**Editorial comments:**

Changes to be made by the author(s) regarding the manuscript:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

* The manuscript was edited using Mircosoft Word and Grammarly.

2. Please shorten the Abstract to no more than 300 words.

* The Abstract is currently at 296 of 300 words.

3. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).

* The manuscript overall was edited for the use of pronouns.

4. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. However, notes should be used sparingly and actions should be described in the imperative tense wherever possible. Please move the discussion about the protocol to the Discussion.

* The manuscript was edited for verb tenses and structure within the Protocol section to make each bullet point a command. “Notes” were retained within sections of the Protocol to add context needed for the action. Lastly, it was unclear to us which section the Editor or the Reviewers were referring to in requesting the move of “the discussion about the protocol to the Discussion”. Therefore, we removed most notes that could be discussed in the Discussion, leaving only two notes in the Protocol.

5. 3.1: Figure 1B only shows three, not four overhead track lighting systems. Please check.

* Indeed, the figure shows only three lights of one light strip. Due to the room size and placement of the lights near the wall, we could not get a photograph that included all the lights. The legend 1B was altered to address this, “(only one of the four overheads lights strips are visible)”.

6. Please note that your protocol will be used to generate the script for the video and must contain everything that you would like shown in the video. Software must have a GUI (graphical user interface) and software steps must be more explicitly explained ('click', 'select', etc.). Please add more specific details (e.g. button clicks for software actions, numerical values for settings, etc.) to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Some examples:

7. 7.1: Please specify the predetermined level of brightness.

* We have tried to address this point more thoroughly, in particular, in the Protocol section addressing the experimental procedure (sec. 7) where the GUI interface will be used during the video.

8. After you have made all the recommended changes to your protocol (listed above), please highlight 2.75 pages or less of the Protocol (including headings and spacing) that identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol.

* This has been completed with new lines highlighted. As the details on the protocol Sec 7 Experimental Procedure have increased while decreasing the amount discussed relative to the development of the Processing program for the video.

9. Please highlight complete sentences (not parts of sentences). Please ensure that the highlighted part of the step includes at least one action that is written in imperative tense.

* This has been adjusted and completed.

10. Please include all relevant details that are required to perform the step in the highlighting. For example: If step 2.5 is highlighted for filming and the details of how to perform the step are given in steps 2.5.1 and 2.5.2, then the sub-steps where the details are provided must be highlighted.

* This has been adjusted and completed.

11. Discussion: As we are a methods journal, please also discuss critical steps within the protocol, any modifications and troubleshooting of the technique, and any limitations of the technique.

* We specifically added two paragraphs towards the end of the discussion that addresses critical steps and perhaps areas where researchers may find issues arise and recommendations on dealing with them.

12. Table of Materials: Please sort the items in alphabetical order according to the name of material/equipment.

* Done

**Reviewers' comments:**

Reviewer #1:

Manuscript Summary:

The manuscript "Integrating visual psychophysical assays within a Y-maze to isolate the role that visual features play in navigational decisions" investigates the role that motion cues play in guiding fish movement decisions. The manuscript is well written and describes some novel protocols in good detail. I only have a concern about the statistical results, which I would like the authors to check.

Major Concerns:

1.1 Line 438: I find it strange that this relationship is so weak (z = 1.937, P = 0.053). From Figure 5A, it appears the relationship between stimuli speed and decision 'accuracy' is particularly strong. I would urge the authors to check these statistics. If these statistics are correct, they should remove the term 'significant' from line 436*.*

* The statistics and the inferences drawn from modeling the data remain unchanged, but we have corrected the figures. Decision accuracy does improve with the speed of the stimuli, but while the relationship is significant the trend is noisy and hence the high P-value. We have corrected an oversight in Fig 5A that is likely the source of the reviewer’s concern. The original graph had standard errors, but since the data are binary and fit with a logistic model it is more appropriate to use bootstrapped 95% confidence intervals to show the inherent variability. This visual change should make the results more intuitive. For consistency, we also use bootstrapped 95% confidence intervals in Fig5B as well, although using standard errors in this graph would still be appropriate given the time data the linear mixed model’s assumptions (normally distributed errors).

Minor Concerns:

1.2 Line 131: *O*dd sentence due to the multiple uses of the term 'record'. Please restructure.

* Changed to: “Install an overhead camera to record the behaviors of the fish and the visual projections from above. Color or black and white (b/w) cameras may be used.”

1.3 Line 369: LDA and RDA are not defined up until here. There are only defined in the figure legend.

* replaced acronyms: “..Log the clock time, start and stop times from the stopwatch, and the subjects’ choice (i.e., left or right).”

Reviewer #2:

Manuscript Summary:

This manuscript introduces a methodology to study how digital and animated visual features, directly projected on a tank below surface using a computer and a short-throw projector, can influence directional direction of fish in a Y-maze. The model species used is the Golden Shiner (*Notemigonus crysoleucas*).

This manuscript is well-written, the protocol is solid, and both the introduction and discussion are well structured and informative. The methodology presented here is innovative, and very promising for behavioral studies in fish. The protocol is rigorous and provides sufficient details for reproducibility.

Despite a few minor concerns that I would like the authors to address, I recommend this article to be accepted under minor revisions.

Major Concerns:

None

Minor Concerns:

2.1. I recommend the authors to include in their protocol some information about the visual stimuli they used. While the authors properly refer to their other papers Lemasson et aL 2018 in SciRep, where I found the information about the visual stimuli (especially how silhouette vanish and reappear), I believe that it is critical to mention this information here again. Especially as the authors present results of fish choice vs silhouettes' speed, I believe it is important to mention, in this protocol, what happens after the silhouettes reach on side of the Y-maze.

* We now include more details related to the projections in the Introduction to help clarify the representative results [lines 88-97]: “...In particular, the directional cue is generated by having a fixed proportion of the silhouettes act as “leaders” and move ballistically towards one arm or another. The remaining silhouettes act as distractors by moving about at random to provide background noise that can be tuned by adjust the leader/distractor ratio. The ratio of leaders to distractors captures the coherency of the directional cues and can be adjusted accordingly. Distractor silhouettes remain confined to the decision area (“DA”, **Figure 1A**) by having the silhouettes reflect off of the boundary. Leader silhouettes, however, are allowed to leave the DA region and enter their designated arm before slowly fading away once the silhouettes traversed 1/3 the length of the arm. As leaders leave the DA, new leader silhouettes take their place and retrace their exact path to ensure that the leader/distractor ratio remains constant in the DA throughout the experiment.”

2.2. It is maybe related to the reviewer's PDF, but the image quality is very low in my PDF. In particular, legends in Figure 1 are unreadable. Authors should improve their images or verify with the editor that this is only related to the reviewer's PDF low resolution.

* Vector file types (.eps) will be uploaded with our edits to avoid any scaling or resolution issues with the journal’s system.

2.3. This is maybe biased by the fact that I am a more a fish biologist rather than an engineer, but I believe that there is some imbalance between the technical aspects of the protocol (before the experiment) and the experiment itself (something like 5 pages vs. 3 pages). I recommend the authors to add more details on the experimental procedure, the visual stimuli (see my minor concern 1 in particular) and the data analysis, while making the information about the designs, equipment, projections and background a bit more concise and more accessible to a non-specialist.

* We appreciate the reviewer’s concern that the protocol used in the methods outweighs our discussion of the experiment and data analysis. The bias, while perhaps unconventional, was by design and aligns with the journal’s mission to focus on communicating the practicalities involved with executing the experiment through a protocol of actionable items. While we retain an emphasis on the protocol, we have added more material to the introduction (see response to 2.1, changes to representative results, and the note added to action 8 of the protocol, Data analysis).

2.4. More detailed comments:

l. 39: missing "of" in "to aid in the understanding the mechanisms"

* Changed to: “Recent advancements in computer visualization have made it possible for empirical testing in the lab where visual features can be controlled and finely manipulated to isolate the mechanisms of social interactions”

l. 261: "the" not needed before "subtracting"

* Removed

l. 522: remove "e" in "CA allows customization of e visual cues"

* Changed to: “CA allows one to customize visual cues (direction, speed, coherency, or morphology), while introducing a level of standardization and repeatability in the desired stimulus that exceeds what can be achieved when using live animals as the stimulant.”