

15^h November 2022

Dear Editor,

You notified us on September 26, 2022 that we were encouraged to review and resubmit a revised version of our manuscript entitled “Distinct impacts of resource restriction and warming on growth and survival”.

We thank the editor and the two reviewers for their constructive comments that significantly improved the manuscript. We believe that the revised version satisfactorily addresses most of those comments (see our detailed point-by-point explanations below) and more concisely explains our main findings, which should be of a broad interest for the *Ecology* readership. In addition to addressing the points made by reviewers, we have also made changes to improve the flow and clarity of the text without any impact on our main findings.

To ease the review process, in the manuscript, we used a blue color font for the sentences that we modified or added.

We look forward to hearing from you at your earliest convenience.

With kind regards,
Simon Bazin on the behalf of all co-authors.

Editor's comment:
26-September-2022

Dear Authors,

The two Reviewers point out that the study is potentially interesting and relatively well prepared. However, they also point some important shortcomings regarding either the results interpretation and discussion, or the methodology description. The solutions for text improvement provided by the Reviewers are clear and sound, therefore I suggest you to revise the text accordingly.

With kind regards,

Aleksandra Walczyńska

Our response:

Dear Dr Walczyńska, thank you for the positive comments on our study. We are excited about the opportunity to resubmit a revised version of our manuscript and believe that the new version of the manuscript addresses all important concerns raised by the reviewers. In particular, we have provided (1) new information about the effects of temperature and food restriction on fecundity, (2) included additional studies and references to terrestrial organisms, and (3) more information on the interpretation of the results in terms of life history strategies. We also took into account the different replicates in the statistical analyses as suggested by reviewer 2. We

clarified the text and we modified the results and discussion following the inclusion of the data on fecundity. We discussed how the effects of temperature on the different life history traits (fecundity, survival, growth) can result in divergent life history strategies under warming. We hope that this makes our manuscript more appealing to a broad range of ecologists. Our detailed responses to all comments made by the reviewers are given below.

Reviewer(s)' Comments to Author:

Referee 1:

Comments to the Author

This empirical paper subsumes various long-standing life history phenomena ('rules') to apply them in the climate change context, most centrally the temperature-size-rule (TSR; i.e. the empirical phenomenon that ectotherms grow to a smaller size at warmer temperatures: Atkinson 1994). A standard laboratory growth experiment was performed on Medaka, an often-used and easy-to-hold small fresh water fish, at 2 temperatures (20 °C & 30 °C) & 2 food treatments (abundant & restricted). The authors argue that such interactive effects of food and temperature on animal life history traits (notably body size, but here also growth, development and longevity) are rarely studied, but this is certainly not true in invertebrates (notably insects, where this is almost standard). Regardless, albeit minimalist in terms of the number of treatments (2x2) of each environmental axis, such an experiment was worth performing in this context in a fish for which it has not been performed before. I think the main 'advance' -- if there is any, as this is certainly not novel as such -- of this study lies in monitoring the entire non-linear growth of the fish (for ca. 1 year) under the 4 treatment combinations (Fig. 2), and in interpreting the results in terms of plastic vs. evolutionary life history adjustments in the climate change context.

Our response: thank you very much for your constructive comments on this study. We agree that the interactive effects of temperature and food on life history traits have been broadly studied in aquatic (primarily cladocerans) and terrestrial (primarily insects) invertebrates. We included these studies and discussed their findings in the revised version (lines 68 – 75, 76 – 83, 108 – 112, 293 – 297, 363 – 365). Nevertheless, all these studies were conducted on small invertebrate species. As a result, we have no information on how temperature and food availability jointly influence life history traits of vertebrate predators, despite the observation that TS responses are amplified in larger species (Forster *et al.* 2012). This is because of importance as body size changes in predatory species can alter the trophic interaction strength and food webs stability (Emmerson and Raffaelli 2004, Sentis *et al.* 2017, Osmond *et al.* 2017). We believe that this is an important knowledge gap and hope that our study can contribute to fill this gap. We clarified this point in the introduction (lines 118 – 127).

Referee 1:

The main result seems to be a significant interaction between temperature and food level, although in the end the (expected) crossing growth trajectories are only evident for temperature (Fig. 1a) and not for food treatment (Fig. 1b), both as expected and well demonstrated before in other species (in this context). As mentioned before, this result is worth publishing for a new species to demonstrate consistency in diversity, but nothing particularly earth-shattering.

Our response: we agree that similar results have been reported before in invertebrate and, as explained above, we clarified that originality of our study lies in the fact that the experiment was performed on a larger predatory species and multiple traits (growth, fecundity, survival) and not on a smaller invertebrate species or a single trait as in most studies. Moreover, we wanted to investigate whether the TSR was maintained under limiting food conditions since the vast majority of studies investigating TSR are conducted under unlimited food conditions.

Nevertheless, as suggested, we compared our results obtained from food limitation to results obtained under hypoxia which can be considered as another limitation (see below for more details).

Referee 1:

The Discussion in the eco-evolutionary life history context is fair and covers all necessary ground by integrating by now classic research from various life history realms (including ageing) in the climate change context, as is fashionable presently. In the end, however, the actual fitness costs & benefits of the various trajectories have not been investigated in a range of realistic environments (beyond assessing final body size and lifespan; e.g. in terms of effects on male mating success or female fecundity).

As mentioned above, I would have liked to see more temperature and food levels being tested to assess likely relevant non-linearities. By now it is well known that part of the problem of the TSR are incomplete temperature ranges being assessed (e.g. Walczynska *et al.* 2016 *JThermBio* //doi.org/10.1016/j.jtherbio.2016.06.006; Blanckenhorn *et al.* 2021 *JThermBio* //doi.org/10.1016/j.jtherbio.2021.103069). So ultimately, the question of whether the TSR is adaptive (or a physiological constraint) and beneficial for the persistence of populations and species in the face of climate change under realistic food limitations could not be answered with this study either (which I wouldn't have expected anyway). The TSR 'puzzle' has not been solved yet. This is merely another (limited) data point.

Our response: we agree that part of the problem with the TSR is the incomplete assessment of temperature ranges and the observation that the TSR pattern may be restricted to non-stressful temperatures that are close to the optima (as reported in the cited studies). We would have been delighted to test more temperatures and assess non-linearity but we were limited to two temperature for logistical reasons, as it is difficult to maintain a large number of vertebrate predators in the laboratory. Our experimental temperatures (i.e. 20 and 30 °C) were considered as non-stressful as they are close from the thermal optimum of this fish species (i.e. 25 °C, Dhillon and Fox 2004). This fish species also has a very wide thermal range (from 0 to 40 °C, Dhillon and Fox 2004). We are thus confident that our results are not driven by response to a stressful temperatures. Our study is nevertheless a first step and opens the way for more "ambitious" studies testing the adaptive value of the TSR across a wide range of temperatures. We further discussed how the combination of life history traits can influence the adaptive value of fish (lines 390 – 397).

Referee 1:

The entire paper is too focussed on fish and the aquatic realm (e.g. Daphnia; but omitting the work by e.g. Roby Stoks' lab on odonates) in terms of citations, so the Discussion should expand more (and compare) (in)to the terrestrial realm in general (e.g. Rohner *et al.* 2017 *EvoDevo* DOI: 10.1111/ede.12223). This is important because TSR patterns differ in general in aquatic vs. terrestrial taxa (Forster *et al.* 2012; Hirst & Forster 2013). For these reasons, I would publish this work in a fish or aquatic journal if this is so desired.

Our response: we agree that the temperature-size responses of aquatic and terrestrial organisms differ and are interesting to compare. This article was not intended to focus only on aquatic taxa. Therefore, as suggested, we included references on the interactive effects of temperature and food availability on life history traits of terrestrial organisms (mainly insects, Clissold and Simpson 2015, Corrêa *et al.* 2021, Frazier *et al.* 2001, Kingsolver *et al.* 2006, Renner and

Zohner 2018, Stillwell *et al.* 2007, Forster *et al.* 2012, Visser *et al.* 2006) in the introduction (lines 68 – 75, 76 – 83, 108 – 112,) and the discussion (293 – 297, 363 – 365).

Referee 1:

Methodologically this paper seems sound (albeit a bit minimalist; see above). What precisely is a replicate in Fig. 2 should be explicitly stated (groups of fish in small tanks vs. individual fish, as mentioned in the Methods on P5, center), as is now not entirely clear. Overall the paper is well presented.

Our response: as fish were not identified individually, a replicate corresponds to the tank in which fish were maintained. We added this information to the legend of figure 2 (lines 257 – 258). In addition, we included the replicate as random factors in our statistical analyses to account for variations among tanks receiving the same treatments.

Referee 2:

Comments to the authors

In their manuscript, Bazin *et al.* investigate how food quantity modulates thermal effects on survival, growth and the resulting body size. I found this to be an interesting study with valuable insights and have provided some comments and suggestions below which I hope the authors will find helpful in improving their manuscript.

Our response: we thank reviewer 2 for the constructive comments that greatly helped to improve the manuscript. Below you will find our answers to your questions and suggestions.

Referee 2:

General comments

In general, the introduction reads well and explains the main differences in how growth performance responds to either temperature or food quantity (e.g. in fig 1). However, I missed a deeper introduction on the difference between proximate mechanisms (which tend to be rooted in physiology) and ultimate mechanisms (which tend to take an evolutionary perspective grounded in fitness). This study investigates both responses in growth (which could be argued to reflect more proximate mechanisms) and survival (which could be argued to be more ultimate). I believe there is scope for integrating both approaches, possibly deriving expectations on the interactive effect of food restriction (increasing survival and hence selecting for late maturation at a larger size) and warming (decreasing survival and hence selecting for early maturation).

Our response: as suggested, we explained more clearly in the introduction the difference between proximate and ultimate mechanisms (lines 48 – 53, 61 – 64) and used this information to formulate hypotheses (lines 133 – 135).

Referee 2:

Line 47: Several earlier studies that have studied the underlying proximate mechanisms have focused on resource limitation in warm conditions, constraining growth rate later in ontogeny. These have mostly focused on oxygen as a limiting resource rather than food and found that T-S responses tend to be amplified under hypoxia, i.e. when resources are more likely to be limiting (Frazier *et al.*, 2001; Hoefnagel & Verberk, 2015). It may be worthwhile to point out if and how responses to growth as a limiting resource are similar or different from oxygen as a limiting resource. Studies on rotifers have also found support for oxygen limitation as an ultimate explanation (Walczyńska *et al.*, 2015).

Our response: we highlighted in the introduction the fact that some studies have investigated the consequences of the interaction between temperature and oxygen (considering oxygen as a limiting resource) on growth patterns and discussed and compared our results with those obtained in these studies (lines 53 – 57, 342 – 344).

Referee 2:

Related to this, I would suggest to replace resource with food throughout the manuscript whenever resource pertains to food (e.g. change resource restriction to food restriction). See also e.g. lines 222, 226, 247.

Our response: modified as suggested.

Referee 2:

Line 52: The TSR is not exclusively defined by asymptotic size, but is also frequently evaluated by size at maturity (which is different from asymptotic size at least in species with indeterminate growth, such as fish). It may be good to emphasize that the TSR is about comparing body size at a comparable life stage.

Our response: as suggested, we have specified that TSR is a comparison of body size at a comparable life stage (lines 344 - 348). Moreover, we analysed size at maturity and discussed the differences between our results at maturity versus asymptotic size (lines 348 – 351).

Referee 2:

Line 128: Given that around 80 fish were monitored per treatment and aquaria held 20-30 fish, does that mean that you had around 4 aquaria replicates for each treatment? If so, did you include tank replicate as a random factor?

Our response: indeed, we had ~3 aquaria replicate per treatment. We included replicate as random factors in the statistical analyses. The results were qualitatively similar although the growth curves were less nested (in response to food condition) than previously in the warm treatments.

Referee 2:

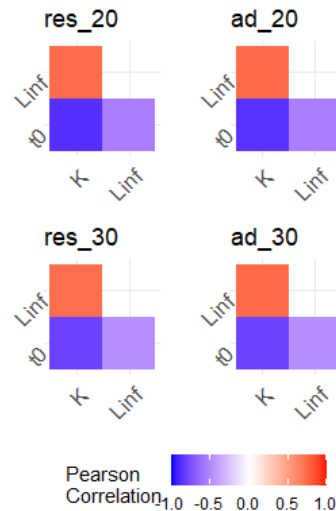
Line 143: indentify should read identified.

Our response: modified as suggested.

Referee 2:

Line 176: Just out of curiosity: was there an autocorrelation between the estimated parameter value for K and L_{∞} within a given treatment? My experience with model fitting is that this can sometimes happen: faster growth to a larger asymptotic size can yield a very similar growth curve (plotting length vs time) as slower growth to a smaller size.

Our response: below is a figure representing the correlations between the K , L_{∞} and t_0 parameters. There is indeed correlation between the parameters, but the correlation is constant between the different conditions. We thus used the same correlation matrix to take into account the random effects.



Referee 2:

Line 190: When are fish adults? In the methods it is stated that they reach maturity after 10-12 weeks at 25C. Presumably, they reach maturity earlier at 30C and later at 20C. If you have information on how temperature and food restriction affected age at maturity, I would suggest to include it so that the size at this age can be compared. Without this information, what is left is comparing asymptotic size, but from the wording and the graph it is unclear how asymptotic size responds. This information is presented in Fig S2, but not referred to in the results.

Our response: we have added the results on age and size at maturity and fecundity, and we have also clarified our interpretations of the TSR amplification under food restriction in the discussion (lines 338 – 340). Moreover, we discussed the differences between our results at maturity versus asymptotic size. When considering size at maturity, food restriction did not appear to amplify TSR in our study as, at each temperature, fish reached sexual maturity at a similar age and size. This suggests that food restriction would not influence medaka’s fitness via its effects on developmental time and size at maturity. We specified this information in the discussion (lines 348 – 351).

Referee 2:

In general, I found the discussion to be somewhat long. I think there is possibilities to reduce it somewhat and also restructure it. Maybe focusing first on growth trajectories (why does food restriction reduce growth more in the cold treatment), then survival, followed by their integration (can we understand responses in size at maturity/asymptotic best from effects of temperature and food on growth or from their effects on survival?), ending with a brief paragraph on implications (for trophic relationships and how to model these).

Our response: we restructured the discussion as suggested. We now first discuss the influence of temperature and food on growth trajectories, then on fecundity and survival. We then integrated these different life history traits to discuss potential life history strategies. We conclude with a paragraph on the implications of our results for modelling the effects of ecological consequences of body size changes. The length of the discussion has not changed significantly but we believe that it reads better to the structural changes we made.

Referee 2:

Line 221: replace Although with Because

Our response: modified as suggested.

Referee 2:

Line 230: See comment about the difference between adult and asymptotic size above.

Our response: see our response above.

Referee 2:

Line 240: I think a recent paper does consider temperature effects of body size in a foodweb context (Lindmark *et al.*, 2022).

Our response: we mentioned the finding of this study (lines 321 – 324).

Referee 2:

Line 246: Note that a recent study showed that effects of mass and temperature on metabolism are interacting such that the effect of temperature varies between large and small fish (Rubalcaba *et al.*, 2020).

Our response: we mentioned the finding of this study (lines 318 – 321).

Referee 2:

Line 259: If growth rates are more limited in warm than in cold, I do not see how the results for Amphiprion larvae are similar.

Our response: the sentence has been removed from the text.

Referee 2:

Line 261: I feel there is more that could be said here? Do you mean that at lower temperatures the stoichiometric ratio's needed change in such a way that food restriction has more severe effects?

Our response: we meant that in the current context of global warming, the effects of nutritional quality on growth (and otherwise) should be increasingly prominent. We clarified this sentence (lines 333 – 338).

Referee 2:

Line 274: I urge the authors to include this unpublished data and use the temperature (and food treatment) specific ages at maturity to make a comparison for size at maturity and whether thermal responses in size at maturity are magnified under food restriction.

Our response: as mentioned above, we included these data.

Referee 2:

Line 280: I presume that food intake was not quantified precisely. Still I wonder if fish exhibited compensatory feeding such that on a feeding day the restricted fish had greater appetite and ate more? Related to this, does this fish species have a stomach which it can use to 'overfeed' and store food?

Our response: we have no evidence for overcompensation but, as we did not quantify food intake, we cannot exclude this possibility. We are not aware about the capacity of medaka stomach for overfeeding.

Referee 2:

Line 281,305: I think the result of a stronger TSR needs to be better argued/supported in the results. Currently, the differences in asymptotic size (Fig S2) are perhaps slightly more

pronounced under food restriction, but the large CI do not make this a convincing argument for a stronger TSR. If the argument is instead based on the stronger reduction in initial growth following food restriction in the cold, then this has to be better explained: Most researchers would evaluate the strength of the TSR based on size alone, not necessarily the growth trajectory.

Our response: we agree with the comment and apologies for the lack of clarity. We clarified in the discussion that our statement about an amplification of TSR is based on the reduction in initial growth in the cold treatment (lines 338 – 340).

Referee 2:

Line 282: Not sure how relevant the work on unicellular organisms is for the fish as the constraints for uptake of resources are quite different.

Our response: we deleted the part about unicellular organisms as suggested.

Referee 2:

Line 293: Note that this review appeared in print in 2021, not 2020.

Our response: modified as suggested.

References

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