

## Supplemental Material for:

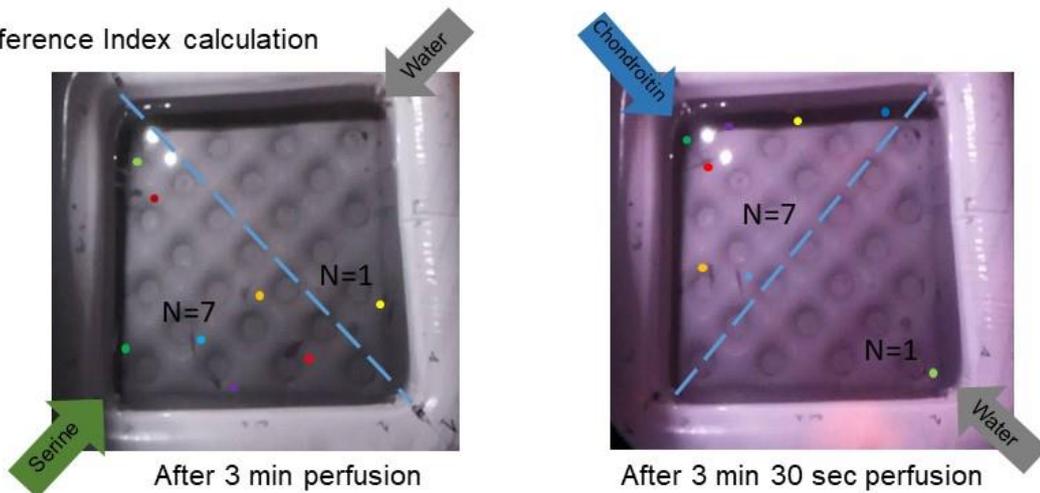
### Diversity of olfactory responses and skills in *Astyanax mexicanus* cavefish populations inhabiting different caves

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A. Blue water perfusion test



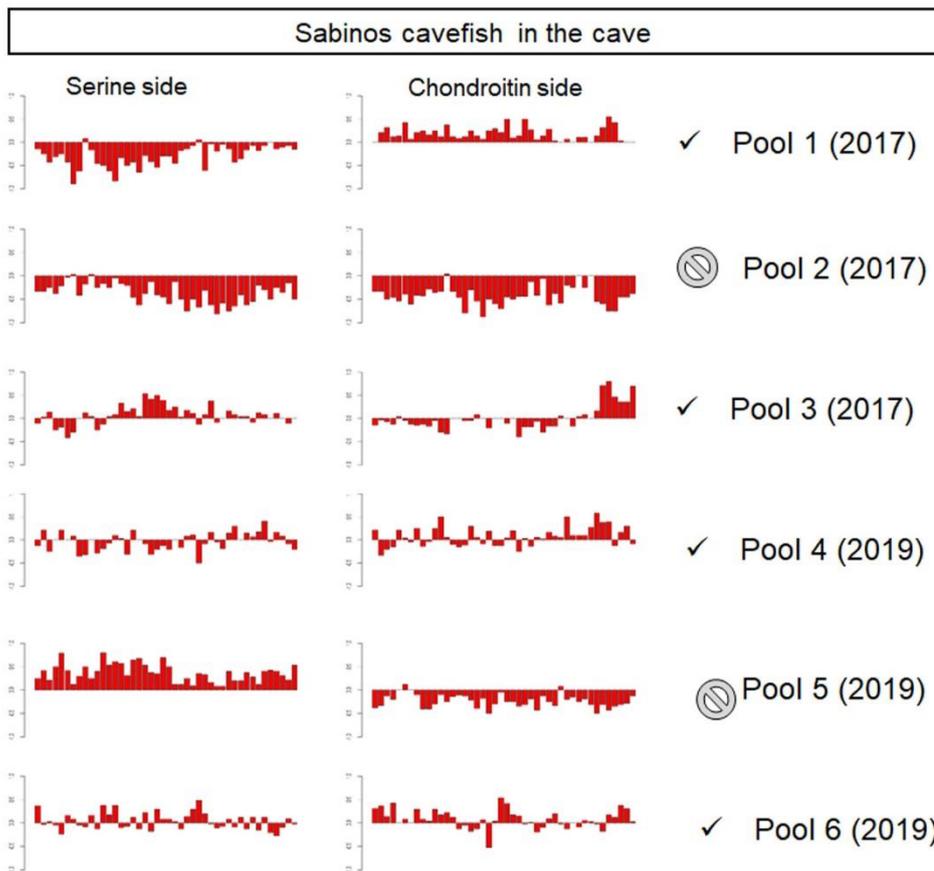
B. Preference Index calculation



**Suppl Fig 1: Rationale for olfactory scoring.**

**A:** Kinetics and extent of diffusion of perfused molecules in the plastic pool setups. 50ml of “blue water” (methylene blue) were perfused at the two opposite corners of a plastic pool. After 6 minutes, at the end of the perfusion, the blue color occupies roughly half of the arena. This was used as a criteria for preference index calculation.

**B:** To calculate PIs (preference Index) every 15 seconds, the number of fish present in the two relevant halves of the arena was counted. Two examples are given. In both, there are 7 fish in the odor side and 1 fish in the control side, thus the  $PI=7-1/8=0.75$



**Suppl Fig 2: Place preference problem in the Sabinos cave.**

Graphs showing the position of the fish along the whole ~one-hour protocol for the n=6 experiments performed in the Sabinos cave. Compare with main Figure 5. The bars represent the preference of fish towards the serine perfusion side (left column) or the chondroitin perfusion side (right column). Plastic pools 2 and 5 recorded in Sabinos were thus excluded from analysis.

**Suppl video 1: behavioral response of Pachón cavefish to chondroitin in laboratory settings.**

**Suppl video 2: behavioral response of Pachón cavefish to chondroitin in natural settings.**