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Letter to Editor

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Validating the use of computer animations in male *Etheostoma zonale*: a comparison of individual response to live and artificial stimuli

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The use of artificial stimuli in tests of animal behavior are common, and as technological advances increase, opportunities for the use of 3D computer animations in behavioral tests are becoming increasingly available (Witte et al. 2017). The importance of easy-to-manipulate, consistent, and reliably performing stimuli is clear, and this is readily achievable with the use of computer animations. Validation of animated stimuli is critical, however, to ensure that behaviors observed in response to artificial, computer-generated stimuli are equivalent to what would be expected if animals were presented with a comparable live stimuli (Woo and Rieucau 2011; Chouinard-Thuly et al. 2017; Powell and Rosenthal 2017). In this study, our aim was to validate the use of computer animations in the banded darter *Etheostoma zonale* by comparing behavior between live and animation trials. Previous work with this species validated the use of video playback studies (Roberts et al. 2017) and motorized models (Williams and Mendelson 2011), showing that preferences for conspecific over heterospecific stimuli in playback and motorized model trials were not significantly different than preferences in live trials. Here, we compared preferences of male *E. zonale* for live conspecific over live heterospecific *E. barrenense* females to preferences for computer animated conspecific females over computer animated heterospecific females (Figure 1A; Supplemental Methods; example animation provided as Supplementary Material V1). *Etheostoma barrenense* is a sympatric congener of the focal species, and several studies have examined preferences for conspecifics and conspecific phenotypes in this species pair (Williams and Mendelson 2010; Roberts and Mendelson 2017; Roberts et al. 2017). We compared the strength of preference (SOP) for conspecific females in live trials with the SOP for conspecific female animations in animation trials. SOP is a standardized metric of preference ranging from +1 to −1, indicating a complete conspecific and heterospecific preference, respectively (from Stalker 1942). A SOP score of zero indicates no preference for either stimulus. We also compared the proportion of time spent with conspecific and heterospecific stimuli in live trials and animation trials.

SOP was significantly different than zero in both live and animation trials. SOP in live trials was $\bar{x} \pm SE = 0.38 \pm 0.16$ (2-tailed *t*-test: $t = 2.47$, $df = 12$, $P < 0.05$) and SOP in animation trials was 0.34 ± 0.12 ($t = 2.78$, $df = 12$, $P < 0.05$). We found no significant difference in SOP between live and animation trials for male *E. zonale* (2-tailed paired *t*-test: $t = 0.29$, $df = 12$, $P = 0.78$; Figure 1B). Males spent a significantly larger proportion of time associating with the conspecific versus the heterospecific stimulus in live trials (2-tailed paired Wilcoxon signed-rank test: $Z = 2.06$, $df = 12$, $P < 0.05$; Figure 1C). Males spent $\bar{x} \pm SE = 41.3 \pm 7.1\%$ of total trial time in the conspecific preference zone compared with $15.2 \pm 4.5\%$ of total trial time in the heterospecific preference zone during live trials. We also found that males spent a significantly larger proportion of time associating with conspecific versus heterospecific stimuli in the animation trials ($Z = 1.99$, $df = 12$, $P < 0.05$; Figure 1D), spending $25.7 \pm 4.5\%$ of total trial time in the conspecific preference zone and $12.4 \pm 3.4\%$ of total trial time in the heterospecific preference zone during animation trials.

Our results therefore validate the use of computer animations to test preference for conspecific over heterospecific stimuli in *Etheostoma zonale*. Model validation is an important first step in the use of any artificial stimulus in behavioral studies. Combined with previous studies, which validate the use of motorized models and video playback stimuli to test preferences in *E. zonale* (Williams and Mendelson 2011; Roberts et al. 2017), our results show that multiple artificial stimuli can reveal preferences that are comparable with preferences for live individuals. Deciding which type of artificial stimulus to use in behavioral studies, therefore, can be guided by the unique advantages and disadvantages of each stimulus type and by the specific questions at hand, rather than limitations in behavioral response to artificial stimuli. This study thus highlights the ability of multiple testing mechanisms to successfully query the behavior of the focal species and provides useful insights into study design, allowing researchers to choose the method of stimulus presentation that best meets the specific needs of the experiment.

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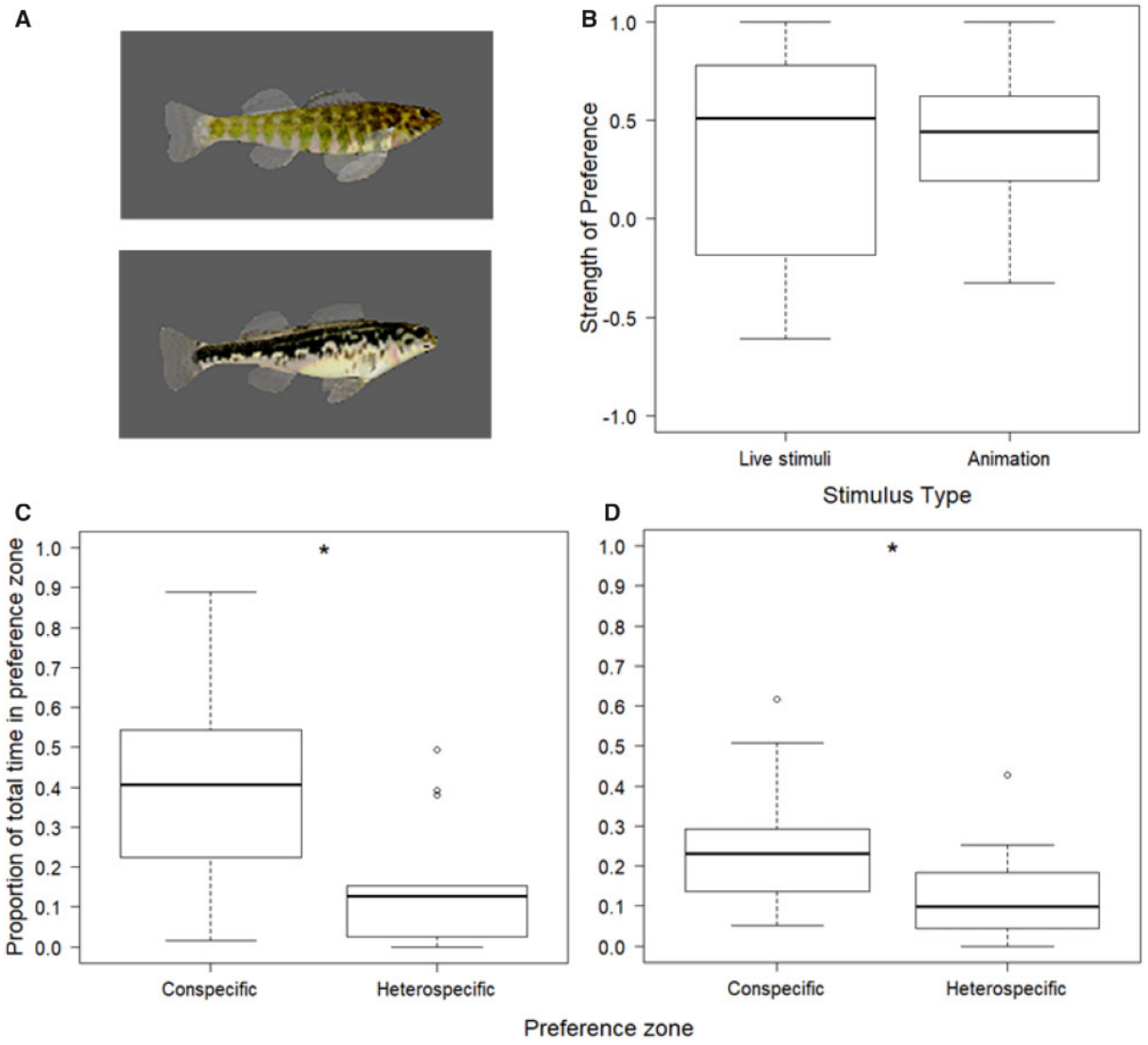

Figure 1. (A) Examples of 3D models used as stimuli in computer animation trials showing a female *E. zonale* (top) and *E. barrenense* (bottom). **(B)** Male SOP for conspecific over heterospecific live fish and for conspecific over heterospecific computer animations in *E. zonale* ($P = 0.78$). **(C)** Proportion of time spent with conspecific and heterospecific females in live and **(D)** computer animation trials. Bars in box plots represent medians, boxes indicate upper and lower quartiles, whiskers show sample minima and maxima, and open circles show outliers for box and whisker plots.
*Significant difference of $P < 0.05$ according to a 2-tailed paired Wilcoxon signed-rank test.

Supplementary Material

Supplementary material can be found at https://academic.oup.com/cz.

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