preys on" and "predaceous" by "predatory" (L453)

Line 424: Add missing s: priority effects.

Done

Line 425: described on some -> described in some.

Done

Line 428: Add missing s: …also affects the larval survival.

Done

Line 431: Add missing s: microorganisms.

Done

Line 450: Remove extra parentheses around reference? And add comma before “e.g.”.

Done

Line 451-453: Do you know which of these two possibilities it is? You don’t have individual-level data, right? What is the interval between breeding attempts for females? Would she both in autumn and that same spring?

We tried to find which one of the two possibility it is… but we don’t have clear results to present. We added some information in the text (L482):

“There is no individual data available for this species and our only attempt to mark adults with visible implant alpha tags was not successful. Preliminary results based on genotyping of eggs, spawned in the same pond at different periods, suggests that females could breed several times in one year but this has to be confirmed (unpublished data). Clearly, this is a line of research to develop in the future if we want to fully understand the evolution of reproduction in this system.”

Line 461: Remove “Poethke et al.” inside parenthesis. Your reference manager might have an option to “suppress author”? Which will only insert “(2016)”.

Done

Line 466: Perhaps add “($0 < c < 1$)” after “mixed breeding strategy”.

Done

Line 469 & 470: Also here adding the symbols used for the metrics is useful – make it “(q=0.43)” and “(c=0.57)”.

Done

Line 489: This Haaland et al. reference isn’t 2020 biorXiv, but now out in J Evol Biol 2021, so can update here and in the reference list. doi:10.1111/jeb.13788

Modified

Line 491: Remove extra space before the comma.

Done

Line 492: Use suppress author again to just make it read “…by Rádai (2020).”

Done

Line 495: The shift from the previous paragraph to this conclusion is very unexpected. Perhaps add something to the beginning of the sentence like “In conclusion, …”?

We changed it.

Line 730 (legend Fig. 6): Change (“solid black line)” to “(black lines)”. Both the lines show the optimal strategies. Also, about using “Evolutionarily stable strategy”: is this in fact an ESS model? I can’t quite tell how you arrive at your selection gradient, or even how the given selection gradient produces the “optimal strategy”. Although it’s straightforward to just optimize the equation on line 276 over c – is this what you do? If so, what do we use the selection gradient for? As you can tell from my confusion, some more explanation either here, or better at the end of the methods and results, would help.

Yes to all questions, our redaction must have been awkward because the reviewer has got this impression he was wrong and there was something more complicated than a simple ESS model. It is indeed a simple ESS model, the selection gradient is the derivative of the fitness function, we look for values of *c* that make it zero (=those that maximize log-fitness). The selection gradient is an intermediate in the computation to find the maximum. We hope our new redaction (see above) makes it clear this is just a straightforward ESS model- as the reviewer rightly understood. We rewrote the opening sentence as “the following model is **an ESS model** derived from Saiah and Perrin 1990 etc…” (L 259)

Line 732: Where did the numbers 4.7% and 3.8% for autumn tadpoles and spring tadpoles come from – have we been shown these elsewhere? I can't see for example from Fig. 4 how the 3.8 number is arrived at. And shouldn’t autumn tadpole survival be lower than that of spring tadpoles (in absence of older competitors) because of the risk of a bad winter?

Those numbers are presented in Mat & Meth section, in the bet-hedging model (L 298).

“We set survival probabilities based on our estimates of survival from egg to metamorphose: s_1 = 0.047 (estimated among breeding events producing offspring that survived until spring) and s_2 = 0.038 (in the absence of autumn tadpoles).”

In this model, the survival of autumn tadpole is a combination of a stochastic year effect (affecting the probability of autumn offspring to persist until spring) and the survival S1. Therefore, it is expected that S1>S2. In addition, we showed in another article (Jourdan-Pineau et al 2012) that autumn juveniles are larger than spring ones and this may further increase their survival until reaching sexual maturity. Therefore, we believe that the S1 might be underestimated (compared to S2).

We also tested a model where S1 is twice S2 and it resulted in flatter curves hence the maintenance of the bimodal strategy is more probable. We therefore consider that our results based on S1 estimate are conservative.

Reviewed by Zoltan Radai, 14 Mar 2022 18:54
Download the review
Summary The authors investigated the presence of a potential bet-hedging strategy in the parsley frog Pelodytes punctatus, in which species a bimodal breeding phenology is observed.

They use both empirical observations and theoretical modelling to test whether or not this phenomenon could indeed be a bet-hedging strategy, which dual approach makes this study quite robust and valuable. Overall, the manuscript is well written and concise, with only a very few errors. The introduction lays out the theoretical background neatly, and presents the study system well. Empirical knowledge on bet-hedging is still limited, and the authors did an excellent job highlighting the relevant knowledge gaps, and the manuscript made a substantial contribution to the scientific literature on the subject. The sampling methods appear to be reasonable and justified. The results are communicated mostly clearly, and the discussion was focused, avoiding over-arching conclusions or speculations. Alongside with the main findings, I think a great strength of the presented results is that it provides strong empirical support for the notion that shorter lifespans increases the selection pressure for mixed strategies. Also, the evidence of inter-cohort competition is a unique and very valuable finding, which puts emphasis on a yet scarcely documented and understood phenomenon with potential relevance to the understanding of the evolutionary ecology of a wide range of species where cohort splitting occurs.

I only noted a few major comments, which should be addressed in my opinion, in order to improve on some segments. Specifically, one of the tested measures (offspring survival from egg to metamorph) should be revised, or at least given a firm rationale and explanation (see Major comments: Methods). In addition, while most of the results are clearly discussed, I think a bit more detail on the likely (autecological) advantages and disadvantages of each (autumn vs. spring) strategy would greatly improve the concluding paragraphs of the discussion, helping readers to appreciate more the nuances of the presented system.

We have expanded the final paragraph as requested (now lines- L535-556).

Major comments

Methods 193-198: I might be just misunderstanding something, but why is it necessary to take the product of hatching rate and metamorph rate? The latter seems to need the former to take place, i.e. they are not independent events. It seems that the former is a time interval like t1-t2, and the second is t1-t3, so taking their product may not be necessary, as the second already takes into account hatching success (i.e. will always be less than (or, at best, equal to) hatching rate). Hence, overall metamorph success [metamorph/eggs] seems sufficient as a proxy for larval survival. Or, alternatively, juvenile survival (using the above time nomenclature: t2-t3) is simply [metamorphs/tadpoles]. This should be clarified, and if needed then corrected, as it would affect some parts of the conclusions (e.g. Discussion l:386).

We made a mistake in the manuscript (also spotted by the first referee, see above). The survival during larval stage is the **ratio** (not the product) of the survival from egg to metamorph and the hatch rate. To simplify the text, we corrected it as follow (L201):

"Finally, we calculated the survival during larval stage as the ratio of the number of metamorphs over the number of small tadpoles. This index could only by estimated in about one third of the breeding events when hatching was successful (i.e. the number of small tadpoles was not null)."

We hope that this now clarifies this point.

264-266: "Overall the mean number of individuals produced per female is s1 when the autumn c cohort doesn't fail and (1 −) s2 when it does" – wouldn't that be the expected proportion of c offspring reaching sexual maturity, rather than the "mean number of individuals"?

Yes, that is perfectly true. We changed it (L275):

"Overall the expected number of offspring reaching sexual maturity is $c\ \ s_1$ *when the autumn cohort doesn't fail and* $(1-c)\ \ s_2$ *when it does."*

371: "The breeding effort in our population was higher in autumn than in spring" – in the "Statistical analyses" season was not mentioned as a predictor for the fitness-related dependent variables, nor was it mentioned in the "Explanatory variables". In their current forms, these sections indicate that no models were fitted on breeding probability, breeding effort, etc., with season as predictor. I think it would be important to include such models (e.g. "breeding effort ~ season") and present their results prior to describing results from the "pond-characteristics" models, as they would likely provide general insight into the dependent variables in question. For instance: how was breeding probability affected by season?

Thanks for this comment, this was a mistake in the text as we indeed did the models with the season effect. We now mentioned "season" as an explanatory variable (L 222).

"Explanatory variables for the breeding probability and breeding effort are the season, depth of the pond as well as the presence of conspecific and inter-specific competitors (larvae of anuran species) and predators (invertebrates and adult newts) in the pond. Except for the depth of the pond, all those explanatory variables were also applied to explain the success (offspring survival) of breeding events."

Table 2 (formerly Annex 2, now included in main text following your comment on the result section) summarizes "all results of the statistical analyses performed to explain the variation of *presence of egg masses*, *number of egg masses*, *hatching success* and *metamorphosis success."* It clearly includes season as an explanatory variable.

We decided not to change the order of results section, however.

This approach may help us to understand why such a bimodal phenology is apparent. Based on the reported results, it might seem that autumn tadpoles gain substantial advantages, but if there is higher variation in autumn whether or not a given pond survives (or, indeed, in larvae survival), this could indicate that throughout this risk spreading strategy, the relatively "low risk, low benefit" spring cohort represents a safer route, but the "higher risk, higher benefit" autumn cohort is a necessity to decrease variance in the long term (lineage, i.e. geometric mean) fitness, hence neither the strategies can dominate the other on the long run.

There is no significant difference in "pond survival" between spring and autumn and if anything, autumn "pond survival" is slightly higher (see lines 324-326), in spite of a higher risk of drought (and hence complete elimination of the cohort) in autumn (lines 327-329). This was perhaps not easy to understand from the previous version due to confusion with variables names

and definitions. We hope this is now clearer. We now discuss this better in the last concluding paragraph of the discussion.

Results

In the results, I think it is important to report all estimates and statistics, even when non-significant, e.g. at lines 328-330. Alternatively, these could be also reported in table(s): continuous trends (if not in interaction with other variables) can be simply reported as appearing in the model summaries, whereas between-group differences (from categorical predictors) can be reported as estimated marginal contrasts (the R-package "emmeans" may be useful for this). Just in case, such marginal contrasts can be acquired as:

model specification, where "x" is a categorical factor

m1 = lm(y ~ x)

getting group-level comparisons and corresponding marginal estimates emmeans(m1, pairwise ~ x)$contrasts

All statistics (even when non-significant) were given in Annex 2: "*Results of the statistical analyses performed to explain the variation of presence of egg masses, number of egg masses, hatching success and metamorphosis success. Bold letters indicate a significant test (p-value < 0.05).*"

We decided to include this Table in the main document, now cited as Table2.

We also added missing statistics when mentioning the proportion of breeding events affected by drought (L 343):

"Drought (pond totally dried up) caused the total failure of 7 breeding events in autumn and of 5 breeding events in spring over the 3 year-survey and the 19 sites (representing 9% and 4.8% of the breeding events, those percentages are not significantly different, $\chi^2_1 = 0.66$, $p = 0.42$)."

Discussion

455-465: while the frequency-dependence indeed appears to be supported by the findings, the cited reference of Gremer and Venable (2014) highlights density-dependence, which was not (explicitly or implicitly) modelled by the authors, therefore I'm not quite certain that this reference is adequate here. Alternatively, the authors could elaborate on how density dependence could also play a role in the observed patterns: for this, it would be useful to fit models on the fitness-associated variables (breeding effort, etc.) of the spring cohort in relation to the estimated conspecific density (i.e. pondsize-corrected number of parsley frogs [originating both from spring and autumn] of ponds). If I'm not mistaken, this was not modelled, only the presence/absence of conspecifics.

This is an interesting comment but, you are right, our model did not include density-dependence. We added this information in the text (L 499):

"Density-dependence was not included in our model and we do not have field data to assess its effect in our populations. This would be a fruitful line of research to improve our understanding of this breeding system."

Minor comments

57: "genotypic" → "genotype"

Modified

90: "insect" → "insects"

Modified

113: "sensible" → "sensitive"

Modified

114: delete "than"

Done

162: delete ")"

Done

207: "undistinguishable" → "indistinguishable"

Ok

233: "apply" → "applied"

Modified

236: was the binomial model quasi-binomial here as well?

It was a binomial mixed model.

264: delete whitespace after "below"

Modified

307: does "spawning probability" correspond to "breeding probability"? If so, please unify throughout the manuscript.

Our variable names were a bit confusing so we decided to rename them. See our response to similar comment by the first reviewer.

308 and others: when writing mean and SE estimates, I think the "x ± y" format may be better (e.g. 0.18 ± 0.02)

Modified

332: no space between "%" and "(", and there is a whitespace between "(" and "0.61"

Modified

337-338: “From the point of view of spring breeders, in 28/57 cases, they found autumn tadpoles in the pond” – not quite clear: does that mean that in 28/57 cases, those females laying eggs in spring shared the ponds with autumn tadpoles? Please clarify.

Your interpretation was right. However, we deleted this sentence which was not useful here. This was related to the idea that female breeding in spring could decide to avoid ponds with autumn tadpole. This was already mentioned in the “breeding phenology” section (L 330):

“The presence of egg masses observed in spring was not affected by the presence of autumn tadpoles (χ^2_1=0.03, p-value= 0.875).”

338-339: “success of spring breeding event” → survival of spring tadpoles?

According to what we indicated above, we changed it to

“the metamorphosis success of spring cohorts”

343: all stage-dependent survival rates for spring tadpoles, or for both spring and autumn tadpoles? In other words: only spring tadpoles were affected by such mixed ponds?

In our manuscript, we tested the effect of being in a mixed pond only for spring tadpoles (i.e. the effect of being with tadpoles of the autumn cohort).

We now added the test of effect of spring tadpoles on autumn tadpoles (L370):

“We also tested if the metamorphosis success of autumn tadpoles might be affected by the presence of spring tadpoles, but this was not the case ($\chi^2$1=2.75, p-value=0.097).”

422: “predates” → “predate”

Modified to “depredates” according to reviewer 1’s advice.

491: “and sensitive to environment , a phenomenon proposed” → delete whitespace after “environment”

Modified