

Exploring Olfactory Cues: Behavioral Responses in Cichlid Juvenile to Food and Amino Acid Stimuli



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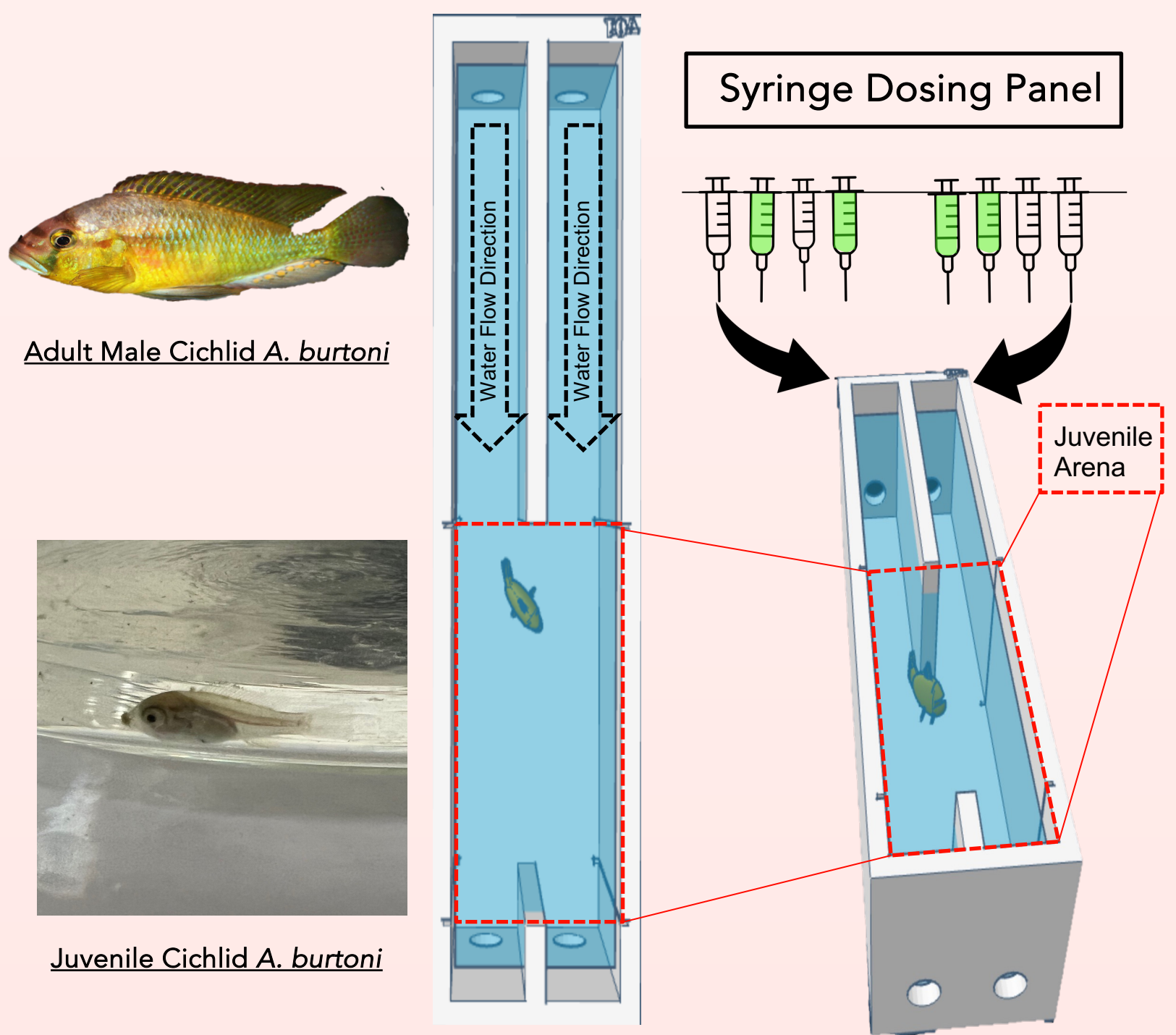
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Background

- *Astatotilapia burtoni* are a member of the diverse cichlid fish family.
- Cichlids are known for a high rate of speciation, driven in part by adaptation to new food sources.
- However, we do not know how cichlids use their chemosensory system to detect food
- Previous research on this has been done in other model fish species but not in cichlids.

Based on previous research proving the responsiveness of adult cichlid fish to olfactory cues, I predict that introducing cichlid juvenile to both food and amino acid cues will evoke a behavioral response, determined by time spent in odor delivered compared to clean water, confirming the ability to detect olfactory cues in cichlid juvenile.



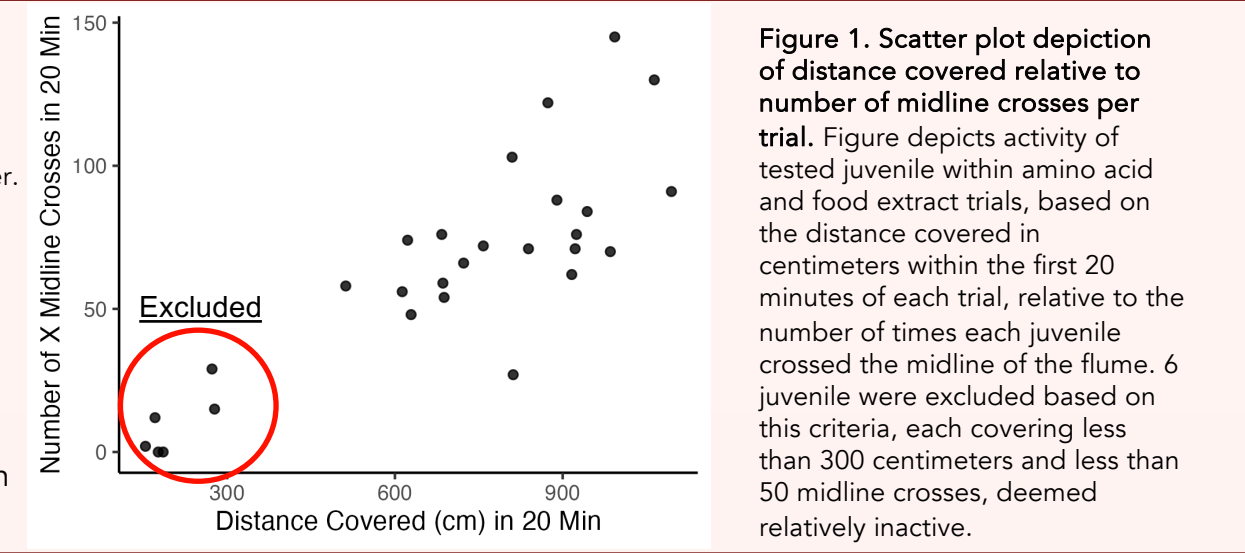
Methods & Results:

Subjects (Aminos): n = 16 model juvenile of the Wild-Type *Astatotilapia burtoni* Cichlid fish species (~ age 13-33 dpf)
 Subjects (Food): n = 9 model juvenile of the Wild-Type *Astatotilapia burtoni* Cichlid fish species (~ age 17-32 dpf)

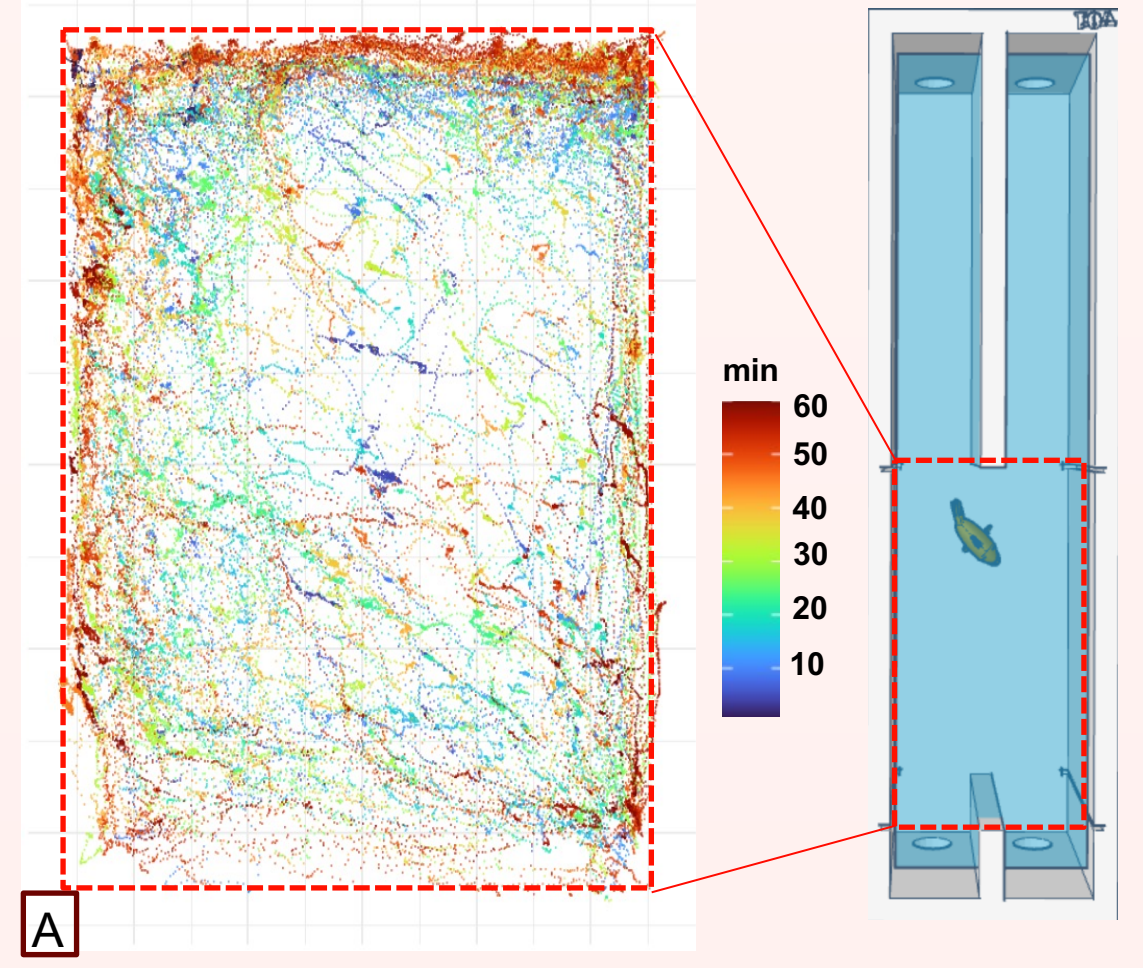
Amino acid mix (Ala, Cys, His, Lys, Met, Phe, Tyr, Val) concentrated at 0.5 mM in flume. Food extract ingredients consist of standard Fish Food Flakes.
 Odor delivered via syringe panel, consisting of 4 syringes per side of the flume. Syringes were used to deliver clean water and food stimuli, based on pre-determined syringes dosing panel order.

Juvenile tested using amino acids were split across two separate waves. Wave 1 consisted of 45 minute intervals, in which odor stimuli was delivered at 3 separate intervals. Wave 2 consisted of 60 minute intervals, in which odor stimuli was delivered at 2 separate intervals. (Flow rate ~ 200 mL/min)
 Juvenile tested using food extract were split across two separate trials. Within the first trial, juvenile were tested in 50 minute intervals. Within the second trial, juvenile were tested in 60 minute intervals. (Flow rate ~ 200 mL/min)

Data analysis obtained from frame-by-frame idTracker software. Visual, graphical, and statistical analysis in "R" (v 4.3.1).
 4 of the juvenile tested in amino acid trials were excluded from final analysis for inactivity; final analysis of amino acid trials consisted of n=12. 2 of the juvenile tested in food trials were excluded from final analysis for inactivity; final analysis of food trials consisted of n=7.



Overhead path trace of fish movement



Horizontal path trace of fish movement

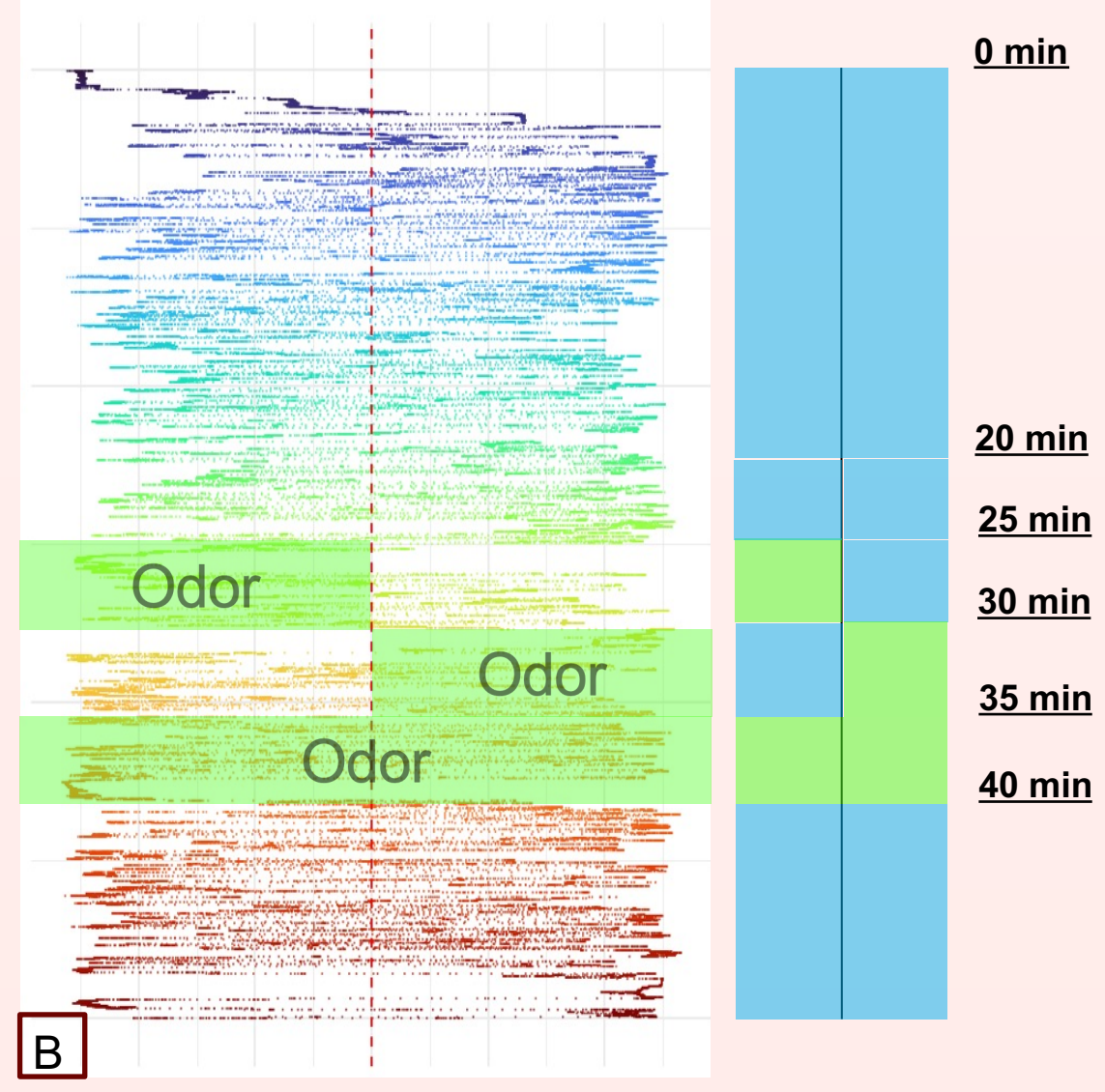
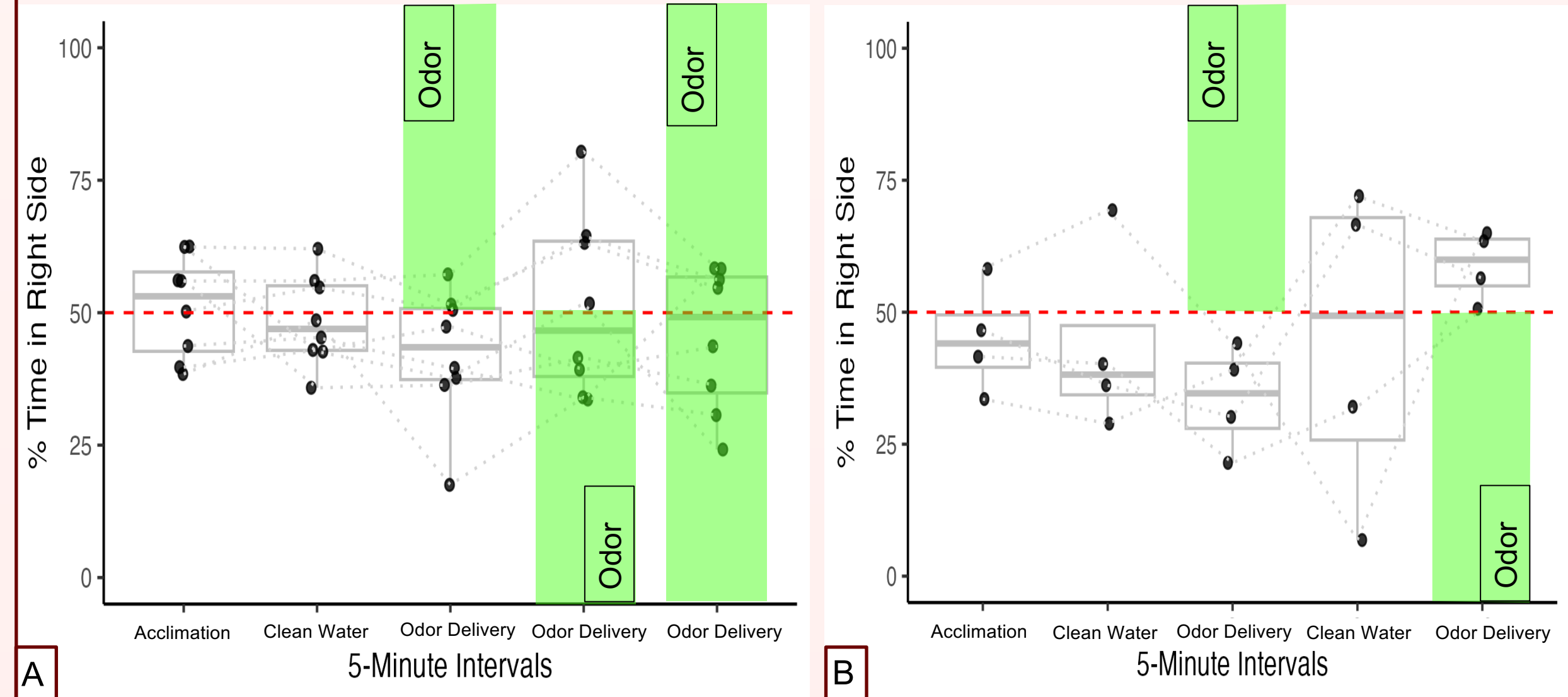


Figure 2A. Overhead path trace of fish movement depicting example of preference displayed in an amino acid trial. Figure depicts position of fish over time. Figure 2B. X-axis path trace of fish side-to-side over time. Figure depicts position side-to-side over time. Legend depicts a timeline and intervals that correspond to time blocks in which clean water and amino acids were delivered to one side or the other in this example. Clean water and odor are represented in blue and green respectively.

No preference for amino acids



Possible preference for food extract

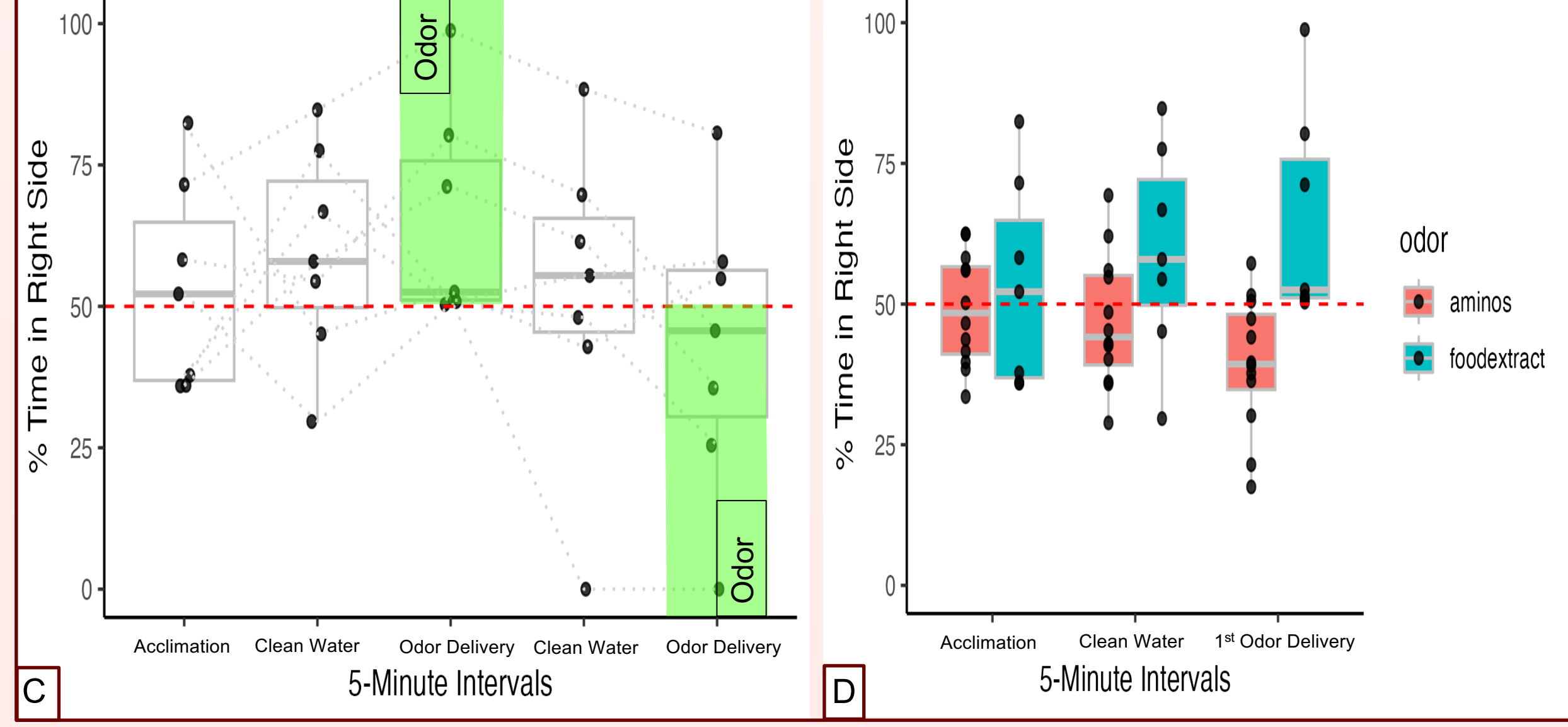


Figure 3. Figure depicting amino acid trials wave 1 and 2, food extract trials, and comparison of amino acid and food extract trials relative to each, per 5-minute interval of interest. 3A. Box plot depicting 5-minute intervals of interest throughout wave 1 of amino acid trials. X-axis represents 5-minute intervals of acclimation, clean water, and odor delivery, with respect to the amount of time the fish spent on either side of the flume during that 5-minute interval, on the Y-axis. Figure 3B. Box plot depicting 5 minute-intervals of interest throughout wave 2 of amino acid trials, axes as above. Figure 3C. Box plot depicting 5 minute-intervals of interest throughout food extract trials. Figure 3D. Box plot comparing amino acid and food extract trials' time spent across left and right side of flume, per 5-minute interval.

Conclusions:

A two-way ANOVA (time block x odor type) revealed a **significant main effect of odor type** ($F = 12.95, p = 0.0007$) and **no main effect of time block**. The interaction effect was not significant ($F = 2.47, p = 0.09$). The presence of a **strong main effect of odor** and the **interaction term** having $p < 0.10$ encouraged us to look more closely at the data.

A Post-Hoc analysis (Tukey's HSD) revealed a **single significant pairwise difference** ($p = 0.0056$) at the comparison of 1st odor deliverance between the two odor conditions.

These data refute our hypothesis that fish would spend more time in odor compared to clean water. However, we find some support for a difference in response between the two odors.

Future Impacts

The following next steps function in exploring several possibilities, making use of this work to better understand chemosensation in Cichlids:

- Use of food extract in future experiments, given its observed difference in behavioral response.
- Increased control of water flow to allow fish juvenile to swim more freely.

Confirming wild-type preference will allow for testing of chemosensation in mutant line *A. burtoni*, to better understand the specific neural mechanisms involving chemosensory signaling.

Acknowledgments

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