Peer Community In
Animal Science

Advancing sustainable aquaculture: behavioral insights for climate-change resilient fishes

Nicolas Bedere [iD] *based on peer reviews by 2 anonymous reviewers*

Cite this recommendation as:
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The study by Lagarde et al. (2025) is part of efforts aiming to meet the challenges of adapting livestock farming, and more specifically of aquaculture, to the effects of climate change. In fact, during heat waves, water temperatures rise and oxygen becomes scarce. Fish have to adapt to these conditions of hyperthermia and hypoxia. Studies have already shown that it is possible to genetically improve these resistances in salmonids (e.g. Debes et al., 2021). However, current methods for phenotyping these resistances rely on exposing fish to extreme conditions until they lose equilibrium, which indicates that the animal experiences severe conditions and raises ethical and animal welfare concerns.

From the aforementioned, there is then a strong interest in using and identifying less invasive phenotypes, such as behavioural changes, that could serve as indicators of fish responses to hyperthermia and hypoxia. Some behaviours show promise in both wild (Campos et al., 2018) and farmed fish (Van Raaij et al., 1996). A former study by the authors of this manuscript suggests that some of these behaviours are sufficiently heritable to consider applying selection on them (Lagarde et al., 2023). Therefore, the main aim of the present study was to test whether behaviour can be used as an indirect selection criterion to improve the adaptation of rainbow trout to heat waves. To achieve this lofty goal, the responses of different isogenic lines of trout to hyperthermia and hypoxia were investigated using new criteria based on behavioural responses and the reference measure of loss of equilibrium.

The results suggest that certain behavioural traits, such as distance travelled and frequency of zone changes, are associated with resistance to these stresses. Moreover, moderate correlations were observed between

certain behavioural variables and resistance to hyperthermia. Indeed, lines that were more resistant to hyperthermia had lower distance travelled and frequency of zone changes during the behavioural test. Other significant and positive correlations were observed between acute hypoxia resistance and certain behavioural variables, likely distance travelled, frequency of zone change and percentage of time spent moving.

These results pave the way for less invasive methods in assessing hyperthermia and hypoxia resistance based on behavioural observations, which could improve the resistance of farmed fish in response to climate change. The study further allows refining the measurements carried out on candidates for selection in order to improve their welfare during evaluation tests.

In conclusion, this study is commendable for its thematic relevance, originality and the potential application of its results to genetic selection of farmed fish.

References:

Campos DF, Val AL, Almeida-Val VMF. 2018. The influence of lifestyle and swimming behavior on metabolic rate and thermal tolerance of twelve Amazon forest stream fish species. Journal of Thermal Biology 72:148–154. https://doi.org/10.1016/j.jtherbio.2018.02.002

Debes P V., Solberg MF, Matre IH, Dyrhovden L, Glover KA. 2021. Genetic variation for upper thermal tolerance diminishes within and between populations with increasing acclimation temperature in Atlantic salmon. Heredity 127:455–466. https://doi.org/10.1038/s41437-021-00469-y

Lagarde H, Phocas F, Pouil S, Goardon L, Bideau M, Guyvarc'h F, Labbé L, Dechamp N, Prchal M, Dupont-Nivet M, Lallias D. 2023. Are resistances to acute hyperthermia or hypoxia stress similar and consistent between early and late ages in rainbow trout using isogenic lines? Aquaculture 562:738800. https://doi.org/10.1016/j.aquaculture.2022.738800

Lagarde H, Lallias D, Phocas F, Goardon L, Bideau M, Guyvarc'h F, Labbé L, Dupont-Nivet M, Cousin X. 2025. Screening for links between behaviour and acute hyperthermia and hypoxia resistance in rainbow trout using isogenic lines. bioRxiv, ver.4 peer-reviewed and recommended by PCI Animal Science https://doi.org/10.1101/2023.10.19.563047

Van Raaij MTM, Pit DSS, Balm PHM, Steffens AB, Van Den Thillart GEEJM. 1996. Behavioral strategy and the physiological stress response in rainbow trout exposed to severe hypoxia. Hormones and Behavior 30:85–92. https://doi.org/10.1006/hbeh.1996.0012

Reviews

Evaluation round #2

DOI or URL of the preprint: https://doi.org/10.1101/2023.10.19.563047
Version of the preprint: 4

Authors' reply, 20 March 2025

Dear Dr. Bédère,
Thank you for your careful review. My answers are directly in the text. I slightly changed the mansucript according to your suggestions, and reposter a new version on biorxiv.
Best regards,
Henri Lagarde

I have noted a few things that you may want to adjust before the recommendation that will fix the version of the preprint:

1.   Both reviewers mentioned that the rationale as well as the way you corrected for body weight was unclear. This is still somewhat the case in the current version of the manuscript.First, there is a confusion between body weight and fork length. Since body weight was not recorded individually, but fork length was, fork length was used as a proxy in the study. I recommand to explain this once (as done L294) and then refer to fork length for clarity for the rest of the paper.

In behaviour test, body weight was individually measured. I changed the manuscript lines 285-286 to make this point more clear. What was not possible was to link behaviour data and body weight data as fish were weighted in groups of 6 fish from the same isogenic (lines 286-287). For behaviour test, i mentionned body weight in lines 353-366 to give some comparison points with the body weight of fish that were measured for resistance to acute hyperthermia and acute hypoxia. I think this part is interesting as it gives some points of comparison between body weight of fish in acute hyperthermia and hypoxia resistance tests (in which we only measured the individual body weight of fish) and body weight of fish in behaviour test (in which we measured the individual body weight but we can not to link it with behaviour data).

Your response to the second reviewer is a bit long, what I understood from your explanation is that body weight and isogenic lines are confounding factors and there was no better way to deal with this than to include both effects in the analyses ; isn't that right ?

It is not exactly the point we wanted to show. In the response to second reviewer we show with figure 1 that body weight and isogenic lines are only partially confused as despite N38h is the heaviest line and A32h the lightest line, some fish from isogenic line N38h are lighter that some fish of line A32h.

In the first response to reviewer 2, I think it will improve to change "We ensured that a part of the fish in the lines of extreme body weight overlapped well, so that the model was able to capture the effect of body weight, which is indeed the case (Figure 1)." by : "We ensured that a part of the fish in the lines of extreme body weight overlapped well, so that there is no total confusion of effect between body weight and isogenic lines, which is indeed the case (Figure 1)." I upload a document containing this update of our response to reviewers.

2.   The font used is not consistent throughout the paper.

I corrected.

Download author's reply

Decision by **Nicolas Bedere** ⓘ, posted 11 March 2025, validated 11 March 2025

Dear Authors,

I have carefully read your responses to the reviewers' comments and the new version of the paper. The paper has improved a lot and I appreciate your efforts as well as those of the two reviewers. I think the preprint can now be recommended. However, I have noted a few things that you may want to adjust before the recommendation that will fix the version of the preprint:

1. Both reviewers mentioned that the rationale as well as the way you corrected for body weight was unclear. This is still somewhat the case in the current version of the manuscript. First, there is a confusion between body weight and fork length. Since body weight was not recorded individually, but fork length was, fork length was used as a proxy in the study. I recommand to explain this once (as done L294) and then refer to fork length for clarity for the rest of the paper. Your response to the second reviewer is a bit long, what I understood from your explanation is that body weight and isogenic lines are confounding factors and there was no better way to deal with this than to include both effects in the analyses; isn't that right?

2. The font used is not consistent throughout the paper.

I leave any edition of the current manuscript to you, please inform me of your decisions so that I can proceed with the recommendation.

Best regards,

Nicolas Bédère

Evaluation round #1

Authors' reply, 21 February 2025

Dear recommender and dear reviewers,

We are very grateful for the time you spent, the quality of your reviews and the interest you showed in our work, which enabled us to revise the paper in depth. In particular, we would like to thank reviewer 1 for the advice to improve the introduction, reviewer 2, whose comments enabled us to improve the statistics section and recommender to pinpoint the most important issues of the submitted version. Sorry for the delay, but this is due to a complete overhaul of the statistical analyses and major changes to the paper.

The main changes are the following:

-    We withdrew from the paper the group behaviour test to focus on the individual behaviour test. In fact, there was too much information in the previous version of this paper and the group behaviour data is less robust than the individual behaviour data due to the software's inability to follow the fish individually from one end of the test to the other.

-    The correlations between behavioural variables and resistances, which in the previous version were the only criterion for selecting variables associated with resistances, have been reinforced by graphs showing the reranking of lines between each behavioural variable and resistances.

-     The individual behaviour data was analysed at the individual level, giving much greater statistical power.

We took most of your comments into account. You will see below our detailed answers.

Sincerely,

Henri Lagarde and co-authors

Decision for round #1 : Revision needed

Dear Authors,

Thank you for submitting your preprint entitled "Links between behaviour and acute hyperthermia and hypoxia resistance in rainbow trout using isogenic lines" for review and recommendation to PCI Animal Science.

I have now received 2 reviews from your peers, both of them seem to have similar questions and recommendations to further improve your manuscript. Based on their comments, I encourage you to consider the following changes:

- Edit the introduction, possibly its order and structure, and clearly state the research questions; We followed the recommendations of the reviewers.

- Provide additional information or explanation on experimental protocols and statistical analyses, one of the reviewers suggests drawing a schematic of the set-up, which is interesting We provided additional information when asked. We simplified the existing schematics.

- Explain in more detail the need for body weight control and discuss further the potential impact of this choice on the results. We added a section about it in the discussion.

I am very grateful to both reviewers for their involvement in these very detailed reviews. Please take the time to address all points and explain if you disagree with some of the comments.

Yours sincerely,

Nicolas Bédère

Review by anonymous reviewer 1, 30 Aug 2024 10:05

Dear authors and managing board of PCI,

General comment :

« Links between behaviour and acute hyperthermia and hypoxia resistance in rainbow trout using isogenic lines » is an interesting and very relevant topic for aquaculture, especially in the current context of climate change. Authors have done a great amount of experiments and appropriately analysed the data.

About the main sections of the manuscript, I find the introduction should be rearranged and made some suggestions for that. Perhaps I missed something or misunderstood, sorry about that if so, but it looks like some results are not enough described in material and methods, and similarly some results which are however discussed do not appear enough detailed in the results section. Below I suggested to clarify the whole experimental setup with a sketch (hyperthermia and hypoxia resistance, individual behaviour, group behaviours in hyperthermia and in hypoxia). The results could also be exposed in a more « ecological » way rather than very close to model formula. In the discussion, many sections are very close to results and should be revised by seeing the results from a broader scope and with more other studies. I think avoiding references to figures, tables and names of variables in the discussion is a first step towards this.

Please find below my detailed suggestions and comments:

L31-33 : the purpose of your study is not to understand the (physiological) mechanisms underlying resistance to hypoxia and hyperthermia; it is to study whether links exist between these resistances and these behavioural traits. I suggest you revise this sentence in this way.

We have rephrased the sentence as recommended by the reviewer as follows (L30-31): « The present paper aims to test whether behaviour may be associated with acute hyperthermia and hypoxia resistance to better characterize these phenotypes. »

L38 : about a « moderate » thermal challenge, see in my comments below, but I did not understand the difference between moderate and acute thermal in your study. Comment below : -you talk of moderate heat stress : is it really moderate and not acute ? However Fig2 shows temperature rising up to 23.0°C very similarly to 23.2°C in acute test referred to line 197

In the revision of the paper, for reasons explained at the beginning of this rebuttal letter, we removed from the first submitted manuscript the group behaviour experiment in which a moderate thermal challenge was applied. For your information, we will, however, try to explain why we called the moderate thermal challenge "moderate". "moderate" and "acute" are context-dependent adjectives without absolute definition. For example, in rainbow trout, acute hyperthermia resistance was defined as resistance to hyperthermia in a one-day challenge by Perry et al. (2005), but in a 1-week challenge by Chen et al. (2021). In our experiments, final water temperature in moderate challenge was much lower than final water temperature in acute challenge and acute hyperthermia challenges (as performed in Lagarde et al. 2023b) led to fish loss of equilibrium, while this was not the case in the moderate hyperthermia challenges.

L38-40 : I suggest to rephrase this way : « The experimental design used 150 fish phenotyped per isogenic line for each resistance trait and 18 fish per isogenic line for behavioural traits, distributed in triplicates. » done (L35-36).

L46 : delete « also » done.

L85 : replace « consist of » with « consist in » done (L87).

L84-89 : I suggest to shift this paragraph about detailed methods coming just after a much broader scope further (see below a proposal of rearrangement of your introduction). Done.

L90 : replace « have » with « has » done (L83).

L93 to L112 : this is not useful in this paper I think. Why developing this physiological unknown part since this study does not address this point. I suggest to delete. Moreover, here you advocate for not selecting for hypoxia or temperature resistant breeds while your work brings new way to select such resistant breeds with behaviour as a proxy.

Agree, this paragraph was deleted.

L112: when you introduce previous studies, if possible, this part could be further developed with other examples (included in other taxa) showing links between such physiological traits and behaviour.

For example, the first paragraph of point 3.3 in the discussion (L607-614) would better fit here. Introduction is the place where to synthesize what is known on the topic you deal with.

Done, as suggested (L105-115).

L115-117 : I suggest to delete the sentence « The present paper aims to test whether behaviour may be linked with acute hyperthermia and hypoxia resistance. ». This is your problematic and should appear at the end of the introduction, not in the middle ; besides it is repeated L135-137.

Agreed, it was deleted.

L126-127 : I suggest to delete « and society has a growing pressure on the scientific community to reduce animal suffering »

Agreed, it was deleted.

L141 : you refer to moderate stress in group test while I understood that it was acute in group test in your study (I detail this point in the following)

In the revision of the paper, for reasons explained at the beginning of this rebuttal letter, we have now removed the group behaviour experiment in which a moderate thermal challenge was applied. The sentence has been rephrased as follows (L134-136): "In the present study, three phenotypes were collected on six isogenic lines, using systematically different individuals: acute hyperthermia resistance, acute hypoxia resistance and individual behaviour. Rearing of fish methods and phenotyping protocols are presented below."

L142 : instead of activity I would speak of movement which is more general and remove the term movement from inside the parenthesis

As we have removed from the revision the group behaviour experiment, the sentence "Measured behaviour phenotypes were related to activity (acceleration, speed, distance travelled, movement), risk-taking (emergence, time spent in a risky zone), and schooling behaviour (inter-individual distance, contacts between fish)." was changed to (L122-124): "The measured behaviour phenotypes were describing activity (maximum acceleration, maximum velocity, distance travelled, movement) and risk-taking (emergence, time spent in a risky zone)."

L146-147 : this is a very detailed methodological consideration that would better fit in the material and methods section.

Deleted.

Your introduction could thus be rearranged by concatening paragraphs this way :
L79-84
L90-93
L84-89
L118-134
L112-117
L607-614

L138-146

L135-137

We thank the reviewer for the suggestion on the rearrangement of the introduction, and have followed it.

L156 : not necessary to repeat fish are homozygous since you have precised they come from homozygous lines This has been done. The sentence now reads (L143-144) "Homozygous isogenic lines are a powerful genetic resource…".

L184 : delete « reached rearing » Done.

L187 : unless I misunderstood, whereas this experiment is presented first, you do not present some of its results before Fig6. I think they should be described before and first in the results section (or completely refer to Lagarde 2023b for these methods/results if they are the same).

This is correct and we thank the reviewer for pointing out a discrepancy in the order in which experiments in the M&M and results have been presented. In the revision of the paper, at the beginning of the Results section, we have now added lines 348-350 to remind that there were significant differences between lines in the resistance phenotypes. These results, detailed in Lagarde et al. 2023b, have also been summarized in a new Table (Table 2).

L196 : the acute hyperthermia challenge is not completely independent from O2 since when water warms up, O2 can lower down to 65% stauration (Lagarde 2023b) ; I guess this can be uncumfortable for trout and should be discussed ?

Indeed, oxygen can interact with temperature on fish hyperthermia resistance. However, as discussed in Lagarde 2023b (end of 4.1, p.9), O2 concentration was most of the time above 80% as we were bubbling pure O2 to control O2 level – this was added L191. As the main objective of the paper is to investigate links between behaviour and resistance phenotypes, we do not think that it is relevant to discuss this point.

L197 : (12.4°C to 23.2°C in three hours) how close is this to the optimum range of temperature for this species ? This optimum temperature would be worth being reminded here.

We have added the following sentence (L189-191): "For comparison, in rainbow trout, the optimum temperature is 16-17°C for growth and 10-13°C for reproduction (MacIntyre et al., 2008)."

L199 : the suitable oxygen saturation range for trout would be worth to be reminded and the temperature during the hypoxia challenge should be reported as well. From Lagarde 2023b, it looks like temperature could change but then just precise the temperature range, underscoring it remained in very suitable values for this species

We have added the following sentence (L196-198): "For comparison, in rainbow trout, at 13°C for 28 days, a drop of oxygen saturation from 90% to 40% decreased the survival and growth by 7% and 10%, respectively (Jiang et al., 2021). In acute hypoxia challenge, the temperature remained in a range suitable for rainbow trout (10-14°C)."

L229-230 :

-about the same six individuals: in the previous individual test, fish could have learned how to behave in this new environment which is very similar in group test. Don't you think this could bias their behaviour in the group test? and so to add a witness group composed of fish not having experienced individual test before and compare its behaviour to groups having experienced would be a guarantee that results are not biased?

In the revision of the paper, for reasons explained at the beginning of this rebuttal letter, we have now removed the group behaviour experiment. Therefore, answering this question is no longer relevant.

- why evaluating behaviour alone at a fixed temperature and then grouping and increasing T? by changing

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two factors at the same time, it is difficult to deconvolute. Fish behaviour can be different alone or in group. There is perhaps a reference supporting this method.

L269: this section 1.5 about behaviour in group only tests acute hyperthermia stress ; we expect here hypoxia test to be described as well.

L297-298 and 304: about group variables, it is not clear wether they are calculated from a barycentre over all fish positions or an average of the metrics calculated for each individual, especially if a single fish cannot be tracked

L309: you only refer to temperature increase while I also expected some experiments on hypoxia stress also

In the revision of the paper, for reasons explained at the beginning of this rebuttal letter, we have now removed the group behaviour experiment. So, answering these questions is no longer relevant.

L291: just for sharing, about the behavioural variables, I think in the future time spent and distance travelled close to the sides of the aquarium could be interesting as well to characterize shy/bold individuals.

Thanks for the advice.

L323: for your information, it seems that some software can track individuals in all cases: idTracker for example. In case you are interested in: https://www.idtracker.es/home: Tracking individuals in a group by automatic identification of unmarked animals. A. Pérez-Escudero, J. Vicente-Page, R.C. Hinz, S. Arganda, G.G. de Polavieja Nature Methods 11(7):743-748 (2014)

We have not tested this software and Ethovision is supposed to avoid losing fish identity. As we have now removed the group behaviour data from the revised version of the manuscript, we'll keep this suggestion for another project.

L366: Between the first two sentences of section 1.8, insert a transition sentence like: "Pearson correlation coefficients between lsmeans of lines for behavioural variables and for resistance to stress were calculated."

Thank you for this suggestion. We have added the sentence (L338-339) as requested and it improves the clarity of the text.

L369: I would not say « slightly »: this is as much as 31% (from 11.6 to 15.2 g). Especially, weight can play on oxygen needs; there are also links between size (and thus weight) and temperature.

We agree with the reviewer and have now removed this small paragraph (lines 369-372 of the initial submission). Instead, this paragraph has been moved to the Results section lines entitled "Fork length and body weight in resistance and behaviour experiments" (L352) – the word "slightly" has been deleted.

L379-385: not sure this paragraph is useful as it repeats figure and table caption; references to these figures and tables are made below and are enough. I suggest to delete this paragraph.

As the individual behaviour data have been re-analysed in the revision of the paper, the corresponding Results section has been reorganised and this paragraph has partly been deleted. We have only kept the sentence "The significance of effects (line, time, fork length and their interactions) on the different behaviour variables is shown in Table 3." See L369-370. Note that Table 2 of the initial submission is now called Table 3.

L396-399: in the description of model effects in general, you could lighten the text and make it more "ecological» this way for example on this paragraph: « In individual test, body weight inflenced only maximal acceleration by increasing it by 2.5 cm.s-2 per gram, i.e. bigger fish had higher acceleration, all things being equal (Table 2). Body weight had no effect in group test. »

Thank you for this suggestion. Following the re-analysis of the individual behaviour data, the results have changed as body length is now significant for all behaviour variables except EMERGENCE and MEANDER. In this part of the results, we have tried to describe them in a more "ecological" way. See lines 379-386.

L404-406: suppress the sentence « The effect of time on these behaviour variables is presented in Fig. S2 and Table S4 » and just replace it by a reference to « (Fig. S2 and Table S4) » after « […] tended to decrease ».

This has been changed. Table S4 is now Table S3 and the Figure S2 has been deleted.

L421: I would replace « activity levels » by « their movement characteristics » which is more general since %MOV did not differ between lines. %MOV is related to activity.

The whole paragraph was rewritten (L398-405).

L433-434: another example of more ecological description of results; this sentence could be rephrased this way: « In the individual test, line effect and time were independent whatever the behaviour variable was (Table 2). »

This sentence was added line 387-388. We have not included "In the individual test", as there is no need to specify now that the group behaviour data were removed from the paper.

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L447: please add a coma after « For other two »

The sentence containing "for other two" was deleted in the new version of the manuscript.

L448: please add a space after N38h

The sentence was deleted in the new version of the manuscript.

L472-473: « Fish staying longer in the risky zone therefore tended to swim longer distances than others. »: I am not sure to well understand this conclusion. PCA just tells that %RISK is uncorrelated to "activity" variables.

The PCA has been removed from the revised version of the manuscript. We now only present the correlations between behavioural variables in Table 5 and this sentence has been deleted.

L479: replace dependant with dependent

This comment concerns a section of the initial paper focusing on the group behaviour data, so this change is no longer relevant as this section has now been deleted.

L518: Looking at Table 3 and Figure 6, I probably misunderstood something:

-are results on individual tests on acute stress, corresponding to the first experiments you described in section 1.2, described somewhere? It looks like they are used in Fig6, but they should be firstly described at the beginning of results section.

- are behavioural experiments and results on acute hypoxia described somewhere?

To clarify the whole experimental setup, I suggest to add a schematic in the material and methods section synthesizing individual and group tests, both on behaviour variables and on resistances (from section 1.3 and 1.2) and both on hyperthermia and hypoxia. Figure 2 is probably a good basis to complete.

As explained before, we have now added a paragraph at the beginning of the Results section to briefly describe the acute hyperthermia and hypoxia tests (results previously published in Lagarde et al 2023b) – see also the new Table 2. Therefore, M&M and Results sections are now presented in the same order.

Also, to make it clearer, we added at the beginning of M&M section (lines 134-136) : "In the present study, three phenotypes were collected on six isogenic lines, using different individuals: acute hyperthermia resistance, acute hypoxia resistance and individual behaviour. Rearing of fish methods and phenotyping protocols are presented below."

Figure 1 already synthetizes all the phenotyping: acute hyperthermia resistance, acute hypoxia resistance and individual behaviour test. Figure 1 and Figure 2 have been revised to remove the group behaviour experiment (withdrawn from the revised paper).

L542: in your discussion, avoid reference to tables and figures and the use of variables' names; it leads to a repeat of the results section. Thank you for these suggestions. We removed references to tables and figures in the Discussion, as well as variables names.

L545-547: this looks more to an introduction than a discussion; rather introduce main results, eg : for some lines, links could been established between behaviour variables and resistance to acute hyperthermia or hypoxia. Behavioural differences were found in isogenic lines and some of them could be linked with resistance to …. Or a synthesis of the main results since they are quite rich and complex, so that you can then discuss them with the help of other study results.

Text was changed to "In this work, we identified links between behaviour variables and resistance to acute hyperthermia e.g. travelling distance and frequency of change between the tanks' zones or resistance to acute hypoxia e.g. travelling distance, frequency of change between the tanks' zones and the percentage of time in movement." (L432-435)

L553: replace was with were
Done (L450).

L557: replace « exploratory behaviour »with « exploration » to avoid repetition of behaviour As the group behaviour experiment has been removed, this modification no longer applies.

L560: about behavioural differences between isogenic lines, I suggest to discuss the weight differences since they are significant. As soon as L193, we wonder about weight since it can play on oxygen needs; there are also links between size (and thus weight) and temperature.

Body weight was corrected in the model and the aim of the paper was not to discuss about the effect of the body weight. However, we added a paragraph about it in the discussion (L437-445).

L562 and L565: replace « our own » with « ours »
Done (L459, the sentence of the second "our own" was deleted).

L568: replace « apart » with « ago »
Done (L462).

L571: in addition to the duration of the group test, you could discuss the influence of the group compared to individual alone. I wonder about this
As the group behaviour experiment has been removed, this modification no longer applies.

L579-584: this is typically too close to a result section for being in the discussion and needs to be more deeply discussed rather than rephrasing results

This section was mixing results obtained during the individual behaviour experiment and the group behaviour experiment, and is therefore not relevant anymore. This paragraph has been deleted. Instead, two sentences have been added to discuss the correlations between the behavioural variables (L464-467): "Behaviour variables reflecting the level of activity of fish (i.e. maximum acceleration, distance travelled, zone change frequency, maximum velocity) were highly correlated together at the isogenic line level. A strong correlation between activity variables was already found in rainbow trout for similar variables (Makaras et al., 2020)."

L589: replace have with has
The sentence containing this mistake was deleted.

L590: use a term like movement metrics or something like that instead of « ACC_MAX, DIST_TRAV and VEL_MAX » which is close to the result section. Done. All behaviour variables names (ACC_MAX, DIST_TRAV, …)

were deleted from the discussion part of the paper.

L595: delete with

This comment refers to group behaviour data and is no longer relevant.

L607-614: I suggest to move this paragraph into the introduction (see my comments above) where we classically cense works linked to the one detailed in the paper to highlight the interest of your work compared to what has already been done.

Agree, we moved it.

L615-619: This is a repeat of detailed results, and this sentence can be deleted; I however do not understand why you start with « In the individual behaviour ». The next sentence would be sufficient with very few changes: « Acute hyperthermia resistance appeared to be associated with a low level of activity and acute hypoxia resistance with a high level of activity. »

Thank you for this suggestion. We have deleted this sentence and have modified the following sentence as suggested. See lines 474-475.

More importantly, I did not see behavioural experiments and/or results at the individual level in what is written before. I probably misunderstood something or it is not clear.

We changed "at the individual level" to "individually" (L299)

L620 and 623-624: I did not see detailed methods/results on behavioural experiments in acute hypoxia conditions. Again, I probably misunderstood something or it is not clear.

This comment refers to group behaviour data and is no longer relevant.

L630: when you say « thus can resist longer acute hyperthermia. », does this mean that resisting to hyperthermia consumes energy? I suggest you give a reference for that here, perhaps some physiological explanation from those you gave in introduction.

The reference of Campos et al. 2018 was in the previous sentence; to increase clarity, we have included the same reference at the end of the sentence line 480.

L633: What you name panick (perhaps prefer enhanced activity?) is interpreted as a way to find a refuge area (more rapid movements to efficiently explore greater areas) when a fish meets bad conditions (see some references in Westrelin, S., Bouletreau, S., and Santoul, F. 2022. European catfish Silurus glanis behaviour in response to a strong summer hypoxic event in a shallow lake. Aquat. Ecol. 56(4): 1127–1142. doi:10.1007/s10452-022-09952-y and also some explanations on the interest of different kinds of strategies). Strategies of fishes selected by evolution to face hypoxia can differ and this could lead to behaviours inadapted in artificial conditions. For example, we could imagine that, in nature, trout has better to try to actively search for a refuge area in case of hypoxic conditions rather than limits its movements hoping for better conditions to appear. In tanks where there is no refuge area, the problem is "artificial " and completely different. You could discuss this.

I discussed about it (L517-537). Thank you for the idea, it is very interesting.

I also wonder how fast some fish that have explored their new environment in the individual test before being gathered for a stressful group test have learned about this environment and thus could adapt their behaviour according to, what would influence their movements. Here some references on fish learning, including spatial one:

Kieffer, J.D. and P.W. Colgan, The role of learning in fish behaviour. Reviews in Fish Biology and Fisheries, 1992. 2(2)

Dunlop, R., S. Millsopp, and P. Laming, Avoidance learning in goldfish (Carassius auratus) and trout (Oncorhynchus mykiss) and implications for pain perception. Applied Animal Behaviour Science, 2006. 97(2)

Thank you for this relevant comment and references. As we have removed from the revised manuscript the group behaviour test, this comment no longer applies.

L639-641: you could please explicit what you mean.

Following your suggestion, we have now rephrased this sentence. The original sentence "However, the results from the present study and Van Raaij et al. (1996) do not conflict since the level of activity was measured in a hypoxia stress challenge in Van Raaij et al. (1996) which was not the case in our experiment." Has been changed to (L490-494): "The Van Raaij et al. experiment, performed with individuals from heterogeneous origins, established a link between behaviour during the hypoxia challenge and the later survival rate which is completely different from our experiments in which we established a link between hypoxia resistance and responses in a behavioural challenge performed in normoxia."

L649: replace of with in

This comment refers to group behaviour data and is no longer relevant.

L696-698: I don't see how these relationships help to understand the mechanisms, especially if you think about physiological mechanisms. I would delete this sentence.

We agree with this comment. In the new version of the paper, this sentence was deleted from the conclusion.

Review by anonymous reviewer 2, 17 Oct 2024 15:54

Answers to questions

Introduction

- Are the research questions/hypotheses/predictions clearly presented? No — the research question is stated in general terms at Line 135, but no specific hypotheses or predictions are written. The following paragraph lists the traits they measured, but not how they expected them to interact. I can also can't find any question or hypothesis for the choice to use both group test and individual tests, as well as the temperature ramping during the group test.

We have added underlying hypothesis just before the mentioned lines (now L126-130). Regarding the other question, we detail the rationale for choosing individual test and according to explanation given above, group test was removed from this study. Following reviewer 1's suggestions, we have also reorganised the Introduction for increase clarity. We hope that these changes address these comments and clarify the research question and hypotheses of the paper.

- Does the introduction build on relevant research in the field? No — Mentions of similar studies are missing from the introduction (but are present in the discussion) (e.g. Van Raaij et al 2016 and Campos, Val & Almeida-Val 2018)

We acknowledge these references could enrich introduction however some papers presenting links between resistances and behaviour were already present in the introduction albeit performed with other species (e.g. Cerqueira et al. 2016 in Nile tilapia; Adriaenssens & Johnsson, 2011 in brown trout; Binder et al., 2016 in bluegill sunfish). In the revised version of the paper, we have added the two mentioned references on rainbow trout (L105-115).

- Are the methods and analyses sufficiently detailed to allow replication by other researchers? - No - See details below. Some important information is missing from the practical methods as well as statistical methods. Details below.

- Are the methods and statistical analyses appropriate and well described? No - Some information is missing from methods (details below), and I'm not sure about the validity of some statistical methods (especially that

of controlling for body weight, details below).

The paragraphs about statistics were rewritten in the material and method part (section starting L296). We discussed about the validity of controlling for body length in the discussion (L437-445).

Summary of the study

In this study, the authors have looked for genetic associations between behaviour and tolerance to both high temperatures (hyperthermia) and low oxygen conditions (hypoxia).

Their case for understanding this is 1) a need to mechanistically understand these tolerance traits and 2) understand how selection for tolerance traits may affect behaviour, and 3) that behavioural traits might be used to test for tolerance to temperature and hypoxia (instead of directly exposing fish to tests for these traits).

The authors studied this question by assessing these traits in six "isogenic lines" of rainbow trout (6 genetically distinct cohorts of genetically identical individuals), and then checking if between-line variation in behaviour correlated with between-line variation in either tolerance (resistance) trait.

Some correlations between tolerance traits and behavioural traits were found. For example, lines with high tolerance to high temperatures displayed a lower swimming activity, and lines with high tolerance to hypoxia displayed higher swimming activity.

Report

The authors have used a novel and interesting study system to look for genetic links between physiology and behaviour, and give findings that can be of high value for research on such associations especially with regards to environmental issues. However, I'm not fully confident in the findings presented here due to some statistical issues (See section on critical issues below).

I am also a little sceptical of the potential for using behavioural assays as a proxy for tolerance traits to temperature and hypoxia. The authors do find a correlation between these traits, but these traits are not measured in the same individuals (-though they are genetically identical). This makes it hard to estimate how predictive these behavioural measures are. However, this limit is clearly stated in the discussion and conclusion. I think the main novel finding of this study is the between-line correlation between these traits, giving a good indication that there is some genetic link between these traits, and showing this in a novel way.

Thank you for this positive report on the novelty of our paper. We fully agree with your remark on the difficulty to estimate how predictive behavioural measures would be of resistance to hyperthermia and hypoxia. We are aware that additional experiments are necessary before operational implementation of our findings. Thank you for acknowledging that we are aware of this limit.

A significant portion of this study is the use of a group test for behaviour (in addition to individual tests), together with a temperature increase during the group test. However, it is not stated in the introduction why this test was done. What was the expected benefit of doing a group test in addition to the individual test? -and also, why include the temperature increase? In the discussion, the temperature increase is only mentioned as something confounding the test's time-factor. The group test and its results are thoroughly discussed in the discussion, but its significance in relation to the individual tests is not described, so it is not clear to me what extra benefit it gives.

As explained in our reply introduction, in the revised version of the manuscript, we have decided to remove the group test because of its lesser statistical robustness compared to the individual test and the impossibility to correct data with weight as for the individual test. Consequently, remark about group test, albeit fully relevant, will not be addressed.

The practical methods are explained fairly clearly in the methods, but a few important details are missing. Details below.

We have tried below to answer every comment / question.

A lot is missing from the descriptions of the statistical methods. In particular, I am missing information on what models were used, exactly how many models were compared in the model selection process, what method was used for determining statistical significance, and some missing p-values (or similar measures).

Details below.

    We have tried below to answer every comment / question.

    It would benefit the manuscript to have a round of careful editing for language, especially focusing on sentence structure, choice of terminology, and consistency in choice of terminology. I've detailed a few issues at the end of the report, but this is not an exhaustive list.

    Thank you for this suggestion. In the previous version we already used a proof-reading service. In the revised version of the paper, we have tried to carefully edit the language. We have however not used a proof-reading service again.

    Critical issues

1) One aspect of the statistical methods makes me worry about the validity of this study's findings. To test for correlations in between variation in behaviour with variation in tolerance to hyperthermia and hypoxia, the authors needed to get estimates of each line's (the six isogenic groups of fish) value for each trait. To get line-specific values for each trait, a linear model was fitted for each trait while using line as a categorical factor in this model (among other factors), and then using those models to estimate least-square means (essentially model predictions) of each trait for each line. In order to control for body-weight differences between lines, these least-square means were calculated for fish at 15.2 grams (the whole study-population mean). However, since the different isogenic lines differed in body weight, the estimated trait values for each line do not necessarily represent the actual trait value for that line (since it is estimated for a fish of a body-weight that is not representative of the line's mean). Overall, I'm not sure I really understand the need to control for body weight. The goal of the study was to look for between-line differences in tolerance to hyperthermia and hypoxia; If they found a difference and also that this difference was associated with a body-weight difference, then that would be an interesting finding in itself and not something they would need to control for.

    -to put this in another way: The lines differed significantly in body weight (line and body-weight is correlated), yet effects of both line and body-weight were included the same models (models for resistance traits). This is especially problematic knowing that body-weight was also associated with the resistance-traits which were being modelled.

    It would help if the authors could:

- Better explain the need to control for body weight and how this relates to their study question.
- If controlling for body weight, give more information about the relationship between isogenic line, body weight and hyperthermia/hypoxia resistance to show that this does not produce any false positive results (and if results differ after controlling for body weight, discuss why).

    Thank you for your detailed comments. We agree with you that dealing with these between-lines differences in body weight / length is complicated and needs to be further explained.

    Body weight is a known component of the resistance to acute hyperthermia and hypoxia (Perry et al., 2005; Nilsson & Östlund-Nilsson, 2008; McKenzie et al., 2021). In the present paper, we wanted to correct for body weight as body weight differed between isogenic lines. Indeed, if we hadn't corrected for body weight, we could not have determined whether the link between isogenic line resistance and behaviour is due to body weight effect or due to isogenic line effect. We have added a whole paragraph on the subject at the beginning of the discussion (L437-445).

    We ensured that a part of the fish in the lines of extreme body weight overlapped well, so that the model was able to capture the effect of body weight, which is indeed the case (Figure 1).

    Figure 1 : Distribution of BW of the lightest and heaviest lines in hyperthermia and hypoxia challenges.

The relationship between body weight and hyperthermia/hypoxia resistance for each isogenic line is given in Table 1. Table 1 presents the BW regression coefficients estimates for each isogenic line, expressed in CTE (cumulative thermal exposure, the phenotype of acute hyperthermia resistance) or CHE (cumulative hypoxia exposure) per gram of fish.

Table 1 : Detailed presentation of the results of acute hyperthermia and acute hypoxia statistical models. CTE is cumulative thermal exposure and CHE is cumulative hypoxia exposure, the phenotypes of resistance used in the present manuscript.

Challenge	Best model fixed effect and covariate	Isogenic line	Isogenic lines' effects estimates[1] (CTE/CHE)	BW regression coefficients estimates (CTE.g-1/CHE.g-1)
Hyperthermia early	Line + several BW regression slopes (one per line)	A02h	6959	-39
		A22h	7113	-67
		A32h	7520	-28
		B45h	7911	-90
		N38h	6097	-15
		R23h	6968	-39
Hypoxia early	Line + several BW regression slopes (one per line)	A02h	43507	
		A22h	52622	
		A32h	33892	
		B45h	16357	
		N38h	32532	
		R23h	48281	

-709

-1535

-1013

277

-298

-972

1Isogenic lines effects are given for fish of 0 gram in this table. To find estimated resistance of isogenic lines at 15 grams (which is used in the present paper), you have to multiply the BW regression coefficients by 15.5 and sum it with Isogenic lines' effects estimates.

The results differ little for isogenic lines in terms of acute resistance to hyperthermia and hypoxia, whether or not they were corrected for the effect of body weight (Table 2).

Table 2 : Estimated isogenic lines resistance to acute hyperthermia and hypoxia resistances at 15 grams, without or with body weight correction.

2)When doing model selection for behavioural traits, exactly how many -and what models were tested?

Line 336 reads: "with vs. without time and/or line and/or body weight and/or the different interactions between these variables"

That is approximately 13 different possible combinations of effects and interactions, and then there is the inclusion/exclusion of weighted terms (x2) then the use of AR1, ARMA (or none) (x3) resulting in a total of 78 different models. Multiply that by the number of traits (x10) gives 780 different models. Is that right?

The following would help:

- A description in the methods of exactly how many models were tested/compared, and exactly which combinations were tested.

Model selection was carried out by following these steps:

1)   Model selection (with vs. without time and/or line and/or fork length and/or the different interactions between these variables) was done using stepAIC function àmodel selection is done automatically by this function. In the initial manuscript we did not use this function, but we think it is clearer now with this function.

2)   Model selection for weighted terms allowing variances to differ among isogenic lines were considered (with vs. without weighted terms) à 2 models were compared with AIC criteria.

3)   Model selection for temporal autocorrelation (AR1/AR2/ARMA(1,1)/ARMA(2,1)) à 2 models were compared with AIC criteria.

The description of model selection was rewritten accordingly (L305-L323).

3)Line 379 says that table 2 shows the significance of model effects, but table 2 just contains a list of which models had the "best structure" for each behaviour variable. There are no significance parameters in this table (like p or F values or confidence intervals etc). This makes me wonder if this table just lists which models (of the many which were compared) had the lowest AIC, but then on some models it says "no significant effect", which seems to indicate that indeed some significance testing was involved?

The following would help:

-A thorough description of what table 2 actually shows.

- A description in the methods of how significance testing was done for these models, and how this relates to table 2

- Specifying exactly which models were used in the end (not just which fixed effects+interactions, but also the usage of terms-weighing and correction for autocorrelation)

- Ideally, the results should also include a table of the models used, what the effect sizes for the different parameters were, and some measure of significance for these (p-values, confidence intervals, or similar)

You are right, table 2 of the initial manuscript (now table 3) did not show the significance of model effects but the best models according to AIC test.

We added the result of F-test in table 3. We also added Table S2 which gives more details about F-statistic value and degrees of freedom of the numerator and denominator.

We added line 322: "The significance of model effects was tested using F-tests with package lmerTest 3.1–3." To explain how significance was tested.

The effects sizes of isogenic line, fork length and time bin are given in Table 4, Table S3 and Table S4, respectively.

4)Why are no p-values (or other measures of statistical significance) presented for the correlations? The main result of this paper hinges on these correlations, so it seems like an odd exclusion, and it would be good if the authors could elaborate on it.

The design of the experiment was not intended to test the significance of correlations, due to the small number of isogenic lines used. The correlations between behaviour variables and resistance phenotypes were only significant for DIST_TRAV and hypoxia (p-value = 0.02), FRQ_CHAN and hypoxia (p-value = 0.01) and MOV% and hypoxia (p-value = 0.02) (Table 3 below).

Table 3 : Correlations and p-value of these correlations using the cor.test() function of package stats

We decided to keep in the discussion the variables for which the correlation between behaviour variables and resistance phenotypes was higher than 0.6 even if not significant as it could reveal a possible link (L412-422). However, we have added a warning about it (L423-428).

Missing from methods
We have added all requested information in the methods section.
- How large were the tanks used to measure hyperthermia tolerance? 0.12 m3 L185
- How large were the tanks used to measure hyperoxia tolerance? 0.12 m3 L185
- How was temperature increased in the hyperthermia tolerance test? With water from a heated buffer tank. L192-193
- Was oxygen being replenished in the test-tank during the hyperthermia tolerance test? -if not, was it being monitored? Yes, oxygen was being replenished in the test-tank during the hyperthermia test. Oxygen saturation was monitored using an oximeter, and maintained by bubbling pure O2. A sentence was added in the M&M line 191.
- How was the temperature increase-rate ensured in the hyperthermia tolerance test? The temperature increase rate was manually controlled by adding water from a heated buffer tank. L192-193
- How was temperature measured (what tool and where in the tank) in the hyperthermia test? Temperature and O2 concentration and saturation were recorded every 5 min during acute hyperthermia and hypoxia challenges using electronic probes (HQ40d, Hach Company, Loveland, CO, USA). (Lagarde et al 2023b) L200-202
- How were oxygen-levels manipulated in the hyperoxia test? by bubbling dinitrogen gas into a saturation column (Lagarde et al 2023b) L195
- How were oxygen levels measured in the hyperoxia test (what tool and where in the tank) ? Temperature and O2 concentration and saturation were recorded every 5 min during acute hyperthermia and hypoxia challenges using electronic probes (HQ40d, Hach Company, Loveland, CO, USA). (Lagarde et al 2023b, 2.4) L200-202
- Were fish exposed to air at any point during transfer between tanks? Describe how fish were transported

between tanks, test-setups, in a way that lets the reader know if they were exposed to air or not. The evening prior to each challenge, fish of the challenged replicate were moved to the indoor challenge tank (0.12 m3) supplied with the same water source as the one used in the respective rearing tanks and left alone for the night for acclimation. (Lagarde et al 2023b, 2.4) L183-186

- How did you determine if a model effect was statistically significant or not? For mixed-effect models this requires some special packages. Which did you use? What was your chosen level of significance (e.g. P > 0.05? -or other?). Function lme in package nlme 3.1–153 (Pinheiro et al., 2021) was used to fit linear mixed models. The chosen level of significance was P>0.05. L223-224

- Line 205: How were fish euthanized? Fish were softly anaesthetized (Tricaïne MS222, 50 mg/L), weighed using digital scales (CP2202S, Sartorius, Germany), and euthanized by an overdose of anaesthetic (Tricaïne MS222, 150 mg/L). (Lagarde et al 2023b, 2.4.1) L238-240.

- Line 394 mentions an ANOVA analysis not mentioned in methods. Missing info on which model is used, r-package, and type of statistical test used. We added L302-304: "Mean fork length differences among isogenic lines were analysed by analysis of variance (ANOVA), using stats package. ANOVA assumptions of normality and homoscedasticity were verified by visual inspection of residual-fit plots." .

- Line 260-261: Missing description of door and remote opening mechanisms in more detail. Are there any pictures of the test setup? How big was the door? How did it open? (sliding? hinged? etc ...). As requested, we have added details and the text now reads as: "Aquariums were divided into two zones: on one side, a zone of size 20 x 8 cm covered by a plate opaque to visible light but transparent to infrared light called the safe zone, and on the other side, an uncovered zone of size 20 x 32 cm called the risky zone (Figure 2 and actual video capture as Fig. S1). Fish were first placed in the safe zone, separated from the risky zone by a door opaque and completely closing the space between the safe and risky zones. Fish were left for 5 minutes in the safe zone, and the door was then lifted using an electric engine remotely controlled allowing fish to freely move between the safe and risky zones. The two engines, each lifting the three doors of the three contiguous tanks, were located on a structure independent from the tanks to prevent vibration and were activated simultaneously to ensure synchrony of the opening." L252-261 Please find below a picture of the setup, that we also provide as Supplementary Figure S1.

   - Line 203: How was loss of equilibrium defined? (e.g. "unable to maintain upright swimming for X seconds»?). We have added line 205: "defined as an inability to maintain upright swimming for 10 seconds".

- Line 217-218: Were any random effects included in these models? (e.g. triplicate?) Thank you for pointing this out. Yes, random effects were included in the models. We have added line 221: "and replicate as a random effect".
- Line 219: What is the measure of significance here? P < 0.05 ? Yes, it was added line 329-330.
- Line 342 Where any r-packages used for AR1 or ARMA(1,1) accounting? We added line 317: "using package nlme 3.1–153".

   Other issues / questions
   L110-111: Are there any examples of how selecting for poorly understood traits could be ineffective or harmful?
   This sentence was removed in the reorganisation of the introduction suggested by reviewer 1. For your information an example is the selection for individual growth that can select the less cooperative and more aggressive animals, leading to a decrease of performance at the farm level. For example in salmon : https://doi.org/10.1006/anbe.2003.2237.

L314: Why were individual-level variables averaged over groups for the individual behaviour tests? I understand it for the group tests but not the individual ones. This seems like an odd exclusion of variation which could have been included in your models.

We agree with your comment. In the initial submission that included individual and group behaviour tests, individual-level data were averaged at the replica level in order to analyse the two datasets in the same way. As explained in the initial version, this was necessary for the group behaviour experiment as the Ethovision software could not follow each fish individually due to frequent swapping of identity when two fish crossed paths. In the revised version of the manuscript, as we have decided to remove the group behaviour experiment, the individual behaviour data were reanalysed at the individual level (6 isogenic lines x 3 replicas x 6 individuals, 108 data points per behaviour variable and time bin) as stated as the beginning of section 1.6 L299-301.

L320: I don't understand this line of reasoning. Here it reads that behavioural variables need to be corrected for fish body weight to disentangle the genetic and body weight effects, but I assume body weight is also affected by genetics? By correcting for body weight, is the data really representative of the genotypes still?

We believe that we have responded to this issue in the Critical issues section (first point). Particularly by adding a new paragraph in the discussion on the need to correct resistance and behaviour phenotypes with body size.

Discussion: The usage of isogenic lines is very interesting, but also very novel within this field, and most readers are likely not going to be familiar with this. It would be good if the authors could include some text in the discussion on how they think this choice of study system could potentially influence the results, and if there is anything else the reader should keep in mind when interpreting the result from such a system.

We think the specificities of the usage of isogenic lines was already mentioned in the paper :
-	Introduction: "The resistance and behaviour phenotypes, measured on different individuals, were related at the level of lines, thanks to the complete genetic homogeneity among fish within an isogenic line."
-	M&M: "Homozygous isogenic lines are a powerful genetic resource, as fish are genetically identical within a line. Therefore, fish from a given line can be considered as replicates of a unique genotype."
We also think we warned the reader about the limit of using six isogenic lines :
-	Discussion: "However, as associations revealed with only six genotypes can give misleading results, these results still need to be validated on more genotypes and different populations."
If you were looking for other types of information, could you please specify your request?

Minor language / text / structure issues
L36: Unclear what is meant by "systematically" different individuals
Systematically was changed by "using different individuals for each phenotype" (L33).

L41: Confused by "related", does this sentence claim that these traits actually are related, or if they were tested for a relation?
Thank you for pointing out this confusion. We have changed the sentence as follows L36-38 "Relations between acute hyperthermia and hypoxia resistance phenotypes and behaviour phenotypes were tested at the level of isogenic lines."

L84-88: The first sentence makes it sound like these are the only methods used for measuring resistance traits. Including an "among others" or similar could fix this.
Done. We have added the word "predominant" line 86. The sentence now reads: "The predominant methods in use...".

L90 and 561: the phrase "genetic determinism" carries a connotation to the "nature vs nurture" debate, while what I think you are actually referring to here is closer to "a genetic component" or "genetic influence".

Corrected. Lines 82, 105 and 456, we have changed "genetic determinism" by "genetic component".

L96: "observed genetic variability" implies you have actually observed variation at the genetic level (i.e. observed variation in the genetic code, allele frequencies, and such); What I think you are actually referring to here is "... about the mechanisms for this genetic influence/component", or similar.

This sentence was removed when the introduction was reorganized, as suggested by reviewer 1.

L115: Not clear what is meant by "extensive behavioural repertoire". This sounds like "the rainbow trout had a lot of different behaviours", but I don't think that is what you mean? (-also not clear how that would suggest that behaviour may be linked to resistance phenotypes).

We agree that the text was not clear and was not expressing our thoughts. Text was modified as follows (L102-105):

"During the course of previous studies, aiming at the evaluation of the resistance to acute hyperthermia and hypoxia of rainbow trout (Dupont-Nivet et al., 2014; Lagarde et al., 2023b), we observed expression of a different range of behaviours between lines suggesting there may be an underlying genetic component for these behaviours."

L118-121: Is this the correct interpretation of this sentence? Consider splitting it up.
"A strong interest in identifying mechanisms (i.e. those underlying resistance to acute hyperthermia and hypoxia)(-and whose phenotyping would be reasonable intrusive, such as behavioural ones) would be to use them (the mechanisms) as predictors to replace the current classical phenotyping methods"

Done. We have rephrased this sentence L91-93: "There is a strong interest in identifying reasonably intrusive phenotypes, such as behavioural ones, that could be used as predictors of resistance to acute hyperthermia and hypoxia, to replace the current classical phenotyping methods."

L118-121: Also, in the context of measuring behavioural traits in lieu of resistance traits, I don't think you can call those behavioural traitn "mechanisms", but rather "proxy traits" or something similar.

Agree, we removed from the article all the part 'understanding the physiological mechanisms" as it was not designed for it.

L123: This sentence mentions "several" drawbacks, but the following paragraph only lists two.
We have removed "several".

L141: "were related to" is ambiguous, it sounds like you either a) tested if beh. vars were related to the following list of traits, b) you found that they were related to the following list of traits, or c) that you measured traits which were related to the following list of traits.

We have rephrased the sentence: "The measured behaviour phenotypes were describing activity (maximum acceleration, maximum velocity, distance travelled, movement) and risk-taking (emergence, time spent in a risky zone)." (L122-124).

L154-154: Give the full name of abbreviated institutions.
Added (L140-143).

L195: Here you first write that each replicate had 50 fish, then that each replicate had 300 fish (make the distinction between replicate and replicate types/names).

Done lines 182-183: "Each replicate (named A, B and C in Fig. 1) consisted of 300 fish, composed of 50 fish from each of the 6 isogenic lines."

L187 and 224: is "experiments" the right word here? (-instead of "test" ?)
We have changed "experiment" to "test".

L236-237: What is "temperature-equilibrated water"? -equilibrated to what?
As the group behaviour experiment was removed, this comment is irrelevant.

L244: Confusion -> confounding
Changed (L243).

L251, 255, 278 : Remove square-symbols in dimensions
Done.

L251: What is system water?
Removed 'system'. System means from the same water source but it is already mentioned in 1.3.

L292 (and other following): Remove "." from equations (replace with space)
Done.

L304: says you measured group variables, but you measured individual variables and averaged them over groups.
As the group behaviour experiment was removed, this comment is irrelevant.

Line 393: overall -> mean
Done (L353).

Line 408: evolutions -> changes (or similar)
Done (L471).

Line 607-614: This paragraph first sounds like you are describing your own results, but then instead lists findings from other studies?
Following Reviewer 1's suggestion, this paragraph was moved to the introduction section.
Download tracked changes file

Decision by **Nicolas Bedere** , posted 17 October 2024, validated 18 October 2024

Dear Authors,

Thank you for submitting your preprint entitled "Links between behaviour and acute hyperthermia and hypoxia resistance in rainbow trout using isogenic lines" for review and recommendation to PCI Animal Science.

I have now received 2 reviews from your peers, both of them seem to have similar questions and recommendations to further improve your manuscript. Based on their comments, I encourage you to consider the following changes:

- Edit the introduction, possibly its order and structure, and clearly state the research questions;
- Provide additional information or explanation on experimental protocols and statistical analyses, one of the reviewers suggests drawing a schematic of the set-up, which is interesting
- Explain in more detail the need for body weight control and discuss further the potential impact of this choice on the results.

I am very grateful to both reviewers for their involvement in these very detailed reviews. Please take the time to address all points and explain if you disagree with some of the comments.

Yours sincerely,

Nicolas Bédère

Reviewed by anonymous reviewer 2, 30 August 2024

Dear authors and managing board of PCI,

General comment :

« Links between behaviour and acute hyperthermia and hypoxia resistance in rainbow trout using isogenic lines » is an interesting and very relevant topic for aquaculture, especially in the current context of climate change. Authors have done a great amount of experiments and appropriately analysed the data.

About the main sections of the manuscript, I find the introduction should be rearranged and made some suggestions for that. Perhaps I missed something or misunderstood, sorry about that if so, but it looks like some results are not enough described in material and methods, and similarly some results which are however discussed do not appear enough detailed in the results section. Below I suggested to clarify the whole experimental setup with a sketch (hyperthermia and hypoxia resistance, individual behaviour, group behaviours in hyperthermia and in hypoxia). The results could also be exposed in a more « ecological » way rather than very close to model formula. In the discussion, many sections are very close to results and should be revised by seeing the results from a broader scope and with more other studies. I think avoiding references to figures, tables and names of variables in the discussion is a first step towards this.

Please find below my detailed suggestions and comments :

L31-33 : the purpose of your study is not to understand the (physiological) mechanisms underlying resistance to hypoxia and hyperthermia; it is to study whether links exist between these resistances and these behavioural traits. I suggest you revise this sentence in this way.

L38 : about a « moderate » thermal challenge, see in my comments below, but I did not understand the difference between moderate and acute thermal in your study.

L38-40 : I suggest to rephrase this way : « The experimental design used 150 fish phenotyped per isogenic line for each resistance trait and 18 fish per isogenic line for behavioural traits, distributed in triplicates. »

L46 : delete « also »

L85 : replace « consist of » with « consist in »

L84-89 : I suggest to shift this paragraph about detailed methods coming just after a much broader scope further (see below a proposal of rearrangement of your introduction).

L90 : replace « have » with « has »

L93 to L112 : this is not useful in this paper I think. Why developing this physiological unknown part since this study does not address this point. I suggest to delete. Moreover, here you advocate for not selecting for hypoxia or temperature resistant breeds while your work brings new way to select such resistant breeds with behaviour as a proxy.

L112 : when you introduce previous studies, if possible, this part could be further developed with other examples (included in other taxa) showing links between such physiological traits and behaviour.

For example, the first paragraph of point 3.3 in the discussion (L607-614) would better fit here. Introduction is the place where to synthesize what is known on the topic you deal with.

L115-117 : I suggest to delete the sentence « The present paper aims to test whether behaviour may be linked with acute hyperthermia and hypoxia resistance. ». This is your problematic and should appear at the end of the introduction, not in the middle ; besides it is repeated L135-137.

L126-127 : I suggest to delete « and society has a growing pressure on the scientific community to reduce animal suffering »

L141 : you refer to moderate stress in group test while I understood that it was acute in group test in your study (I detail this point in the following)

L142 : instead of activity I would speak of movement which is more general and remove the term movement from inside the parenthesis

L146-147 : this is a very detailed methodological consideration that would better fit in the material and methods section.

Your introduction could thus be rearranged by concatening paragraphs this way :

L79-84

L90-93

L84-89

L118-134

L112-117

L607-614

L138-146

L135-137

L156 : not necessary to repeat fish are homozygous since you have precised they come from homozygous lines

L184 : delete « reached rearing »

L187 : unless I misunderstood, whereas this experiment is presented first, you do not present some of its results before Fig6. I think they should be described before and first in the results section (or completely refer to Lagarde 2023b for these methods/results if they are the same).

L196 : the acute hyperthermia challenge is not completely independent from O2 since when water warms up, O2 can lower down to 65% stauration (Lagarde 2023b) ; I guess this can be uncumfortbale for trout and should be discussed ?

L197 : (12.4°C to 23.2°C in three hours) how close is this to the optimum range of temperature for this species ? This optimum temperature would be worth being reminded here.

L199 : the suitable oxygen saturation range for trout would be worth to be reminded and the temperature during the hypoxia challenge should be reported as well. From Lagarde 2023b, it looks like temperature could change but then just precise the temperature range, underscoring it remained in very suitable values for this species

L229-230 :

-about the same six individuals : in the previous individual test, fish could have learned how to behave in this new environment which is very similar in group test. Don't you think this could bias their behaviour in the group test ? and so to add a witness group composed of fish not having experienced individual test before and compare its behaviour to groups having experienced would be a guarantee that results are not biased ?

- why evaluating behaviour alone at a fixed temperature and then grouping and increasing T ? by changing two factors at the same time, it is difficult to deconvolute. Fish behaviour can be different alone or in group. There is perhaps a reference supporting this method.

-you talk of moderate heat stress : is it really moderate and not acute ? However Fig2 shows temperature rising up to 23.0°C very similarly to 23.2°C in acute test referred to line 197

L269 : this section 1.5 about behaviour in group only tests acute hyperthermia stress ; we expect here hypoxia test to be described as well.

L291 : just for sharing, about the behavioural variables, I think in the future time spent and distance travelled close to the sides of the aquarium could be interesting as well to characterize shy/bold individuals.

L297-298 and 304 : about group variables, it is not clear wether they are calculated from a barycentre over all fish positions or an average of the metrics calculated for each individual, especially if a single fish can not be tracked

L309 : you only refer to temperature increase while I also expected some experiments on hypoxia stress also

L323 : for your information, it seems that some software can track individuals in all cases : idTracker for example. In case you are interested in: `https://www.idtracker.es/home`: Tracking individuals in a group by automatic identification of unmarked animals. A. Pérez-Escudero, J. Vicente-Page, R.C. Hinz, S. Arganda, G.G. de Polavieja Nature Methods 11(7):743-748 (2014)

L366 : Between the first two sentences of section 1.8, insert a transition sentence like : "Pearson correlation coefficients between lsmeans of lines for behavioural variables and for resistance to stress were calculated."

L369 : I would not say « slightly » : this is as much as 31% (from 11.6 to 15.2 g). Especially, weight can play on oxygen needs; there are also links between size (and thus weight) and temperature.

L379-385 : not sure this paragraph is useful as it repeats figure and table caption; references to these figures and tables are made below and are enough. I suggest to delete this paragraph.

L396-399 : in the description of model effects in general, you could lighten the text and make it more "ecological" this way for example on this paragraph: « In individual test, body weight inflenced only maximal acceleration by increasing it by 2.5 cm.s-2 per gram, i.e. bigger fish had higher acceleration, all things being equal (Table 2). Body weight had no effect in group test. »

L404-406 : suppress the sentence « The effect of time on these behaviour variables is presented in Fig. S2 and Table S4 » and just replace it by a reference to « (Fig. S2 and Table S4) » after « [...] tended to decrease ».

L421 : I would replace « activity levels » by « their movement characteristics » which is more general since %MOV did not differ between lines. %MOV is related to activity.

L433-434 : another example of more ecological description of results ; this sentence could be rephrased this way : « In the individual test, line effect and time were independent whatever the behaviour variable was (Table 2). »

L447 : please add a coma after « For other two »

L448 : please add a space after N38h

L472-473 : « Fish staying longer in the risky zone therefore tended to swim longer distances than others. » : I am not sure to well understand this conclusion. PCA just tells that %RISK is uncorrelated to "activity" variables.

L479 : replace dependant with dependent

L518 : Looking at Table 3 and Figure 6, I probably misunderstood something :

-are results on individual tests on acute stress, corresponding to the first experiments you described in section 1.2, described somewhere ? It looks like they are used in Fig6, but they should be firstly described at the beginning of results section.

- are behavioural experiments and results on acute hypoxia described somewhere ?

To clarify the whole experimental setup, I suggest to add a schematic in the material and methods section synthesizing individual and group tests, both on behaviour variables and on resistances (from section 1.3 and 1.2) and both on hyperthermia and hypoxia. Figure 2 is probably a good basis to complete.

L542 : in your discussion, avoid reference to tables and figures and the use of variables' names; it leads to a repeat of the results section.

L545-547 : this looks more to an introduction than a discussion ; rather introduce main results, eg : for some lines, links could been established between behaviour variables and resistance to acute hyperthermia or hypoxia. Behavioural differences were found in isogenic lines and some of them could be linked with resistance to .... Or a synthesis of the main results since they are quite rich and complex, so that you can then discuss them with the help of other study results.

L553 : replace was with were

L557 : replace « exploratory behaviour »with « exploration » to avoid repetition of behaviour

L560 : about behavioural differences between isogenic lines, I suggest to discuss the weight differences since they are significant. As soon as L193, we wonder about weight since it can play on oxygen needs; there are also links between size (and thus weight) and temperature.

L562 and L565 : replace « our own » with « ours »

L568 : replace « apart » with « ago »

L571 : in addition to the duration of the group test, you could discuss the influence of the group compared to individual alone. I wonder about this

L579-584 : this is typically too close to a result section fo being in the discussion and needs to be more deeply discussed rather than rephrasing results

L589 : replace have with has

L590 : use a term like movement metrics or something like that instead of « ACC_MAX, DIST_TRAV and VEL_MAX » which is close to the result section

L595 : delete with

L607-614 : I suggest to move this paragraph into the introduction (see my comments above) where we classically cense works linked to the one detailed in the paper to highlight the interest of your work compared to what has already been done.

L615-619 : This is a repeat of detailed results, and this sentence can be deleted ; I however do not understand why you start with « In the individual behaviour ». The next sentence would be sufficient with very few changes : « Acute hyperthermia resistance appeared to be associated with a low level of activity and acute hypoxia resistance with a high level of activity. »

More importantly, I did not see behavioural experiments and/or results at the individual level in what is written before. I probably misunderstood something or it is not clear.

L620 and 623-624: I did not see detailed methods/results on behavioural experiments in acute hypoxia conditions. Again, I probably misunderstood something or it is not clear.

L630 : when you say « thus can resist longer acute hyperthermia. », does this mean that resisting to hyperthermia consumes energy ? I suggest you give a reference for that here, perhaps some physiological explanation from those you gave in introduction.

L633 : What you name panick (perhaps prefer enhanced activity ?) is interpreted as a way to find a refuge area (more rapid movements to efficiently explore greater areas) when a fish meets bad conditions (see some references in Westrelin, S., Bouletreau, S., and Santoul, F. 2022. European catfish Silurus glanis behaviour in response to a strong summer hypoxic event in a shallow lake. Aquat. Ecol. 56(4): 1127–1142. doi:10.1007/s10452-022-09952-y and also some explanations on the interest of different kinds of strategies). Strategies of fishes selected by evolution to face hypoxia can differ and this could lead to behaviours inadapted in artificial conditions. For example, we could imagine that, in nature, trout has better to try to actively search for a refuge area in case of hypoxic conditions rather than limits its movements hoping for better conditions to appear. In tanks where there is no refuge area, the problem is "artificial " and completely different. You could discuss this. I also wonder how fast some fish that have explored their new environment in the individual test before being gathered for a stressful group test have learned about this environment and thus could adapt their behaviour according to, what would influence their movements. Here some references on fish learning, including spatial one :

Kieffer, J.D. and P.W. Colgan, The role of learning in fish behaviour. Reviews in Fish Biology and Fisheries, 1992. 2(2)

Dunlop, R., S. Millsopp, and P. Laming, Avoidance learning in goldfish (Carassius auratus) and trout (Oncorhynchus mykiss) and implications for pain perception. Applied Animal Behaviour Science, 2006. 97(2)

L639-641 : you could please explicit what you mean.

L649 : replace of with in

L696-698 : I don't see how these relationships help to understand the mechanisms, especially if you think about physiological mechanisms. I would delete this sentence.

(Apologies in advance for any potential typos, this peer-review turned out rather long and I might have missed some of my own errors)Answers to questions

Title and abstract

- Does the title clearly reflect the content of the article? **Yes**

- Does the abstract present the main findings of the study? **Yes**

Introduction

- Are the research questions/hypotheses/predictions clearly presented? **No** — the research question is stated in general terms at Line 135, but no specific hypotheses or predictions are written. The following paragraph lists the traits they measured, but not how they expected them to interact. I can also can't find any question or hypothesis for the choice to use both group test and individual tests, as well as the temperature ramping during the group test.

- Does the introduction build on relevant research in the field? **No** — Mentions of similar studies are missing from the introduction (but are present in the discussion) (e.g. Van Raaij et al 2016 and Campos, Val & Almeida-Val 2018)

Materials and methods

- Are the methods and analyses sufficiently detailed to allow replication by other researchers? - **No** - See details below. Some important information is missing from the practical methods as well as statistical methods. Details below.

- Are the methods and statistical analyses appropriate and well described? **No** - Some information is missing from methods (details below), and I'm not sure about the validity of some statistical methods (especially that of controlling for body weight, details below).

Results

- In the case of negative results, is there a statistical power analysis (or an adequate Bayesian analysis or equivalence testing)? **Not applicable**

- Are the results described and interpreted correctly? **Yes**

Discussion

- Have the authors appropriately emphasized the strengths and limitations of their study/theory/methods/argument? **Yes**

- Are the conclusions adequately supported by the results (without overstating the implications of the findings)? **Yes**Summary of the study

In this study, the authors have looked for genetic associations between behaviour and tolerance to both high temperatures (hyperthermia) and low oxygen conditions (hypoxia).

Their case for understanding this is 1) a need to mechanistically understand these tolerance traits and 2) understand how selection for tolerance traits may affect behaviour, and 3) that behavioural traits might be used to test for tolerance to temperature and hypoxia (instead of directly exposing fish to tests for these traits).

The authors studied this question by assessing these traits in six "isogenic lines" of rainbow trout (eight genetically distinct cohorts of genetically identical individuals), and then checking if between-line variation in behaviour correlated with between-line variation in either tolerance (resistance) trait.

Some correlations between tolerance traits and behavioural traits were found. For example, lines with high tolerance to high temperatures displayed a lower swimming activity, and lines with high tolerance to hypoxia displayed higher swimming activity.**Report**

The authors have used a novel and interesting study system to look for genetic links between physiology and behaviour, and give findings that can be of high value for research on such associations especially with regards to environmental issues. However, I'm not fully confident in the findings presented here due to some statistical issues (See section on critical issues below).

I am also a little sceptical of the potential for using behavioural assays as a proxy for tolerance traits to temperature and hypoxia. The authors do find a correlation between these traits, but these traits are not

measured in the same individuals (-though they are genetically identical).   This makes it hard to estimate how predictive these behavioural measures are. However, this limit is clearly stated in the discussion and conclusion. I think the main novel finding of this study is the between-line correlation between these traits, giving a good indication that there is some genetic link between these traits, and showing this in a novel way.

A significant portion of this study is the use of a group test for behaviour (in addition to individual tests), together with a temperature increase during the group test. However, it is not stated in the introduction why this test was done. What was the expected benefit of doing a group test in addition to the individual test? -and also, why include the temperature increase? In the discussion, the temperature increase is only mentioned as something confounding the test's time-factor. The group test and its results are thoroughly discussed in the discussion, but its significance in relation to the individual tests is not described, so it is not clear to me what extra benefit it gives.

The practical methods are explained fairly clearly in the methods, but a few important details are missing. Details below.

A lot is missing from the descriptions of the statistical methods. In particular , I am missing information on what models were used, exactly how many models were compared in the model selection process, what method was used for determining statistical significance, and some missing p-values (or similar measures). Details below.

It would benefit the manuscript to have a round of careful editing for language, especially focusing on sentence structure, choice of terminology, and consistency in choice of terminology. I've detailed a few issues at the end of the report, but this is not an exhaustive list. **Critical issues**

1)

One aspect of the statistical methods makes me worry about the validity of this study's findings. To test for correlations in between variation in behaviour with variation in tolerance to hyperthermia and hypoxia, the authors needed to get estimates of each line's (the six isogenic groups of fish) value for each trait. To get line-specific values for each trait, a linear model was fitted for each trait while using line as a categorical factor in this model (among other factors), and then using those models to estimate least-square means (essentially model predictions) of each trait for each line. In order to control for body-weight differences between lines, these least-square means were calculated for fish at 15.2 grams (the whole study-population mean). However, since the different isogenic lines differed in body weight, the estimated trait values for each line do not necessarily represent the actual trait value for that line (since it is estimated for a fish of a body-weight that is not representative of the line's mean). Overall, I'm not sure I really understand the need to control for body weight. The goal of the study was to look for between-line differences in tolerance to hyperthermia and hypoxia; If they found a difference and also that this difference was associated with a body-weight difference, then that would be an interesting finding in itself and not something they would need to control for.

-to put this in another way: The lines differed significantly in body weight (line and body-weight is correlated), yet effects of both line and body-weight were included the same models (models for resistance traits). This is especially problematic knowing that body-weight was also associated with the resistance-traits which were being modelled.

It would help if the authors could:
- Better explain the need to control for body weight and how this relates to their study question.
- If controlling for body weight, give more information about the relationship between isogenic line, body weight and hyperthermia/hypoxia resistance to show that this does not produce any false positive results (and if results differ after controlling for body weight, discuss why).

2)

When doing model selection for behavioural traits, exactly how many -and what models were tested?

Line 336 reads: "with vs. without time and/or line and/or body weight and/or the different interactions between these variables"

That is approximately 13 different possible combinations of effects and interactions, and then there is the inclusion/exclusion of weighted terms (x2) then the use of AR1, ARMA (or none) (x3) resulting in a total of 78 different models. Multiply that by the number of traits (x10) gives 780 different models. Is that right?

The following would help:

- A description in the methods of exactly how many models were tested/compared, and exactly which combinations were tested.

3)

Line 379 says that table 2 shows the significance of model effects, but table 2 just contains a list of which models had the "best structure" for each behaviour variable. There are no significance parameters in this table (like p or F values or confidence intervals etc). This makes me wonder if this table just lists which models (of the many which were compared) had the lowest AIC, but then on some models it says "no significant effect", which seems to indicate that indeed some significance testing was involved?

The following would help:

- A thorough description of what table 2 actually shows.
- A description in the methods of how significance testing was done for these models, and how this relates to table 2
- Specifying exactly which models were used in the end (not just which fixed effects+interactions, but also the usage of terms-weighing and correction for autocorrelation)
- Ideally, the results should also include a table of the models used, what the effect sizes for the different parameters were, and some measure of significance for these (p-values, confidence intervals, or similar)

4)

Why are no p-values (or other measures of statistical significance) presented for the correlations? The main result of this paper hinges on these correlations, so it seems like an odd exclusion, and it would be good if the authors could elaborate on it.**Missing from methods**

- How large were the tanks used to measure hyperthermia tolerance?
- How large were the tanks used to measure hyperoxia tolerance?
- How was temperature increased in the hyperthermia tolerance test?
- Was oxygen being replenished in the test-tank during the hyperthermia tolerance test? -if not, was it being monitored?
- How was the temperature increase-rate ensured in the hyperthermia tolerance test?
- How was temperature measured (what tool and where in the tank) in the hyperthermia test?
- How were oxygen-levels manipulated in the hyperoxia test?
- How were oxygen levels measured in the hyperoxia test (what tool and where in the tank) ?
- Were fish exposed to air at any point during transfer between tanks? Describe how fish were transported between tanks, test-setups, in a way that lets the reader know if they were exposed to air or not.
- How did you determine if a model effect was statistically significant or not? For mixed-effect models this requires some special packages. Which did you use? What was your chosen level of significance (e.g. $P > 0.05$? -or other?)
- Line 394 mentions an ANOVA analysis not mentioned in methods. Missing info on which model is used, r-package, and type of statistical test used.
- Line 260-261: Missing description of door and remote opening mechanisms in more detail. Are there any pictures of the test setup? How big was the door? How did it open? (sliding? hinged? etc ...)
- Line 203: How was loss of equilibrium defined? (e.g. "unable to maintain upright swimming for X seconds" ?)
- Line 205: How were fish euthanized?
- Line 217-218: Were any random effects included in these models? (e.g. triplicate?)
- Line 219: What is the measure of significance here? $P < 0.05$?
- Line 342 Where any r-packages used for AR1 or ARMA(1,1) accounting?

Other issues / questions

L110-111: Are there any examples of how selecting for poorly understood traits could be ineffective or harmful?

L314: Why were individual-level variables averaged over groups for the individual behaviour tests? I understand it for the group tests but not the individual ones. This seems like an odd exclusion of variation which could have been included in your models.

L320: I don't understand this line of reasoning. Here it reads that behavioural variables need to be corrected for fish body weight to disentangle the genetic and body weight effects, but I assume body weight is also affected by genetics? By correcting for body weight, is the data really representative of the genotypes still?

Discussion: The usage of isogenic lines is very interesting, but also very novel within this field, and most readers are likely not going to be familiar with this. It would be good if the authors could include some text in the discussion on how they think this choice of study system could potentially influence the results, and if there is anything else the reader should keep in mind when interpreting the result from such a system.**Minor language / text / structure issues**

L36: Unclear what is meant by "systematically" different individuals

L41: Confused by "related" , does this sentence claim that these traits actually are related, or if they were tested for a relation?

L84-88: The first sentence makes it sound like these are the only methods used for measuring resistance traits. Including an "among others" or similar could fix this.

L90 and 561: the phrase "genetic determinism" carries a connotation to the "nature vs nurture" debate, while what I think you are actually referring to here is closer to "a genetic component" or "genetic influence".

L96: "observed genetic variability" implies you have actually observed variation at the genetic level (i.e. observed variation in the genetic code, allele frequencies, and such); What I think you are actually referring to here is "... about the mechanisms for this genetic influence/component", or similar.

L115: Not clear what is meant by "extensive behavioural repertoire". This sounds like "the rainbow trout had a lot of different behaviours", but I don't think that is what you mean? (-also not clear how that would suggest that behaviour may be linked to resistance phenotypes).

L118-121: Is this the correct interpretation of this sentence? Consider splitting it up.
"A strong interest in identifying mechanisms (i.e. those underlying resistance to acute hyperthermia and hypoxia)(-and whose phenotyping would be reasonable intrusive, such as behavioural ones) would be to use them (the mechanisms) as predictors to replace the current classical phenotyping methods"

L118-121: Also, in the context of measuring behavioural traits in lieu of resistance traits, I don't think you can call those behavioural traitn "mechanisms", but rather "proxy traits" or something similar.

L123: This sentence mentions "several" drawbacks, but the following paragraph only lists two.

L141: "were related to" is ambiguous, it sounds like you either a) **tested if** beh. vars were related to the following list of traits, b) you **found that** they were related to the following list of traits, or c) that you **measured traits which** were related to the following list of traits.

L154-154: Give the full name of abbreviated institutions.

L195: Here you first write that each replicate had 50 fish, then that each replicate had 300 fish (make the distinction between replicate and replicate types/names)

L187 and 224: is "experiments" the right word here? (-instead of "test" ?)

L236-237: What is "temperature-equilibrated water"? -equilibrated to what?

L244: Confusion -> confounding

L251, 255, 278, : Remove square-symbols in dimensions

L251: What is system water?

L292 (and other following): Remove "." from equations (replace with space)

L304: says you measured group variables, but you measured individual variables and averaged them over groups.

Line 393: overall -> mean

Line 408: evolutions -> changes (or similar)

Line 607-614: This paragraph first sounds like you are describing your own results, but then instead lists findings from other studies?