Submission date. 21/09/2025

Associate editor's decision after peer review (19/10/2025).
Dear Mr. Pinto:
Manuscript ID NI-2025-0160 entitled "Sex differences in landmark-based spatial learning in the sailfin tetra Crenuchus spilurus" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.
The reviewer(s) have recommended publication, but also suggest some revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.
To revise your manuscript, log into https://mc04.manuscriptcentral.com/ni-scielo and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.
You may also click the below link to start the revision process (or continue the process if you have already started your revision) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.
*** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***
https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=883929ee83cd47e5b0b95248582dfaad
You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text. Once the revised manuscript is prepared, upload BOTH versions (the tracked and a clean) and submit them through your Author Center.
When submitting your revised manuscript, you will be able to respond to the comments in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please reply POINT TO POINT all the suggestions of the reviewers and be as specific as possible in your response to the comments.
IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.
Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your revised manuscript should be submitted before 19-Dec-2025. If it is not possible for you to submit your revision by this date, we may have to consider your paper as a new submission.
Once again, thank you for submitting your manuscript to the Neotropical Ichthyology and I look forward to receiving your revision.
Sincerely,
Dr. Eliane Gonçalves de Freitas
Associate Editor, Neotropical Ichthyology
eliane.g.freitas@unesp.br

Anonymous reviewer #1
Recommendation. Minor Revision
Comments. This is a nice paper addressing cognitive sex differences in a new species of fish. I like that the biology of the species allows for a precise hypothesis testing. It is overall a good experimental work, I just made a few suggestions on points that for me are inaccurate or could be improved.

Comments
“in navigational performance.”
I am not an expert of spatial cognition but this terminology sounds odd to me. I would stick in the whole text with spatial learning.
“Restricting water column height functions as a stimulus for fish ”
Happy to see that this worked well for your species!
“(four females of C. spilurus)”
I think that the fact that the reward consisted of female fish may have determined sex difference in motivation. This should probably be discussed.
“To stimulate entry into the test area, we gently inserted a plastic brick at the opposite end of the shelter, directing the fish toward the initial corridor.”
This protocol is not clear to me. Were you pushing the fish somehow? Please provide details.
“route correction”
This variable is not explained, it is just mentioned twice, in the abstract and in a title. I think I understood it (and I liked very much the idea of testing the correction) but it should be explicitly described.
“although the interaction for females approached the significance threshold (Fig. 3B).”
So probably females learned but males did not?
“Crenuchus spilurus can use visual landmarks”
“Although both sexes showed evidence of learning across trials,”
The accuracy measure is usually more robust that then time measure, which can be affected also by motivation, habituation, etc. And according to the accuracy measure males did not learn…
“Females were more efficient in adapting to spatial changes in the task, particularly when it was necessary to correct choices based on new visual information.”
I would say here that females may be more flexible and inhibit better wrong choices. Please note that this is relatively known effect in other fish species. I think this should be mentioned and discussed with opportune references. I suggest two original papers and one review on this effect:
Lucon-Xiccato, T., & Bisazza, A. (2014). Discrimination reversal learning reveals greater female behavioural flexibility in guppies. Biology Letters, 10(6), 20140206.
Lucon-Xiccato, T., Bisazza, A., & Bertolucci, C. (2020). Guppies show sex and individual differences in the ability to inhibit behaviour. Animal Cognition, 23(3), 535-543.
Lucon-Xiccato, T. (2022). The contribution of executive functions to sex differences in animal cognition. Neuroscience & Biobehavioral Reviews, 138, 104705.
There are a couple of additional studies on sex differences in spatial abilities in fish. Please check if they should be add to the reference list:
Miletto Petrazzini, M. E., Bisazza, A., Agrillo, C., & Lucon-Xiccato, T. (2017). Sex differences in discrimination reversal learning in the guppy. Animal Cognition, 20(6), 1081-1091.
Carbia, P. S., & Brown, C. (2020). Seasonal variation of sexually dimorphic spatial learning implicates mating system in the intertidal Cocos Frillgoby (Bathygobius cocosensis). Animal cognition, 23(4), 621-628.
Fabre, N., García-Galea, E., & Vinyoles, D. (2014). Spatial learning based on visual landmarks in the freshwater blenny Salaria fluviatilis (Asso, 1801). Learning and Motivation, 48, 47-54.
Jacobs (1996) proposed the “gender- dispersal hypothesis,”
Gender is more a cultural things, in contrast with sex. This seem an hypotheses to explain human sex differences, are we sure it applies to fish that do not have cultural gender differences as we intend them?

Overall this is a nice contribution to the literature of cognitive sex differences in fish, I am looking forward to see it published!
Best,
Tyrone Lucon-Xiccato

Anonymous reviewer #2
Recommendation. Major Revision
Comments. General consideration
I found this is a very interesting work. I have some comments and suggestions for the authors. My major concern is that results section is quite reduced and shows little information. I suggest this needs to be improved in a great way, adding more information, more figures, tables... I suggest including more variables analyzed. Authors also can consider adding videos as part of results or supplementary material, in order to enrich the importance of the study. In the discussion section, I make specific suggestions refering to this. I also suggest a general revision of the language.
Line number in the PDF restarts in each page, so I will try to refer otherwise.
Introduction
-" Animals use multiple sensory cues as sources of directional information to orient themselves and navigate (Akre, Johnsen, 2014; Green et al., 2020), in addition to integrating past experiences into spatial
behavior, which leads to adaptation and learning processes (Kotrschal, Taborsky, 2010)." : This sentence needs language edition.
- "Spatial orientation in environments where sensory cues are constantly changing poses a
challenge that favors the evolution of navigational abilities requiring attention to signal variation, risk assessment, memory of stimuli, and inferences from incomplete information,
thereby enabling adaptive behavior in different contexts": also this sentence needs language edition.
-Sentence starting with: "In terms of spatial orientation,…": This sentence is not clear enough. What does it mean that navigation strategies can be categorized at behavioral and neural levels...? With the next sentences one can understand the meaning, but the first sentence needs to be clear enough.
-Sentence starting with: "This behavioral requirement is correlated with increased volume in brain regions associated with spatial…" : This sentence is also unclear. What does it mean that males "who do not share this ecological demand in those species"? Please edit this sentence and correct language so that your idea is clear for the reader.
-Sentence starting with: "Conversely, in territorial species…": In which vertebrate group is this evidence? Fish? Birds, mammals? Or in different species of several groups?
-Sentence starting with: "In this study, we investigated potential sex differences in the spatial abilities of the sailfin tetra Crenuchus spilurus…": I would suggest first to give information on the species, and then stating which is the aim of this study.
Also, if moved somewhere later on in this same paragraph, this sentence can be better expressed as: "The aim of this study is to assess potential sex differences in the spatial abilities of the sailfin tetra C. spilurus."
-Since this is an original manuscript and not a review, citing Figures in the introduction should be avoided. Figures should be included in M&M or Results, but not in the introduction, since the introduction refers to previous background leading to the aim and hypothesis of the present study.
-Regarding the figure, it is not clear to me... Is Fig. 1A-B also part of Pires et al., 2016? Or are these photos in this study for the first time? If they are in this study for the first time, please conider including them in results, with the corresponding explanation in a paragraph in that section.

-When authors say: “The C. spilurus population used in this study…”. Are other populations in other environments? With higher turbidity ? Or usually Amazonian streams are similar to the sites chosen here...?
-Sentence starting with: “Thus, mate choice is mutual and based on visual cues…” From the way it is written, somehow it gives the idea that mate choice can be exclusively based on visual cues (even if you don't use the term). I understand this is not your intention. Is there any evidence on other sensitive cues (e.g. olfaction)? Even if there is no evidence in other sensitive cues, mate choice is also probably related to pheromones, mechanosensory cues, and/or others... Please consider editing this sentence.
-In the sentence starting with: “Males are territorial…” you refer to paternal behavior. How is paternal care in this species? Even if this is not the main topic in this study, maybe it is worth mentioning how the male takes care of the eggs/larvae, which behavior he performs, if there are different paternal stages, etc.
-By the end of the introduction authors state the hypothesis, which is very clear. Maybe this can be a good point to include here the aim of this study (instead of in the first sentence of the paragraph, and immediately followed by the hypothesis).
Materials and Methods:
- Housing: For how long were fish housed in the laboratory before performing the experiments?
-Why were males and females housed separately? Don't you think is more "natural" (or at least more similar to their biological condition) if animals of both sexes were housed together?
-Authors explain that aggressive interactions were rarely observed in the holding tanks. Do you think if males and females were housed together, you would observe higher male aggressive interactions?
-Regarding the lack of dissolved organic carbon. Can authors please explain the importance on this? Just as a reply, not in the main text. Has this been established by this method in previous manuscripts of your group, or others?
-During which months were experiments performed? Is this 12:12 hs cycle constant throughout all year long? Or is there a natural shift in sunrise and sunset? These questions are related to the question I ask later on, regarding at which time were experiments performed.
-Apparatus: you use plastic bricks, why did you choose green?
-Regarding PVC tube as a reward: Is the PVC tube a reward with equal salience for males and females?
Considering male aggression, and considering that parental care in this species is performed by the male and not the female, is it possible that PVC tubes are "more positive" reward for males, and that for females PVC may represent a more neutral reward?
To avoid this, why did you prefer using a PVC reward instead of just food?
-Regarding four females as a reward I have a similar question. In this case, this social stimuli is always four females (for male and for female focal fish). So, if focal fish is a male, then this social stimuli is definitely a positive social stimuli, more like a positive reward. But if the focal fish is a female, finding four other female is also probably positive (because for fish it is less stressing being with others than in isolation), but probably with a different salience than in the case of focal males (for example during mate choice in this species, are there competitions among females to be chosen by a reproductive male?).
Do you think this probable difference in salience of reward (in PVC tube and also in these four females) can bring sex differences in results observed after the protocol?
-Procedure: At what time were experiments performed?
-Where is the fish while the spatial task was reconfigured? Isn't it stressful to remain in the apparatus, while the spatial task was being reconfigured? Even if there might be visual isolation, vibrations don't you think vibrations might be stresful?

-Sentence starting with: " When correct choices…". This sentence needs edition.
-When authors explain that the procedure was repeated 5 times: In the same day? Can you please specify the time window in which experiments were performed? It seems like each experiment lasted around 6 hours?
Results:
-Results section is quite reduced and shows very little information. I suggest this needs to be enriched in a great way, adding more information, more figures, tables... I suggest including more variables analyzed. Authors also can consider adding videos as part of results, in order to enrich the importance of the study. In the discussion section, I make specific suggestions refering to this.
- Time to solve the task: this is interesting. Maybe it would be better to first refer to the lack of significant interaction, and then explaining the statistical significance for the test sequence and then for the sex?
Discussion:
- I understand the first paragraph intends to summarize the most important findings of this study, which is a good idea, gives strength to the discussion and helps organizing ideas. However if this is the case, I suggest authors should say how is it that results here suggest that "males and females process and integrate spatial information" differently. In particular, which variables analyzed here are different between sexes? Do males take more time to solve the task? Do they show reduced accuracy? Whichever your findings were, I suggest authors should make it clear briefly here in the first paragraph in which variables males were different from females, and in which direction (higher or lower). And then, start discussing in the following paragraphs.
- In the paragraph starting with: "Although both sexes showed …" you refer to female mobility. "In
C. spilurus, this hypothesis is supported by direct behavioral observations (during snorkeling
sessions) of greater female mobility in the natural environment". This is a very interesting hypothesis. However, I suggest that this is something important to check in your experiments here.
I understand that authors suggest that the fact that females completing the task faster might be related to them having greater mobility (from evidence in nature). I suggest authors should seriously considering assessing female (and male) mobility in these videos, in these experiments from this study. This will greately enrich the findings of this study with more quantified measures and more figures/graphs. Authors can track individual animals and quantify distance moved per frame and speed. THis way, by comparing male and female distance moved per frame, it will really improve the impact of this study. If authors don't have access to a software or tracker, maybe please consider ToxTrac (https://sourceforge.net/projects/toxtrac/) which is a free software.
-Later on authors state: "This combination of hesitation and efficient learning may reflect the "asset protection" principle (Clark, 1994), according to which individuals with high reproductive value adopt more conservative, but not necessarily less effective, strategies in challenging contexts." This is an interesting hypothesis. From here, I understand author suggest that males of this species have " higher reproductive value" than females of this species. Is this correct? Please explain this rationale better. Why do you consider females in this species have lower reproductive value than males?
Also, going deeper in this rationale. Females also might show a degree in their reproductive status (prespawning, postspawning, etc). To further explore this hypothesis, authors might also consider including GSI from females, as part of results (maybe a new analysis and figure?).
For example, if I understood the authors intentions in this sentence well, prespawning females can adopt a more conservative strategy (longer resolution times?) than postspawning females...?

-Later on, authors state: “Thus, the differences observed in this study can be understood as the outcome of natural selection acting on distinct cognitive profiles adapted to the specific demands of each sex within its ecological niche.” It is a very interesting hypothesis, but I suggest it should be stated in a more conditional way.
-“ Females demonstrate greater flexibility and accuracy in spatial tasks based on predominantly
allocentric navigation,…” . I suggest authors should state this more conditional: "Females demonstrate greater flexibility and accuracy in spatial tasks suggesting a predominantly allocentric navigation, whereas males appear .... " etc.
-“… whereas males appear to rely primarily on egocentric navigation and explore the environment using more cautious and conservative strategies”. Same here. While this is definitely a very interesting hypothesis, it doesn't seem that the fact that males take longer to learn necessarily means that they are more cautious and conservative. They just take longer and are less accurate, and based on previous evidence and hypothesis, one can suggest that this could be related to a possible cautious strategy (or not, but it is definitely something worth speculating about, for sure). So, I suggest using a more conditional phrase here as well: "... whereas the fact that males show lower accuracy and longer resolution times can suggest that they may rely primarily on..." etc.
-“These findings support the hypothesis that spatial cognition in fishes is adaptive, sensitive to ecological and social context, and shaped by distinct selective pressures between sexes.” This looks somehow confusing to me... In the introduction, authors say the hypothesis of this work: " we hypothesized that male C. spilurus would display greater spatial navigational ability than females." But here, they include a much broader hypothesis, as a larger framework. Which is very interesting as well, but it is not exactly the one presented in the introduction.
Also, probably evidence from results in this study is not enough to say that this study supports the hypothesis that spatial cognition in fish is adaptative, sensitive to ecological and social context. Experiments here do not test this broad hypothesis.
One can discuss results from these experiments by contrasting with evidence in the nature, and in ecological or social context etc, and discuss a possible ecological or evolutionary meaning of this. But I don't see clearly how the findings here support this very broad hypothesis. I suggest authors should consider narrowing the hypothesis and conclusions of this study, and suggesting future experiments to study whether " spatial cognition in this species is adaptative, sensitive to ecological and social context."
-Figure legend 3: Figures need to have a general title, before jumping into what's A and what's B.
-Figure legend 3: I suggest instead of showing just the mean values and SEM, authors should include for each time point all individual values as dots, then putting them together as a bar and with a horizontal line showing the mean value. Or maybe authors can figure out a better way, but I think showing individual values of each experiment is important, rather than just showing the mean and SEM.
-Figure legend 3: Please consider adding significance to the graph, with asterisks or letters, or somehow. Also, information on which statistical test was perform might also be included in the legend.
-Figure legend 4: Also in this graph, please consider adding individual data as dots showing each experiment, not just the mean value as a dot. Also please include asterisks or letters in case there is significance, and also please add which statistical test was performed in this case in the legend.

Author’s Rebuttal Letter (06/12/2025).
Dear editors,
We were pleased to receive the reviewers’ evaluation of our manuscript titled “Sex differences in landmark-based spatial learning in the sailfin tetra Crenuchus spilurus”

(manuscript number: NI-2025-0160). We carefully considered all the comments and suggestions provided and have outlined below the revisions made in response to each point. We have addressed every comment individually. The reviewers' insights were highly valuable, and we sincerely appreciate their constructive feedback on our revised submission.

Best regards,

MSc Kalebe da Silva Pinto, on behalf of all the authors
Graduate Program in Biology
Instituto Nacional de Pesquisas da Amazônia – INPA.MCTI, Brazil

Point-by-point replies:

Reviewer comments:

Reviewer #1:

This is a nice paper addressing cognitive sex differences in a new species of fish. I like that the biology of the species allows for a precise hypothesis testing. It is overall a good experimental work, I just made a few suggestions on points that for me are inaccurate or could be improved.

Comments

"in navigational performance."

I am not an expert of spatial cognition but this terminology sounds odd to me. I would stick in the whole text with spatial learning.

R: Thank you for your comment. We agree that "spatial learning" is a more appropriate term in this context. Accordingly, we replaced "navigational performance" and "cognitive performance" with "spatial learning" throughout the text where applicable.

"Restricting water column height functions as a stimulus for fish "

Happy to see that this worked well for your species!

R: In a previous study (Pinto et al., 2021), this same technique proved to be useful, which is why we applied it again in the present work. We emphasize that this methodology was inspired by other studies previously cited in the manuscript.

"(four females of C. spilurus)"

I think that the fact that the reward consisted of female fish may have determined sex difference in motivation. This should probably be discussed.

R: In this species, females are indeed effective social motivators for males, as males are typically active in seeking and courting potential reproductive partners. In contrast, females are more social and tend to remain in shoals with other females. Using males as a reward could have been counterproductive: male subjects might perceive other males as competitors, which could induce aggressive or avoidance behavior, while female subjects might not respond positively either, as they may not be motivated to reproduce or may not find the presented male attractive.

However, to make our rationale for using only females as a reward clearer, we have added a brief explanation in the Methodology section.

"To stimulate entry into the test area, we gently inserted a plastic brick at the opposite end of the shelter, directing the fish toward the initial corridor."

This protocol is not clear to me. Were you pushing the fish somehow? Please provide details.

R: There was a shelter in the habituation sector connected to the maze (see Figure 2). The placement of the plastic brick did not involve pushing or forcing the fish. Instead, it gently blocked the opposite end of the shelter, encouraging the fish to swim slowly toward the task entrance. Once at the task entrance, individuals were completely free to proceed into the next sectors of the task at their own pace.

"route correction"

This variable is not explained, it is just mentioned twice, in the abstract and in a title. I think I understood it (and I liked very much the idea of testing the correction) but it should be explicitly described.

R: We agree that the variable route correction was insufficiently defined in the original manuscript. We have now clarified this concept by adding a detailed description in both the Procedure and Statistical Analyses sections, explicitly explaining how route correction was operationalized and quantified in our experiment.

“although the interaction for females approached the significance threshold (Fig. 3B).”

So probably females learned but males did not?

R: Thank you for this question. Although the interaction for females approached the significance threshold ($p = 0.055$), it did not reach statistical significance as defined in our statistical procedures. Therefore, we cannot conclude that females definitively learned the task. However, this near-significant interaction suggests a possible trend toward improved performance across trials in females. We have revised the Results to report this trend explicitly and have clarified in the Discussion that this pattern represents suggestive, but not conclusive, evidence of learning in females.

“Crenuchus spilurus can use visual landmarks”

“Although both sexes showed evidence of learning across trials,”

The accuracy measure is usually more robust that then time measure, which can be affected also by motivation, habituation, etc. And according to the accuracy measure males did not learn…

R: Although both sexes reduced their resolution time across trials, only females showed an increase in accuracy. Therefore, we have revised the manuscript to clarify that learning based on the visual landmark was evident only in females. We also modified sentences that previously implied that both sexes learned the task, and we now emphasize the discrepancy between the two performance measures.

“Females were more efficient in adapting to spatial changes in the task, particularly when it was necessary to correct choices based on new visual information.”

I would say here that females may be more flexible and inhibit better wrong choices. Please note that this is relatively known effect in other fish species. I think this should be mentioned and discussed with opportune references. I suggest two original papers and one review on this effect:

Lucon-Xiccato, T., & Bisazza, A. (2014). Discrimination reversal learning reveals greater female behavioural flexibility in guppies. Biology Letters, 10(6), 20140206.

Lucon-Xiccato, T., Bisazza, A., & Bertolucci, C. (2020). Guppies show sex and individual differences in the ability to inhibit behaviour. Animal Cognition, 23(3), 535-543.

Lucon-Xiccato, T. (2022). The contribution of executive functions to sex differences in animal cognition. Neuroscience & Biobehavioral Reviews, 138, 104705.

There are a couple of additional studies on sex differences in spatial abilities in fish. Please check if they should be add to the reference list:

Miletto Petrazzini, M. E., Bisazza, A., Agrillo, C., & Lucon-Xiccato, T. (2017). Sex differences in discrimination reversal learning in the guppy. Animal Cognition, 20(6), 1081-1091.

Carbia, P. S., & Brown, C. (2020). Seasonal variation of sexually dimorphic spatial learning implicates mating system in the intertidal Cocos Frillgoby (Bathygobius cocosensis). Animal cognition, 23(4), 621-628.

Fabre, N., García-Galea, E., & Vinyoles, D. (2014). Spatial learning based on visual landmarks in the freshwater blenny Salaria fluviatilis (Asso, 1801). Learning and Motivation, 48, 47-54.

R: We agree that the superior performance of females is likely associated with greater behavioral flexibility and enhanced inhibitory control, and we recognize that this pattern has been documented in several fish species. Following the reviewer’s suggestion, we revised the text to explicitly frame our findings within this broader literature and to highlight the role of inhibitory processes in female performance.

We have incorporated all three suggested references (Lucon-Xiccato & Bisazza, 2014;

Lucon-Xiccato et al., 2020; Lucon-Xiccato, 2022) into the discussion and expanded the paragraph to emphasize the links between behavioral inhibition, cognitive flexibility, and sex differences in teleost fish cognition.
Additionally, we examined the other studies recommended by the reviewer and found them relevant to our discussion of sexually dimorphic spatial abilities. We therefore integrated Miletto Petrazzini et al. (2017), Carbia & Brown (2020), and Fabre et al. (2014) where appropriate to strengthen the comparative context of our findings.
"Jacobs (1996) proposed the "gender- dispersal hypothesis,"
Gender is more a cultural things, in contrast with sex. This seem an hypotheses to explain human sex differences, are we sure it applies to fish that do not have cultural gender differences as we intend them?
R: We agree that the use of the term "gender" may evoke culturally grounded interpretations that do not apply to non-human animals, including fish. Although Jacobs (1996) formulated her proposal primarily within the context of mammals and birds, groups in which sex differences may interact with social factors, her central argument is evolutionary rather than cultural. In essence, the author suggests that sex-specific patterns of dispersal and reproductive roles generate different spatial demands, leading to divergent selective pressures on cognitive abilities. These mechanisms are biological and do not depend on human cultural constructions of gender.
Nevertheless, we have replaced "gender-dispersal hypothesis" with sex-based dispersal hypothesis, a more appropriate term that avoids conceptual ambiguity. Additionally, although Jacobs did not discuss fish directly, the general principle that sex-specific ecological pressures shape spatial cognition is applicable across a wide range of taxa, including teleosts. Several studies already show that sex differences in dispersal, territoriality, and reproductive strategies can lead to cognitive differences between males and females in fish.
Overall this is a nice contribution to the literature of cognitive sex differences in fish, I am looking forward to see it published!
Best,
Tyrone Lucon-Xiccato
Reviewer #2
I found this is a very interesting work. I have some comments and suggestions for the authors. My major concern is that results section is quite reduced and shows little information. I suggest this needs to be improved in a great way, adding more information, more figures, tables... I suggest including more variables analyzed. Authors also can consider adding videos as part of results or supplementary material, in order to enrich the importance of the study. In the discussion section, I make specific suggestions refering to this. I also suggest a general revision of the language. Line number in the PDF restarts in each page, so I will try to refer otherwise.
Comments:
Introduction
"Animals use multiple sensory cues as sources of directional information to orient themselves and navigate (Akre, Johnsen, 2014; Green et al., 2020), in addition to integrating past experiences into spatial behavior, which leads to adaptation and learning processes (Kotrschal, Taborsky, 2010)." : This sentence needs language edition.
R: Thank you for your comment. The language was edited.
"Spatial orientation in environments where sensory cues are constantly changing poses a challenge that favors the evolution of navigational abilities requiring attention to signal variation, risk assessment, memory of stimuli, and inferences from incomplete information, thereby enabling adaptive behavior in different contexts": also this sentence needs language edition.
R: The language was edited.
Sentence starting with: "In terms of spatial orientation,…": This sentence is not clear

enough. What does it mean that navigation strategies can be categorized at behavioral and neural levels...? With the next sentences one can understand the meaning, but the first sentence needs to be clear enough.
R: The sentence was edited.
Sentence starting with: “This behavioral requirement is correlated with increased volume in brain regions associated with spatial…” : This sentence is also unclear. What does it mean that males "who do not share this ecological demand in those species"? Please edit this sentence and correct language so that your idea is clear for the reader.
R: The sentence and language were edited.
Sentence starting with: “Conversely, in territorial species…”: In which vertebrate group is this evidence? Fish? Birds, mammals? Or in different species of several groups?
R: We have now revised the sentence to specify that enhanced spatial learning in the more territorial sex has been documented across several vertebrate groups, including fishes, birds, and mammals.
Sentence starting with: “In this study, we investigated potential sex differences in the spatial abilities of the sailfin tetra Crenuchus spilurus…”: I would suggest first to give information on the species, and then stating which is the aim of this study.
R: We reorganized the paragraph so that the biological and ecological information about Crenuchus spilurus comes first, followed by the study aims. The paragraph was rewritten as requested.
Also, if moved somewhere later on in this same paragraph, this sentence can be better expressed as: “The aim of this study is to assess potential sex differences in the spatial abilities of the sailfin tetra C. spilurus.”
R: The paragraph was rewritten as requested.
Since this is an original manuscript and not a review, citing Figures in the introduction should be avoided. Figures should be included in M&M or Results, but not in the introduction, since the introduction refers to previous background leading to the aim and hypothesis of the present study.
R: We have removed the reference to Fig. 1 from the Introduction. The figure is now cited in the Material and Methods section (“Subjects”).
Regarding the figure, it is not clear to me... Is Fig. 1A-B also part of Pires et al., 2016? Or are these photos in this study for the first time? If they are in this study for the first time, please conider including them in results, with the corresponding explanation in a paragraph in that section.
R: We confirm that Fig. 1A-B reproduces images from Borghezan et al. (2019), with proper permission, showing an adult male (A) and an adult female (B) of Crenuchus spilurus. The figure is cited to illustrate sexual dimorphism and dichromatic traits relevant to the study, and since it is not original to this manuscript, we have removed its reference from the Introduction and cite it appropriately in the Material and Methods section under “Subjects”.
When authors say: “The C. spilurus population used in this study …”. Are other populations in other environments? With higher turbidity ? Or usually Amazonian streams are similar to the sites chosen here...?
R: The geographical distribution of C. spilurus covers basins representing all major Amazonian water types (white, black, and clear waters; Sioli 1984). However, the species occurs specifically in streams, which, even within whitewater basins, never exhibit the turbidity of the main channels. Because this terminology could cause confusion for readers unfamiliar with this distinction, we have revised the paragraph to make the environmental context unambiguous.
Sentence starting with: “Thus, mate choice is mutual and based on visual cues…” From the way it is written, somehow it gives the idea that mate choice can be exclusively based on visual cues (even if you don't use the term). I understand this is not your intention. Is there any evidence on other sensitive cues (e.g. olfaction)? Even if there is

no evidence in other sensitive cues, mate choice is also probably related to pheromones, mechanosensory cues, and/or others... Please consider editing this sentence.
R: Our intention was not to imply that mate choice in C. spilurus is exclusively based on visual cues. While the visual component is well documented in the species, other sensory modalities may also contribute to mate assessment, as is common in many fishes. To avoid suggesting exclusivity, we revised the sentence to clarify that visual cues are among the cues involved, without excluding the potential role of other sensory channels.
In the sentence starting with: "Males are territorial…" you refer to paternal behavior. How is paternal care in this species? Even if this is not the main topic in this study, maybe it is worth mentioning how the male takes care of the eggs/larvae, which behavior he performs, if there are different paternal stages, etc.
R: Male parental behavior in this species is well described in previous studies: males guard the clutch inside the shelter, fan the eggs to improve oxygenation, and maintain the nest by removing debris until larval dispersal. These behaviors are stable across reproductive events and are tightly associated with the territoriality and site fidelity already mentioned in the introduction.
However, we respectfully believe that a description of paternal care would not directly contribute to the objectives of the present study, which focuses specifically on spatial navigation and potential sex differences in the use of visual landmarks. We therefore opted not to expand the text with additional reproductive details to maintain an introduction centered on the ecological and behavioral factors directly relevant to our hypotheses.
Nonetheless, we thank the reviewer for the suggestion and hope that this clarification addresses the underlying concern.
By the end of the introduction authors state the hypothesis, which is very clear. Maybe this can be a good point to include here the aim of this study (instead of in the first sentence of the paragraph, and immediately followed by the hypothesis).
R: The paragraph was rewritten as requested.
Materials and Methods:
Housing: For how long were fish housed in the laboratory before performing the experiments?
R: In revising the Methods section to clarify the housing period, we realized that the original manuscript contained a simple date error. The correct collection date is 28 December 2021, not 2022. The first experimental trial was conducted on 13 April 2022, and the final trial on 27 September 2022. This timeline has now been corrected in the Methods section.
Why were males and females housed separately? Don't you think is more "natural" (or at least more similar to their biological condition) if animals of both sexes were housed together?
R: Males and females were housed separately to prevent aggressive interactions and reproductive attempts that commonly occur when both sexes are kept together in confined environments. In Crenuchus spilurus, males establish and defend small territories around shelters, and the presence of females can intensify territorial disputes and courtship displays, potentially compromising animal welfare and introducing uncontrolled social dynamics before testing. Our goal was to ensure standardized housing conditions and minimize stress before the experiments. Importantly, separation was used only as a temporary laboratory management strategy and did not alter the ecological relevance of the study, as all individuals had similar housing conditions before testing.
Authors explain that aggressive interactions were rarely observed in the holding tanks. Do you think if males and females were housed together, you would observe higher male aggressive interactions?

R: When males and females are housed together, a higher frequency of male–male aggressive interactions would likely occur. In C. spilurus, male aggression is closely linked to territorial defense, particularly around shelters or potential spawning sites. In mixed-sex groups, males tend to compete more intensely both for territories and for mating opportunities, which naturally elevates aggression levels. We emphasize that the purpose of the acclimation period was not to replicate natural social dynamics, but rather to maintain stable, low-stress conditions that would not differentially influence the sexes before the experiments.

Regarding the lack of dissolved organic carbon. Can authors please explain the importance on this? Just as a reply, not in the main text. Has this been established by this method in previous manuscripts of your group, or others?

R: Dissolved organic carbon (DOC) helps replicate the natural water conditions of C. spilurus, which inhabits forest streams rich in organic matter. Although these data are not published, we have empirical observations in our laboratory showing that tanks supplemented with DOC exhibit a higher frequency of spawning events in this species. While preliminary, this suggests that maintaining DOC improves welfare by supporting more natural physiological and behavioral conditions. The practice of adding leaves to restore DOC has been used in previous studies from our group, including Pinto et al. (2020) and Pires et al. (2021).

During which months were experiments performed? Is this 12:12 hs cycle constant throughout all year long? Or is there a natural shift in sunrise and sunset? These questions are related to the question I ask later on, regarding at which time were experiments performed.

R: Experiments were conducted between April and September. Light conditions in the laboratory followed the natural illumination entering through the windows, and on experimental days, artificial lights remained on from 08:00 to 18:30 to ensure consistent visibility during setup and testing. Because Manaus is located near the equator, seasonal variation in day length is minimal, so the light–dark cycle experienced by the fish showed only minor fluctuations throughout the study period.

Apparatus: you use plastic bricks, why did you choose green?

R: We used green plastic bricks for the maze because green is a common color in the natural habitat of Crenuchus spilurus, making the apparatus ecologically relevant and visually familiar to the fish, while also minimizing potentially stressful visual stimuli.

Regarding PVC tube as a reward: Is the PVC tube a reward with equal salience for males and females?

R: PVC tubes serve as ecologically relevant shelters for both sexes. While males use them more frequently for territory defense and nesting, females also use the tubes as refuges, a behavior commonly observed in the habituation tanks. Therefore, the tubes provide a salient and meaningful resource for both males and females, even if usage frequency differs.

Considering male aggression, and considering that parental care in this species is performed by the male and not the female, is it possible that PVC tubes are "more positive" reward for males, and that for females PVC may represent a more neutral reward? To avoid this, why did you prefer using a PVC reward instead of just food?

R: We acknowledge that PVC tubes may serve as a more “positive” reward for males, given their role in territorial defense and parental care. However, during the habituation period, females were frequently observed using the tubes as refuges, making them familiar and ecologically relevant objects for both sexes. Thus, PVC tubes function as meaningful resources for females as well, even if their motivational value may differ slightly between sexes. We chose not to use food as a reward because its motivational value can quickly decrease due to satiety; even if a fish accepts food in one trial, it may lose interest in subsequent trials.

Regarding four females as a reward I have a similar question. In this case, this social stimuli

is always four females (for male and for female focal fish). So, if focal fish is a male, then this social stimuli is definitely a positive social stimuli, more like a positive reward. But if the focal fish is a female, finding four other female is also probably positive (because for fish it is less stressing being with others than in isolation), but probably with a different salience than in the case of focal males (for example during mate choice in this species, are there competitions among females to be chosen by a reproductive male?). Do you think this probable difference in salience of reward (in PVC tube and also in these four females) can bring sex differences in results observed after the protocol?
R: We agree that this social stimulus has different ecological meanings for each sex: for males, the presence of four females represents a reproductive opportunity, whereas for females it primarily provides social companionship and refuge, consistent with the shoaling tendency of C. spilurus. However, although the salience of the stimulus likely differs between males and females, it is a positive reward for both. Therefore, we do not believe that this difference in salience could influence or account for the sex differences observed in our results. Finally, there is no evidence of competition among females for access to males in this species.
Procedure: At what time were experiments performed?
R: Experiments were conducted between 10:00 and 18:00. To provide full transparency, we will include a supplementary table listing the exact dates and times of all experimental sessions.
Where is the fish while the spatial task was reconfigured? Isn't it stressful to remain in the apparatus, while the spatial task was being reconfigured? Even if there might be visual isolation, vibrations don't you think vibrations might be stresful?
R: During apparatus reconfiguration, the fish remained in the initial sector, which is physically and visually separated from the maze. Thus, the fish did not stay inside the apparatus while the task was being rearranged. Although vibrations were not completely isolated, the reconfiguration involved only removing the glass plate and gently switching Lego pieces, a quick procedure that does not generate strong or sudden vibrations. Given the physical separation and the minimal manipulation involved, we consider it unlikely that this process caused significant stress to the fish.
Sentence starting with: " When correct choices…". This sentence needs edition.
R: The sentence and language were edited.
When authors explain that the procedure was repeated 5 times: In the same day? Can you please specify the time window in which experiments were performed? It seems like each experiment lasted around 6 hours?
R: All five trials for each individual were conducted on the same day. Each trial was separated by a 1-hour interval, and the tests lasted on average 5 minutes, followed by 5 minutes of access to the social reward and approximately 3 minutes for apparatus reconfiguration. Thus, the full sequence of five trials for each fish lasted approximately 5 to 6 hours. We have updated the Methods section to explicitly state that all trials for each individual were performed within a single day.
Results:
Results section is quite reduced and shows very little information. I suggest this needs to be enriched in a great way, adding more information, more figures, tables... I suggest including more variables analyzed. Authors also can consider adding videos as part of results, in order to enrich the importance of the study. In the discussion section, I make specific suggestions refering to this.
R: We appreciate the reviewer's concern regarding the level of detail in the Results section and the suggestion to expand it with additional analyses and visual elements. Our study focused on three predefined performance measures: resolution time, accuracy, and route correction. Each is directly linked to our hypotheses about landmark-based learning and sex differences in spatial navigation. For this reason, we prefer to retain the original analytical structure rather than introduce additional variables not included in

our experimental design or a priori predictions.
However, to strengthen the presentation of our results and increase transparency, we have incorporated several additions as recommended:
1. We added Table 1, summarizing all main effects and interactions for each performance measure.
2. We included two new supplementary figures (S1 and S2) showing individual-level data distributions for resolution time and accuracy across trials and sectors.
3. We provided a supplementary video illustrating key aspects of the experimental procedure, as suggested.
We hope the reviewer agrees that the results are now presented with greater clarity and completeness, without extending the analyses beyond what our design and hypotheses support.
Time to solve the task: this is interesting. Maybe it would be better to first refer to the lack of significant interaction, and then explaining the statistical significance for the test sequence and then for the sex?
R: The section “Time to solve the task” has been reformulated.
Discussion:
I understand the first paragraph intends to summarize the most important findings of this study, which is a good idea, gives strength to the discussion and helps organizing ideas. However if this is the case, I suggest authors should say how is it that results here suggest that "males and females process and integrate spatial information" differently. In particular, which variables analyzed here are different between sexes? Do males take more time to solve the task? Do they show reduced accuracy? Whichever your findings were, I suggest authors should make it clear briefly here in the first paragraph in which variables males were different from females, and in which direction (higher or lower). And then, start discussing in the following paragraphs.
R: We have now revised the paragraph accordingly. Specifically, we state that males took longer to complete the task, did not improve in accuracy across trials, and therefore did not learn the landmark-based rule, whereas females showed clear accuracy improvement and faster task completion.
In the paragraph starting with: “Although both sexes showed …” you refer to female mobility. “In
C. spilurus, this hypothesis is supported by direct behavioral observations (during snorkeling
sessions) of greater female mobility in the natural environment”. This is a very interesting hypothesis. However, I suggest that this is something important to check in your experiments here.
R: This question will be answered in detail in the next review.
I understand that authors suggest that the fact that females completing the task faster might be related to them having greater mobility (from evidence in nature). I suggest authors should seriously considering assessing female (and male) mobility in these videos, in these experiments from this study. This will greately enrich the findings of this study with more quantified measures and more figures/graphs. Authors can track individual animals and quantify distance moved per frame and speed. THis way, by comparing male and female distance moved per frame, it will really improve the impact of this study. If authors don't have access to a software or tracker, maybe please consider ToxTrac (https://sourceforge.net/projects/toxtrac/) which is a free software.
R: We would like to clarify that the term mobility in our discussion refers to ecological mobility in the natural habitat, that is, documented sex differences in space use and exploratory range in the wild (Pires et al. 2016), rather than to locomotor activity during the experimental trials. We hypothesize that differences in ecological demands may shape cognitive specializations, not that females performed better because they moved more inside the apparatus.

Quantifying locomotor activity (e.g., distance moved per frame) inside our maze would therefore address a different question, related to activity levels rather than spatial learning strategies, and would not directly test the ecological mechanism we discuss. Moreover, locomotor activity is known to vary with motivation, neophobia, or coping style, and does not reliably reflect spatial-learning abilities.

To avoid misunderstanding, we have now revised the text to explicitly state "ecological mobility in the natural habitat" when referring to field observations. We agree that future studies designed specifically to compare locomotor activity, exploration patterns, or fine-scale movement dynamics between sexes would be valuable, but these analyses lie beyond the scope of the present study.

Later on authors state: "This combination of hesitation and efficient learning may reflect the "asset protection" principle (Clark, 1994), according to which individuals with high reproductive value adopt more conservative, but not necessarily less effective, strategies in challenging contexts." This is an interesting hypothesis. From here, I understand author suggest that males of this species have " higher reproductive value" than females of this species. Is this correct? Please explain this rationale better. Why do you consider females in this species have lower reproductive value than males?

R: Our intention was not to suggest that males have intrinsically higher reproductive value than females. Instead, our rationale was based on a context-dependent interpretation of the asset protection principle. In C. spilurus, males invest heavily in reproduction by defending territories, courting females, and providing exclusive paternal care. Because males guard nests and invest substantial energy in protecting eggs, they may behave more cautiously in unfamiliar or potentially risky situations. Under this framework, "high reproductive value" refers to the value of the male's current reproductive investment (territory, nest site, potential brood), rather than to a higher intrinsic value of males compared to females. We have revised the text to clarify that the principle applies to the male's parental investment and current reproductive assets, not to a sex-wide difference in intrinsic reproductive value.

Also, going deeper in this rationale. Females also might show a degree in their reproductive status (prespawning, postspawning, etc). To further explore this hypothesis, authors might also consider including GSI from females, as part of results (maybe a new analysis and figure?).

For example, if I understood the authors intentions in this sentence well, prespawning females can adopt a more conservative strategy (longer resolution times?) than postspawning females...?

R: We agree that reproductive status and GSI could provide valuable information about how reproductive investment modulates behavior. However, in the present study, we did not record body mass or gonad mass, nor did we classify females according to reproductive stage. Because the fish are no longer available, these measurements cannot be obtained retrospectively. For this reason, we cannot test this hypothesis with the current dataset, but we fully agree that this is a promising direction for future research.

Later on, authors state: "Thus, the differences observed in this study can be understood as the outcome of natural selection acting on distinct cognitive profiles adapted to the specific demands of each sex within its ecological niche." It is a very interesting hypothesis, but I suggest it should be stated in a more conditional way.

R: The sentence has been reformulated.

"Females demonstrate greater flexibility and accuracy in spatial tasks based on predominantly

allocentric navigation,…". I suggest authors should state this more conditional: "Females demonstrate greater flexibility and accuracy in spatial tasks suggesting a predominantly allocentric navigation, whereas males appear .... " etc.

R: The sentence has been reformulated.

"… whereas males appear to rely primarily on egocentric navigation and explore the

environment using more cautious and conservative strategies". Same here. While this is definitely a very interesting hypothesis, it doesn't seem that the fact that males take longer to learn necessarily means that they are more cautious and conservative. They just take longer and are less accurate, and based on previous evidence and hypothesis, one can suggest that this could be related to a possible cautious strategy (or not, but it is definitely something worth speculating about, for sure). So, I suggest using a more conditional phrase here as well: "... whereas the fact that males show lower accuracy and longer resolution times can suggest that they may rely primarily on..." etc.
R: The sentence has been reformulated.
"These findings support the hypothesis that spatial cognition in fishes is adaptive, sensitive to ecological and social context, and shaped by distinct selective pressures between sexes." This looks somehow confusing to me... In the introduction, authors say the hypothesis of this work: " we hypothesized that male C. spilurus would display greater spatial navigational ability than females." But here, they include a much broader hypothesis, as a larger framework. Which is very interesting as well, but it is not exactly the one presented in the introduction.
Also, probably evidence from results in this study is not enough to say that this study supports the hypothesis that spatial cognition in fish is adaptative, sensitive to ecological and social context. Experiments here do not test this broad hypothesis.
R: The sentence has been reformulated.
One can discuss results from these experiments by contrasting with evidence in the nature, and in ecological or social context etc, and discuss a possible ecological or evolutionary meaning of this. But I don't see clearly how the findings here support this very broad hypothesis. I suggest authors should consider narrowing the hypothesis and conclusions of this study, and suggesting future experiments to study whether " spatial cognition in this species is adaptative, sensitive to ecological and social context."
R: The sentence has been reformulated.
Figure legend 3: Figures need to have a general title, before jumping into what's A and what's B.
R: The legend has been reformulated.
-Figure legend 3: I suggest instead of showing just the mean values and SEM, authors should include for each time point all individual values as dots, then putting them together as a bar and with a horizontal line showing the mean value. Or maybe authors can figure out a better way, but I think showing individual values of each experiment is important, rather than just showing the mean and SEM.
R: We have added a new figure to the Supplementary Material showing individual data points (jittered) alongside boxplots for each sex and trial. The main figure in the manuscript remains unchanged because the original format provides clearer visualization of group-level patterns, but the supplementary figure now makes all individual values available for inspection.
-Figure legend 3: Please consider adding significance to the graph, with asterisks or letters, or somehow. Also, information on which statistical test was perform might also be included in the legend.
R: We prefer to keep the figure focused on the visual representation of the data, as all statistical analyses, test results, and significance values are already fully described in the Results section. Including statistical annotations directly in the figure could reduce clarity and visual readability without adding new information. Therefore, we opted to maintain Figure 3 without significance markers while ensuring that all relevant statistical outcomes remain clearly detailed in the main text.
-Figure legend 4: Also in this graph, please consider adding individual data as dots showing each experiment, not just the mean value as a dot. Also please include asterisks or letters in case there is significance, and also please add which statistical test was performed in this case in the legend.

R: We will keep Figure 4 focused on mean values and confidence intervals to preserve visual clarity and consistency with the other main figures. However, following the reviewer's suggestion, we have now added an asterisk to indicate the significant effect observed. All statistical tests and significance outcomes are already fully reported in the Results section, so including full test details in the legend would duplicate information already provided in the text.
However, to meet your request for visualization of individual-level data, we will include an additional figure in the supplementary material showing the distribution of all individual data points (boxplots with jittered observations) for the accuracy analysis.

Associate editor's decision after peer review (15/12/2025).
Dear Mr. Pinto:
Manuscript ID NI-2025-0160.R1 entitled "Sex differences in landmark-based spatial learning in the sailfin tetra Crenuchus spilurus" which you submitted to the Neotropical Ichthyology, has been reviewed. The comments of the reviewer(s) are included at the bottom of this letter.
The reviewer(s) have recommended publication, but also suggest some minor revisions to your manuscript. Therefore, I invite you to respond to the reviewer(s)' comments and revise your manuscript.
To revise your manuscript, log into https://mc04.manuscriptcentral.com/ni-scielo and enter your Author Center, where you will find your manuscript title listed under "Manuscripts with Decisions." Under "Actions," click on "Create a Revision." Your manuscript number has been appended to denote a revision.
You may also click the below link to start the revision process (or continue the process if you have already started your revision) for your manuscript. If you use the below link you will not be required to login to ScholarOne Manuscripts.
*** PLEASE NOTE: This is a two-step process. After clicking on the link, you will be directed to a webpage to confirm. ***
https://mc04.manuscriptcentral.com/ni-scielo?URL_MASK=2ff0cf75c8274d3185f5b13c1e44eec2
You will be unable to make your revisions on the originally submitted version of the manuscript. Instead, revise your manuscript using a word processing program and save it on your computer. Please also highlight the changes to your manuscript within the document by using the track changes mode in MS Word or by using bold or colored text. Once the revised manuscript is prepared, upload BOTH versions (the tracked and a clean) and submit them through your Author Center.
When submitting your revised manuscript, you will be able to respond to the comments in the space provided. You can use this space to document any changes you make to the original manuscript. In order to expedite the processing of the revised manuscript, please reply POINT TO POINT all the suggestions of the reviewers and be as specific as possible in your response to the comments.
IMPORTANT: Your original files are available to you when you upload your revised manuscript. Please delete any redundant files before completing the submission.
Because we are trying to facilitate timely publication of manuscripts submitted to the Neotropical Ichthyology, your revised manuscript should be submitted before 15-Jan-2026. If it is not possible for you to submit your revision by this date, we may have to consider your paper as a new submission.
Once again, thank you for submitting your manuscript to the Neotropical Ichthyology and I look forward to receiving your revision.
Sincerely,
Dr. Eliane Gonçalves de Freitas
Associate Editor, Neotropical Ichthyology
eliane.g.freitas@unesp.br

Anonymous reviewer #1
Recommendation. Minor Revision
Comments. General consideration
I found this is a very interesting work. In this revision, authors have assessed many of the previous concerns and provided an improved version of the manuscript. I still have some concerns or suggestions.
Line number in the PDF restarts in each page, so I will try to refer otherwise.
Introduction
-Authors provided more information on the species as suggested, which I believe highly improves the introduction and highlights the importance of this species as very appealing model to test this hypothesis. I just suggest that the following sentence needs to be edited: "During reproduction, males display their hypertrophied fins and perform bold courtship behavior, whereas females indicate receptivity through abdominal darkening, and mate choice is mutual, with visual cues to play a key role…"etc.
Materials and Methods:
- Previous Figure 1 was moved to M&M, which I believe is much more appropriate.
-I have concerns on the way animals were kept in only males, and only female tanks. Authors reply that in mixed tanks, higher frequency of male–male aggressive interactions are observed, linked to territorial defense. They suggest that: "… the purpose of the acclimation period was not to replicate natural social dynamics, but rather to maintain stable, low-stress conditions that would not differentially influence the sexes before the experiments". I understand the purpose of the acclimation period and that the aim of the work is not to replicate natural social dynamics. But how can we be sure that this only male or only female tank assure lower stress, if this is not a natural condition…? I understand that authors know that in only males, the aggressive interactions decrease, but how do we know if these reduced aggression is "less stressful" when compared to mixed sex tanks…? Maybe there is lower aggression, but this could be because the animals are so stressed that they cannot even explore the tank (as if they were freezing, which of course I assume is not the case, I am just giving an extreme example to clarify my point here).
I understand the rationale of this explanation, but somehow I understand we cannot be certain about this, unless cortisol is determined in each condition. I understand this only male or only female tanks was the way acclimation was performed for this work, but I believe the rationale for this may need to be clearer in the manuscript… So, if authors have evidence to state that this condition assures lower stress than in mixed tanks, these references need to be cited in the first sentence in Housing: "In the laboratory, males and females were housed separately in aquaria". Otherwise, if there is no evidence to show that these only males and only female tanks assure lower stress than mixed tanks, then I suggest that at least some of these can be discussed in the discussion…
-Regarding the dissolved carbon, authors explain that: "The practice of adding leaves to restore DOC has been used in previous studies from our group, including Pinto et al. (2020) and Pires et al. (2021)." I suggest this can be included in the manuscript.
-Authors now include a table with all individual data. I am not sure this is necessary… But besides this, I suggest that in the main text a very brief explanation stating that exp were conducted between 10:00 and 18:00.
-I believe it is very interesting discussing the salience of the PVC and the female reward. My question was: "Do you think this probable difference in salience of reward (in PVC tube and also in these four females) can bring sex differences in results observed after the protocol?" The reply was: "We agree that this social stimulus has different ecological meanings for each sex: for males, the presence of four females represents a reproductive opportunity, whereas for females it primarily provides social companionship and refuge, consistent with the shoaling tendency of C. spilurus. However, although the salience of the stimulus likely differs between males and females, it is a positive reward for

both. Therefore, we do not believe that this difference in salience could influence or account for the sex differences observed in our results. Finally, there is no evidence of competition among females for access to males in this species." To me, this is a very valid reply and this is a very interesting point that needs to be somehow included in the discussion.
-Regarding new supplementary figures 1 and 2, please include in the legends what is the horizontal line, the boxplots and the whiskers.
-In the reply authors explain the issue about "mobility" and it is convincing. But I really emphasize that the way they managed to include this issue in the discussion is really convincing and highly improves this point in the discussion.
-The conclusions were edited and I believe they are now more accurate and convincing, reflecting the results in this study.

Anonymous reviewer #1
Recommendation. Accept
Comments. I have read the revised version of the manuscript and examined the authors' responses. I have no further comments for the authors. I maintain my initial opinion that this is an interesting study and that it can be considered for publication.
Sincerely,
TLX

Author's Rebuttal Letter (19/12//2025).
Dear Editors,
We thank you for the opportunity to submit a revised version of our manuscript entitled "Sex differences in landmark-based spatial learning in the sailfin tetra *Crenuchus spilurus*" (Manuscript ID NI-2025-0160.R1). We have carefully addressed all comments raised in the second round of review and revised the manuscript accordingly. Below, we provide a detailed, point-by-point response explaining how each comment was addressed and indicating the corresponding changes in the manuscript. We appreciate the reviewers' continued engagement and constructive suggestions, which have helped us further improve the clarity and presentation of our study.
Best regards,
MSc Kalebe da Silva Pinto, on behalf of all authors
Graduate Program in Biology
Instituto Nacional de Pesquisas da Amazônia (INPA/MCTI), Brazil
Point-by-point replies:
Reviewer comments:
Reviewer #1:
I found this is a very interesting work. In this revision, authors have assessed many of the previous concerns and provided an improved version of the manuscript. I still have some concerns or suggestions.
Comments
Authors provided more information on the species as suggested, which I believe highly improves the introduction and highlights the importance of this species as very appealing model to test this hypothesis. I just suggest that the following sentence needs to be edited: "During reproduction, males display their hypertrophied fins and perform bold courtship behavior, whereas females indicate receptivity through abdominal darkening, and mate choice is mutual, with visual cues to play a key role…"etc..
R: Thank the reviewer for the positive feedback. As suggested, the sentence was edited.
Regarding the dissolved carbon, authors explain that: "The practice of adding leaves to restore DOC has been used in previous studies from our group, including Pinto et al. (2020) and Pires et al. (2021)." I suggest this can be included in the manuscript.
R: We included in the Methods section that the procedure of adding leaves to restore

dissolved organic carbon has been previously adopted in studies from our group, citing Pinto et al. (2020) and Pires et al. (2021).

I have concerns on the way animals were kept in only males, and only female tanks. Authors reply that in mixed tanks, higher frequency of male–male aggressive interactions are observed, linked to territorial defense. They suggest that: "… the purpose of the acclimation period was not to replicate natural social dynamics, but rather to maintain stable, low-stress conditions that would not differentially influence the sexes before the experiments". I understand the purpose of the acclimation period and that the aim of the work is not to replicate natural social dynamics. But how can we be sure that this only male or only female tank assure lower stress, if this is not a natural condition…? I understand that authors know that in only males, the aggressive interactions decrease, but how do we know if these reduced aggression is "less stressful" when compared to mixed sex tanks…? Maybe there is lower aggression, but this could be because the animals are so stressed that they cannot even explore the tank (as if they were freezing, which of course I assume is not the case, I am just giving an extreme example to clarify my point here).

I understand the rationale of this explanation, but somehow I understand we cannot be certain about this, unless cortisol is determined in each condition. I understand this only male or only female tanks was the way acclimation was performed for this work, but I believe the rationale for this may need to be clearer in the manuscript… So, if authors have evidence to state that this condition assures lower stress than in mixed tanks, these references need to be cited in the first sentence in Housing: "In the laboratory, males and females were housed separately in aquaria". Otherwise, if there is no evidence to show that these only males and only female tanks assure lower stress than mixed tanks, then I suggest that at least some of these can be discussed in the discussion…

R: We agree that same-sex housing does not necessarily imply lower physiological stress, as stress-related hormones were not measured in this study. Our intention was not to infer stress levels, but to clarify the rationale behind housing males and females separately during the acclimation period. Specifically, this procedure was adopted to reduce overt male–male aggressive interactions associated with territorial defense and to standardize pre-experimental conditions, following housing protocols previously used in experimental studies with Crenuchus spilurus (Pinto et al., 2020; Pires et al., 2021). Therefore, we have revised the Housing section.

"Authors now include a table with all individual data. I am not sure this is necessary… But besides this, I suggest that in the main text a very brief explanation stating that exp were conducted between 10:00 and 18:00.

R: As suggested, we added a brief statement in the Procedure section specifying that all experimental trials were conducted between 10:00 and 18:00. In addition, following the reviewer's observation, we have removed Supplementary Table S1, as the individual-level data were not essential to the main text.

-I believe it is very interesting discussing the salience of the PVC and the female reward. My question was: "Do you think this probable difference in salience of reward (in PVC tube and also in these four females) can bring sex differences in results observed after the protocol?" The reply was: "We agree that this social stimulus has different ecological meanings for each sex: for males, the presence of four females represents a reproductive opportunity, whereas for females it primarily provides social companionship and refuge, consistent with the shoaling tendency of C. spilurus. However, although the salience of the stimulus likely differs between males and females, it is a positive reward for both. Therefore, we do not believe that this difference in salience could influence or account for the sex differences observed in our results. Finally, there is no evidence of competition among females for access to males in this species." To me, this is a very valid reply and this is a very interesting point that needs to be somehow included in the discussion.

R: We addressed this issue in the Discussion, acknowledging potential differences in reward salience while explaining why such differences are unlikely to account for the observed sex differences in learning and navigation performance.
-Regarding new supplementary figures 1 and 2, please include in the legends what is the horizontal line, the boxplots and the whiskers.
R: We have revised the legends of Supplementary Figures S1 and S2 to explicitly describe the meaning of the horizontal line, boxplots, and whiskers, as requested.
Reviewer #2
I have read the revised version of the manuscript and examined the authors' responses. I have no further comments for the authors. I maintain my initial opinion that this is an interesting study and that it can be considered for publication.

Associate editor's decision after peer review (26/01/2026).
Dear Mr. Pinto:
It is a pleasure to accept your manuscript entitled "Sex differences in landmark-based spatial learning in the sailfin tetra Crenuchus spilurus" in its current form for publication in the Neotropical Ichthyology.
Congratulations for the acceptance of your article, and be aware on the following topics:
1. Publication Fee
NI will charge a publication fee if none of the co-authors is an active SBI member. This measure is essential to strengthen SBI and thus ensure the continuity of our journal, scientific society, and biannual meetings. SBI is not limited to Brazilians but is open to anyone interested in freshwater and marine Neotropical fishes. More details on SBI are available at https://www.sbi.bio.br/. Please email tesouraria.sbi@gmail.com to confirm whether any of your co-authors is a current SBI member and to activate your SBI membership if needed. Otherwise, if you will cover the publication fee of R$1.000, please inform us at the same email. For authors outside Brazil, the fee will be converted to US dollars based on the official exchange rate on the date of payment. The only exceptions to this fee are invited articles.
2. Science Communication and Social Media
NI actively promotes published articles to both academic colleagues and the general public, including science journalists. To support this, we create social media posts and require images and/or videos of fish related to your work. If your article does not include such images, please send a photo of a representative fish species, preferably alive in its natural habitat. If you do not have your own photo, you may provide a link to an online image, along with the source, author, and, if applicable, authorization for its use. We also publish video summaries of articles in Portuguese on our Instagram (@neoichth). We ask you to designate one author to record a short video using a mobile phone, following the attached instructions. The video should include visual materials (photos, graphics) and a script for subtitles to enhance accessibility. Please email the completed material to neoichth@nupelia.uem.br within 30 days. For now, please provide the name and email address of the author responsible for recording the video.
Additionally, if your article is taxonomic in nature and has been submitted to Zoobank, it is your responsibility to update the manuscript's status on Zoobank once it has been published.
All of the above information and materials are mandatory for the publication of your article, including the scientific dissemination component, which is crucial in the current climate of science denial and misinformation. If you have any questions, please feel free to contact us at neoichth@nupelia.uem.br.
Please respond to this e-mail within five working days to let us know you are aware of all the important points mentioned above.
Thank you for your fine contribution. On behalf of the Editors of the Neotropical

Ichthyology, we look forward to your continued contributions to the Journal.
Sincerely,
Dr. Eliane Gonçalves de Freitas
Associate Editor, Neotropical Ichthyology
eliane.g.freitas@unesp.br

Anonymous reviewer #1
Recommendation. Accept
Comments. I have read the revised the new version of the manuscript, together with the authors' responses. I have no further comments, and I would like to congratulate the authors for their efforts in this manuscript and in improving the discussion. I understand this work is ready for publication.

HOW TO CITE THIS ARTICLE

- **Pinto KS, Zuanon J, Pires THS.** Sex differences in landmark-based spatial learning in the sailfin tetra *Crenuchus spilurus* (Characiformes: Crenuchidae). Neotrop Ichthyol. 2026; 24(2):e250160. https://doi.org/10.1590/1982-0224-2025-0160

SBI Official Journal of the Sociedade Brasileira de Ictiologia