Chemosensory Evolution in the Blind Mexican Cavefish

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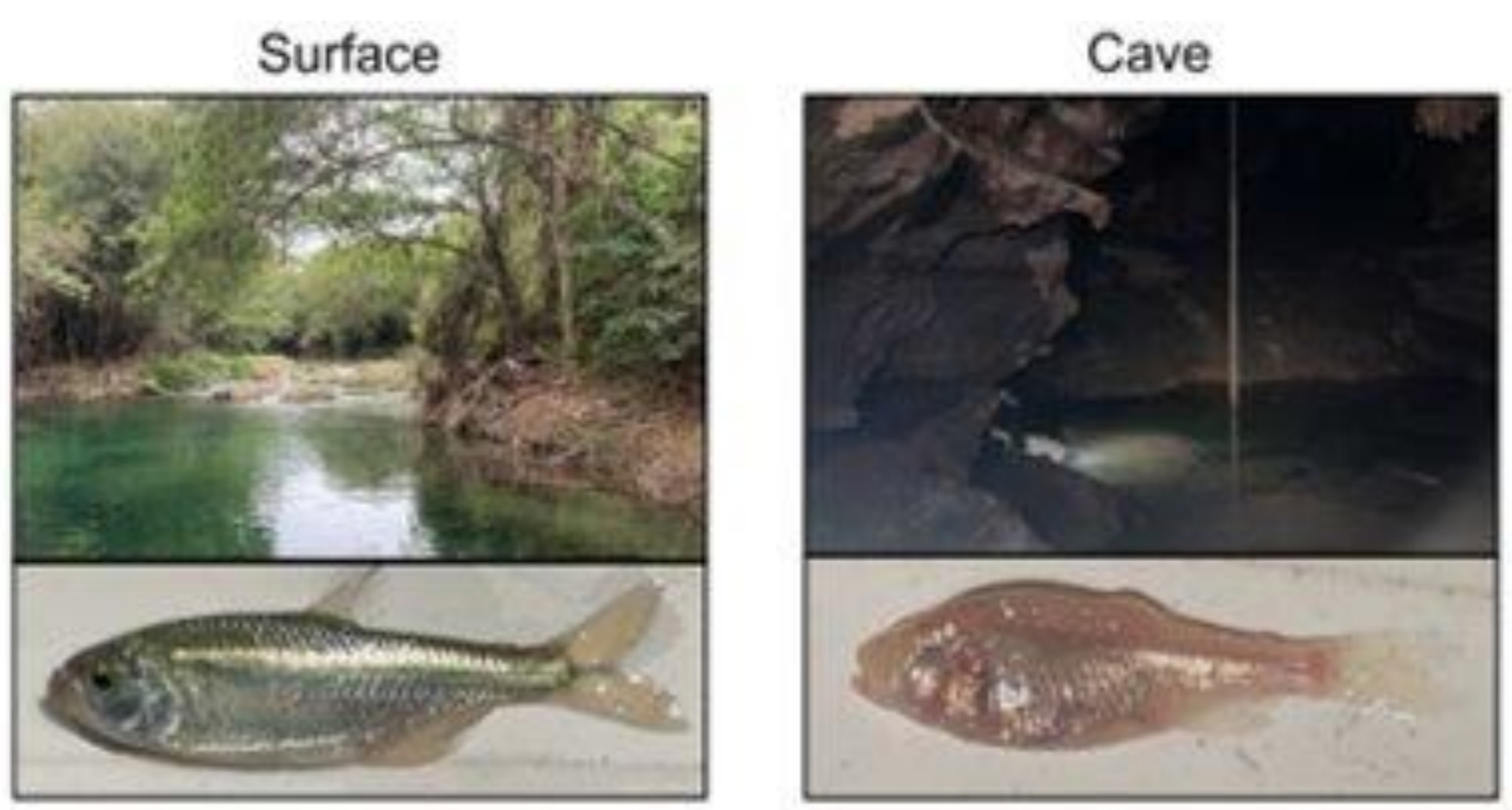
Abstract

Having evolved in a cave environment, blind Mexican cavefish (*Astyanax mexicanus)* have developed enhanced non-visual senses to compensate for their lack of vision. This study investigates the behavioral changes in response to odorants as a result of this change in sensory input. The study involves comparing the behavioral response of blind Mexican cavefish and closely related surface fish, who have maintained sight and physical eyes, to various concentrations of different odorants. The study evaluates behavioral responses to odorants in multiple contexts, in both adults and juveniles. By recording their behavioral movements in these different conditions, our goal is to find differences in the chemosensory response of blind Mexican cavefish compared to surface fish. We have found increased behavioral response to both attractive and aversive odorants in cavefish relative to surface conspecifics, in the form of alterations in their proximity to the stimulus source. This study provides a basis to evaluate the behavioral adaptations that have emerged as a result of adaptation to the cave environment, and will aid in understanding how organisms adapt to extreme conditions such as a complete absence of light. This study also provides insight into the behavioral effects of circuit-level and molecular adaptations, which can reveal how evolutionary processes shape organismal function.

Introduction and Background



Environmental and phenotypic differences in *A. mexicanus*. Representative environments and morphotypes of surface-adapted (left) and cave-adapted (right) *A. mexicanus*. Cave environments are characterized by the absence of light and minimal trophic input; the cave morphs exhibiting troglobitic phenotypes including eye loss, lack of pigmentation, reduced metabolic rate, and starvation resistance (Figures adapted from Moran et al., 2023)

A. mexicanus can be found in freshwater throughout America and Central America in two forms: dwelling in caves and closer to the surface. Adapting to a dark cave environment, *A. mexicanus* dwelling in caves have evolved to survive without visual capabilities and physical eyes (2). To make up for their lack of vision, blind Mexican cavefish have developed enhanced olfactory senses essential for survival, detecting predators, danger, and communication signals. Previous studies have found that the chemosensory systems of cavefish have acquired larger chemosensory organs, providing their enhanced olfactory capabilities (1). However, the behavioral evolution of the blind Mexican cavefish has yet to be discovered. Through larval and adult behavioral testing, the manifestation of the cavefish's sensitive olfactory system is further assessed for aversive and attractive tendencies relative to surface fish.

Methods

Adult Chemosensory Behavioral Trials

The adult behavioral chemosensory tests were administered to observe the effects of various olfactory stimuli on the natural behavior of both cavefish and surface fish in a controlled setting. Four plastic rectangular tanks were filled with fish water and four subjects (1:1 cavefish to surface fish) (Figure 1).

In more localized trails, we used four different chambers that were 3-D printed to isolate the diffusion of the released stimuli, allowing for clearer tracking of attraction and avoidance (Figure 2).

We prepared various stock solutions concentrated with aversive and attractive amino acid stimuli such as alanine, TetraMin, and cysteine. In MatLab, a code was developed that released the chemical stimuli through tubing into each chamber. The first 15 minutes of the recorded dark trials were controlled and stimuli-free. After 15 minutes, the MatLab code opened each chamber's tubing, releasing the chemical stimuli and recording the subjects' responses. Once the trial was completed, we ran the video through a Python code to track the movement of the subjects and then another code to translate their responses into quantitative data on Excel. Their x-y positions and locomotion in relation to the stimuli were then analyzed to determine responses to different odorants.

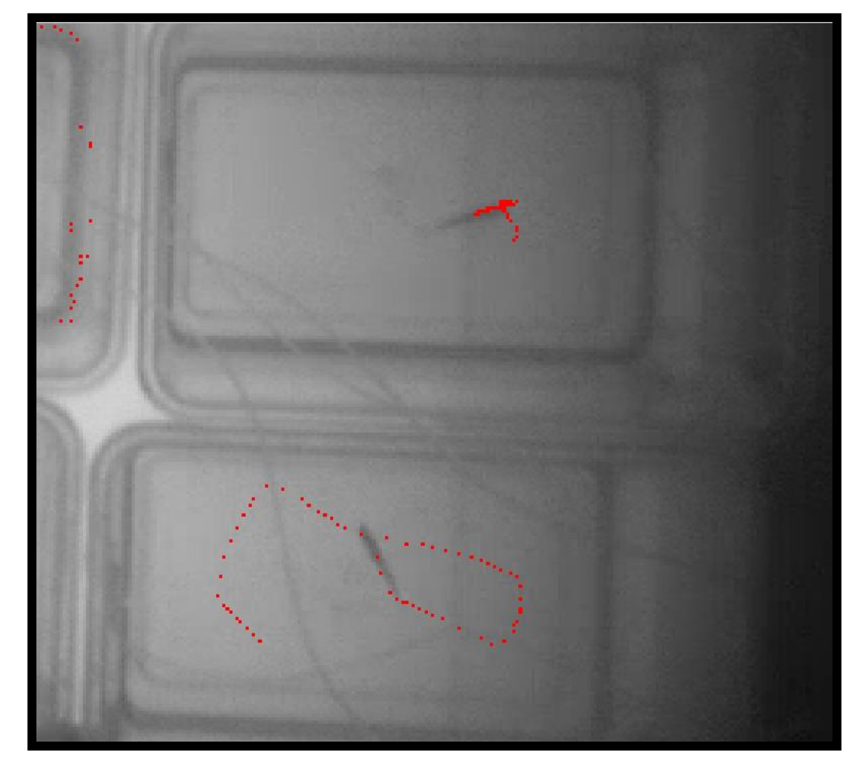


Figure 1: Adult trial rectangular tanks

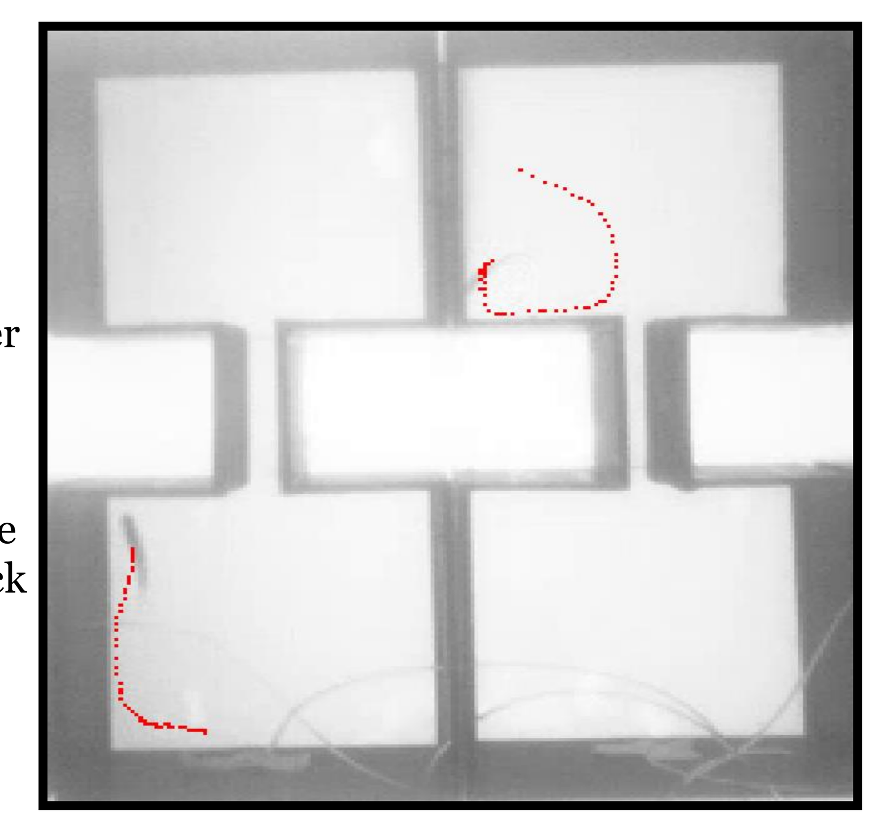


Figure 2: Adult trial localized chamber

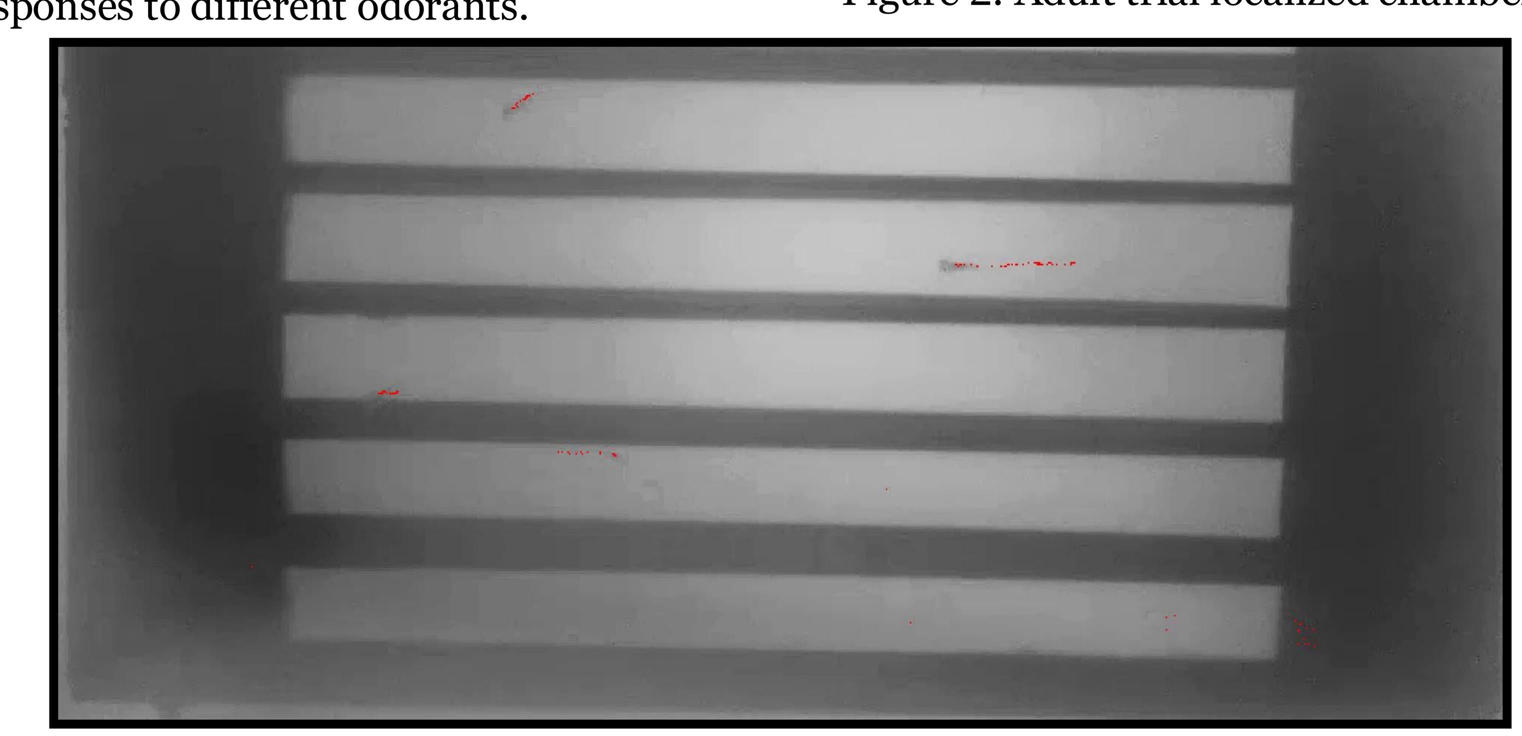


Figure 3: High-salt environment larval test

Larval High-Salt Environment Chemosensory Behavioral Trials

We also performed high-salt environment larval tests to analyze the behaviors of the larval forms of the fish. An assay was developed for close observation of larval cavefish and surface fish aversion in a 3% NaCl agarose gradient (3). We designed another 3-D chamber for trails in OpenSCAD containing 6 separated lanes with two agar pads on each end (Figure 3). One agar pad had a control of fish water and the other contained fish water and the salt stimulus. The same steps from the first experimental design were repeated to retrieve quantitative data for analysis using Python and Excel.

Results

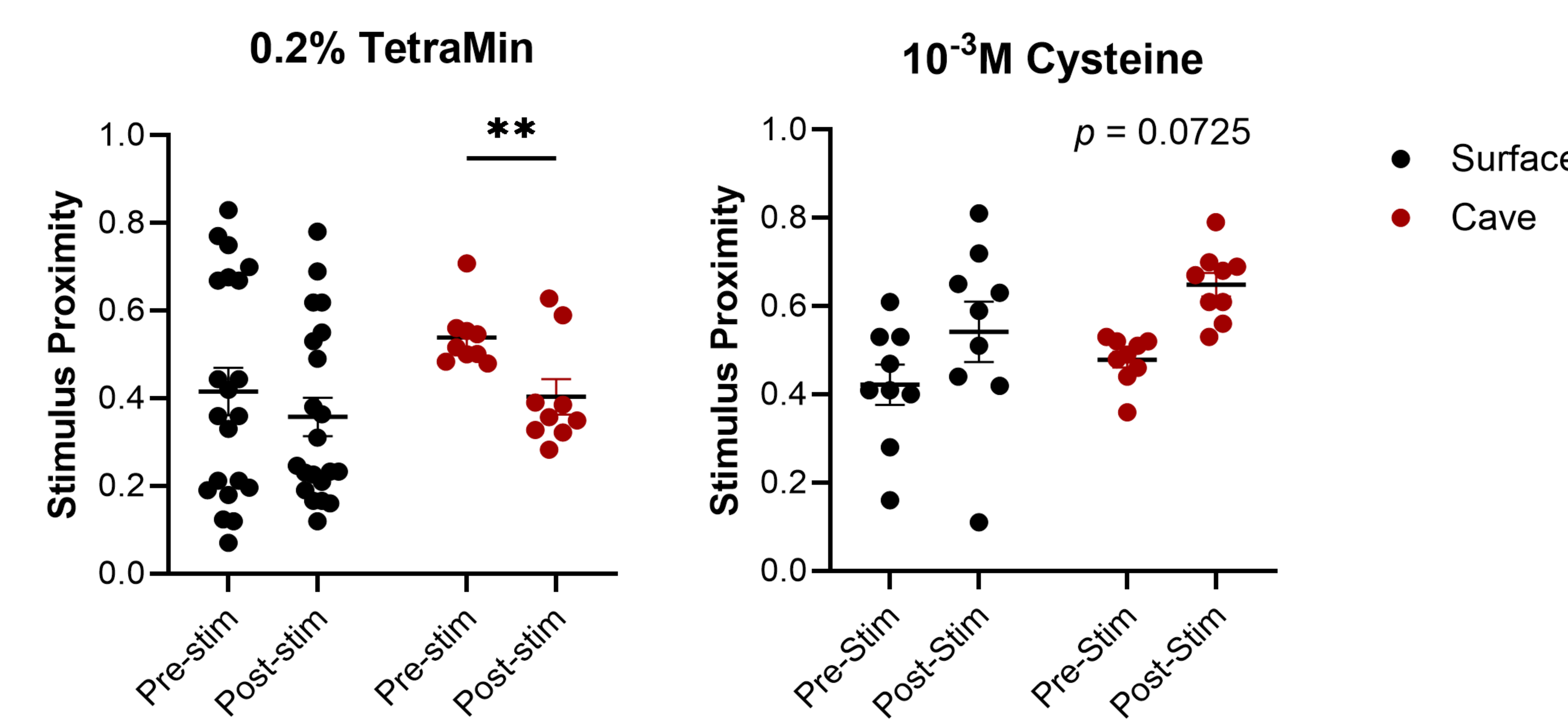


Figure 4: Mean position of the subjects both before and after application of an attractive stimulus (0.2% TetraMin extract), or an aversive stimulus (10^{-3}M cysteine).

Discussion

Our results from the adult chemosensory behavioral testing indicate that blind Mexican cavefish have a more pronounced response to odorant stimuli, swimming towards an attractive stimuli and away from an aversive one. While surface fish also exhibit a behavioral response to odorants, their reaction in terms of proximity to the source of odorant is less notable. Blind Mexican cavefish demonstrate a more distinct and stronger behavioral response to these odorants, as reflected in their movement and positioning relative to the odorant source. This supports our hypothesis that blind Mexican cavefish have a heightened chemosensory response, which may be due to compensation for their absence of sight. Further research can be carried out to distinguish the relative contributions of neuroplasticity and evolved mutations.

In our second experiment, we were unable to determine a prominent behavioral variation between the blind Mexican cavefish and the surface fish regarding their proximity to the source of odorant over time. Both types of larval fish seemed to have shown a preference of being closer to the agarose gel containing the control fish water, however, the differences between both types of larval fish were very slim.

Future Directions

- To continue our study and understand the evolution of chemosensory responses of blind Mexican cavefish, we have already begun researching their molecular adaptations and neurological morphology.
- We have performed whole mount SV2 and TH cell immunohistochemical antibody stains to assess the development of the olfactory bulb and glomeruli of larvae at 3,7, 14, 21, and 28 days post-fertilization.
- Using Fiji (ImageJ), we have quantified TH cells in samples to analyze olfactory activity during early cavefish development.
- We are in the process of constructing a 3-D representation of the morphology of the olfactory bulb from SV2 stain results using Fiji software.

References