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Evolutionary modifications of *Astyanax* larval prey capture (LPC) in a dark environment

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ABSTRACT

Feeding strategies of an organism depend on the multimodal sensory processing that most efficiently integrates available visual, chemosensory, and/or mechanoreceptive cues as part of their environmental adaptation. The blind cavefish morph of *Astyanax mexicanus* has developed sensory-dependent behaviors to find food more efficiently than their eyed, surface-morph counterparts while in darkness. In the absence of light, adult cavefish have evolved enhanced behaviors, such as vibration attraction behavior (VAB), and changes in feeding angle. Here, we identified evolved differences in cavefish larval prey capture (LPC) behavior. In the dark, LPC is more efficient in cavefish than in surface fish. Furthermore, different cave populations express laterality in their LPC and strike towards prey preferentially located on their left or right sides. This suggests the occurrence, to some extent, of divergent LPC evolution among cave populations. While LPC can be triggered exclusively by a vibration stimulus in both surface and cavefish, we provide evidence that LPC is, at least partially, a multimodal sensory process different from adult VAB. We also found that a lack of food may exacerbate the laterality of LPC. Thus, we proposed a mathematical model for explaining laterality based on a balance between: (1) enlarged range of foraging field (behavioral or perceptive) due to asymmetry, (2) food abundance, and (3) disadvantages caused by laterality (unequal lateral hydrodynamic resistance when swimming, allocation of resources for the brain and receptors, and predator escape).

Keywords: *Astyanax*; Cave; Troglóbite; Neuromast; Prey; Asymmetry

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INTRODUCTION

Animals have developed a diverse array of foraging and prey-capture mechanisms. Each species possesses a variety of sensory systems attuned to locate their particular food sources (Catania, 2012; Daghfous et al., 2012; Moss, 1996). While many species, including humans, rely heavily on vision to find food, others must rely on alternative sensory inputs due to residing in dark habitats. Many nocturnal species depend almost exclusively on auditory cues (Payne, 1971; Wagner et al., 2013), while other species, including rodents, rely largely on olfaction (Rattazzi et al., 2015; Yao et al., 2016). Notably, cave-dwelling organisms must adapt to the sole use non-visual cues.

The blind Mexican tetra (*Astyanax mexicanus*) is an emerging model system for evolutionary study (Keene et al., 2016). Significant advances have been made in understanding the genetic basis of adaptation to extreme environments, such as continuous darkness in caves. Throughout their evolutionary history, *Astyanax* cave-dwelling fish species have experienced regression of their eyes and pigmentation, and the improvement in their mechanosensory lateral line, olfactory and taste bud chemosensors, and foraging behavior as adaptations to cope with the perpetual darkness (Blin et al., 2020; Devos et al., 2021; Hinaux et al., 2016; Gross et al., 2009; Jeffery, 2005; Keene et al., 2016; Protas et al., 2006; Varatharasan et al., 2009; Wilkens, 1988; Yamamoto & Jeffery, 2000; Yamamoto et al., 2004, 2009; Yoshizawa et al., 2010).

There are two *Astyanax* morphs, i.e., eyed surface morph and eyeless cave morph. The cave morph is restricted to 33 known limestone caves in the Sierra de El Abra region of northeastern Mexico, and two caves in Guerrero in southcentral Mexico (Espinasa et al., 2020; Miranda-Gamboa et al., 2023). The closely related surface morph is broadly distributed throughout surface streams and lakes in Mexico.

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Both morphs remain inter-fertile, making the species well-suited for experimental manipulations (Elipot et al., 2014). Many of the cavefish populations are located within hydrological systems that were connected during their evolutionary history (Espinasa et al., 2014), with some gene flow present among these cave populations (Herman et al., 2018). Indeed, enrichment of troglomorphic phenotype quantitative trait loci (QTL) in genomic regions displaying low divergence among caves strongly implies the potential transfer of critical genetic regions associated with cave phenotypes through gene flow within the El Abra region (Herman et al., 2018). Despite this gene flow, certain cave populations are isolated enough to have undergone independent troglomorphic evolution. For example, regarding the evolution of depigmentation, some cave populations carry different and independent mutations in their *Oca2* (Protas et al., 2006) and *Mc1r* genes (Gross et al., 2009).

The dietary preferences of *Astyanax* cavefish change between the post-larval and adult stages, from aquatic crustaceans (e.g., water fleas, copepods, ostracods, and isopods) in fry to partially decomposed material, guano, and detritus in mud during adulthood (Espinasa et al., 2017). Even in the early fry stage, shortly after yolk depletion, cavefish display enhanced skills in capturing prey when young larvae must find their own food. When paired, 25-day-old cavefish are more successful than surface fish competing for *Artemia* nauplii in the dark (Espinasa et al., 2014). Despite the presence of many enhanced sensory modalities in *Astyanax* cavefish, their precise contributions to evolved differences in feeding behavior at different ages remain poorly understood.

Larval prey capture (LPC) behavior is characterized by a fast-striking motion towards the prey within tens of milliseconds. Serial time-lapse images of single prey capture events have revealed that surface fish, under light conditions, strike mostly at a target directly in front of them, bending the most caudal region of their tail (Lloyd et al., 2018). In zebrafish, this movement is classified as a J turn (McElligott & O'Malley, 2005). Conversely, surface fish in dark conditions and cavefish in both light and dark conditions strike mostly at prey on their side, using a C-bend turn that involves turning the head towards adjacent prey (Lloyd et al., 2018). The most significant difference between the two morphs is that strike distance is significantly greater in cavefish compared to surface fish, suggesting that cavefish may have improved their ability to detect prey in the dark (Lloyd et al., 2018).

In surface fry with gentamicin-induced lateral line ablation or presented with non-moving dead *Artemia*, prey capture is abolished in the dark (Lloyd et al., 2018). This supports the notion that, for surface fish under dark conditions, the detection of *Artemia* movements by the lateral line induces LPC. In contrast, LPC in cavefish appears to exhibit greater complexity. Although lateral line ablation or presentation of dead *Artemia* reduces both strike angle and distance in cavefish, significant LPC still occurs, with successful strikes on prey (Lloyd et al., 2018). These findings suggest that while the lateral line plays a critical role in LPC in both surface and cavefish under dark conditions, cavefish appear to possess additional and enhanced sensory processing mechanisms.

In the current study, we explored the primary sensory modality used in LPC, as well as the type of stimulus to which LPC is attuned in cavefish larvae. We further characterized the differences and similarities between LPC and adult fish vibration attraction behavior (VAB). Finally, we developed a

mathematical model to explain why and when laterality, such as that observed in LPC and VAB, can enhance foraging behaviors.

MATERIALS AND METHODS

Fish rearing and maintenance

Animal husbandry was carried out as described previously (Borowsky, 2008). Most work was conducted at the German Sumbre Laboratory at the Institut de Biologie de l'ENS (IBENS), CNRS, France, except for strike behavior at live *Artemia* by surface fish in the dark, which was performed at the Johanna Kowalko Laboratory at the Department of Biological Sciences, Lehigh University, USA. All experiments performed at the German Sumbre Laboratory were approved by Le Comité d'Éthique pour l'Expérimentation Animale Charles Darwin (APAFIS#27495-2020100614519712 v14). Several specimens were sourced from Sylvie Rétaux at the Paris-Saclay Institute of Neuroscience, CNRS, and the University Paris-Saclay, France. Research involving *Astyanax mexicanus* was conducted under Sylvie Rétaux's authorization number 91–116. The animal facility of the Paris-Saclay Institute of Neuroscience received authorization (B91272108) from the Veterinary Services of Essonne, France, in 2021. Fish were housed at 21 ± 1 °C under a 14:10 h light-dark cycle. All fry used for experiments were fed live *Artemia* nauplii starting at 6 days post-fertilization (dpf). This study used three populations: Pachón cave, Tinaja cave, and a surface population derived from the Choy River. Descriptions of the cave and surface localities can be found in Elliott (2018).

Artemia preparation

Approximately 24 h prior to the behavioral experiments, brine shrimp cysts (*Artemia salina*, S.K.) were added to a plastic container with 1.2 L of water at a salinity of 25–30 ppt, pH of 7.5–8.5, and temperature of 28 °C, with constant aeration. Immediately prior to testing, *Artemia* shrimp were rinsed with fresh water and placed into the recording chambers. Only newly hatched *Artemia* nauplii (1st instar stage) were used in behavioral experiments to ensure consistency in vibrational stimuli.

Previous authors have observed that nauplii use antennal strokes at frequencies between 4.1 and 7.2 Hz for swimming (Kirchner et al., 2014), but the motion of the two main antennae is not the sole source of liquid displacement. Movements of smaller antenna pairs and other body protrusions also contribute to the water vibration signals detected by *Astyanax* larvae. Using optical tracking of plasmonic nanoparticles, the same authors showed that the actual vibrations generated by random nauplii had a frequency maximum between 3.0 and 7.2 Hz, suggesting that antennae strokes are the main component. Given that not all nauplii that swim near *Astyanax* larvae trigger a strike, we calculated the frequency of strokes used by specific nauplius individuals captured by *Astyanax* larvae. Using videos of *Astyanax* larvae successfully striking at the nauplii (see below), the seconds immediately before the strikes were analyzed, and nauplius strokes were counted. Thus, we identified the preferred stroke frequency of nauplii that triggers a strike.

Recording of LPC behavior

An iPhone 12 Mini (iOS v15.5) attached to a tripod was used for recording (1080p HD) under illuminated conditions. A FLIR Grasshopper3 USB 3.0 camera (Teledyne FLIR LLC, USA)

fitted with a zoom lens (75 mm DG Series Fixed Focal Length Lens, Edmund Optic Worldwide, USA, stock #: 88-607) was used for dark conditions. Videos were recorded using the FLIR Spinnaker SDK program, Teledyne FLIR LLC, USA, (SpinView GUI, v2.5.0.80). Recordings were performed in a dark chamber illuminated with an infrared LED light source (940 nm). Dark recordings were filmed from below, similar to previous experiments (Lloyd et al., 2018). Thus, the right/left responses were inverted compared to the light recordings. All recordings were performed at 60 frames/s.

For recordings of LPC behavior of live prey, single fish were placed in a 9 cm diameter Petri dish filled with ~20 mm of water to constrict the larvae into a single focal plane. Fry were allowed to acclimate for 2 min before the start of the experiment. Approximately 30 *Artemia* nauplii were used to record feeding behavior, and fish were imaged until they completed at least four successful strikes.

For recordings of LPC behavior on a vibrating glass rod, microinjection needles were made from glass capillaries with a Narishige PC-10 Dual-Stage Glass Micropipette Puller (Narishige International, USA). Borosilicate glass capillaries are heated and pulled to obtain extremely fine needles, similar to those used for cell injection. The tip of the glass rod had a diameter of 0.15 mm, about half the size of *Artemia* nauplii. Vibration stimulus was generated using this glass rod attached to an audio speaker (8 Ohm, 0.1 W, 38 mm speaker) that produced 0, 5, 10, and 35 Hz with a function generator (TTi TG210 2 MHz). The peak-to-peak voltage was set to 21 V (vibration amplitudes were not normalized and therefore increased with frequency). The axis of the vibration was in the horizontal plane. Individual fish were placed in a 9 cm diameter Petri dish filled with 20 mm of water or a 3.5 cm diameter Petri dish filled with 3 mm of water. Fry were allowed to acclimate in the experimental room for at least 2 h and then gently transferred to the Petri dishes, where they further acclimated for 2 min before introducing the glass rod. The age of the fry tested was 16–21 dpf. An individual was deemed to have expressed LPC if it struck the glass rod one or more times during 2.5 min of stimulation.

Quantification of LPC behavior

Measurements were taken in the recording frame before the initiation of movement towards the prey, similar to Lloyd et al. (2018). Total length of the fry was measured as well as the X and Y coordinates of the edge of the nauplius closest to the fish. The X/Y intersect was standardized to the midline of the fish, below the eyes, and close to the center of the head. Measurements of each strike were plotted against the total fish length and strike distance was calculated, noting whether the strike was to the right or left side. Only successful prey captures were used, and any recording with fewer than four feeding events was excluded from analysis.

Effect of nutrition on the level of laterality was tested in genetically similar fry (siblings) but given different amounts of food. Half of a clutch of Pachón fry were fed every day from 6–14 dpf ($n=39$) while the other half of the siblings were fed on days 6–10 dpf, then tested on 14 dpf ($n=32$), after four days of fasting.

Neomycin ablation of lateral line

In *Astyanax* cavefish, both canal and superficial neuromasts are vulnerable to antibiotic ototoxicity (Van Trump et al., 2010), which can disrupt fry capture of live *Artemia* (Lloyd et al., 2018). Fry at 16–21 dpf were treated with neomycin

(500 $\mu\text{mol/L}$; Sigma, USA) for 1 h, rinsed twice in fresh water after treatment, and left in new fresh water for 5 h (Harris et al., 2003, Privat et al., 2019) before testing LPC behavior towards a vibrating glass rod.

Statistical analysis

The Mann-Whitney U test was used to calculate whether the average distance to prey that triggered a strike was the same between Pachón cavefish vs. surface fish, Tinaja cavefish vs. surface fry, and Tinaja cavefish left-side vs. right-side strikes. The Mann-Whitney U test was also used to calculate whether the number of strikes towards the right or left out of four successful LPC behaviors differed between the Pachón cavefish, Tinaja cavefish, and surface fish. The Wilcoxon signed-rank test was used to determine whether a population had a different side preference for striking other than the hypothetical average of a 1:1 ratio between sides. To evaluate the effects of nutrition level on laterality, the minimum sample size required to significantly differentiate between two means, considering specific variances in the population, was calculated following Zar (2010).

RESULTS

What type of stimulus activates LPC?

Our results showed that the *Artemia* nauplii that preferentially triggered successful strikes from *Astyanax* larvae exhibited an antennae stroke frequency of 8.8 Hz (± 1.3 standard deviation (SD), $n=15$). These observations corroborate the findings of Lloyd et al. (2018) that surface fish in the dark and given live prey display a stereotyped strike preference towards their side using a C-bend turn (Figure 1A). This behavior involves initially turning the head towards adjacent prey (Figure 1B), followed by opening of the mouth, presumably for water suctioning (Figure 1C), which is then expelled through the gills (Figure 1D).

However, moving *Artemia* nauplii may generate a plethora of signals (visual, chemosensory, mechanosensory), with multimodal sensory processing potentially activating LPC. To elucidate whether vibrations can singlehandedly trigger LPC, surface larvae aged 16–21 dpf were presented with a 0.15 mm glass rod, similar in size to *Astyanax* fry prey, vibrating at 5–35 Hz. Their response was then compared to LPC induced by live prey. The vibrating glass rod elicited the same stereotyped strike response in surface fish, i.e., biting the glass rod following a C-turn maneuver. Of note, even in illuminated conditions, where surface fish can likely discern that the source of the vibrations does not resemble typical prey, such as a microcrustacean, the larvae still engaged in LPC (Figure 1E–H).

In response to the presentation of a vibrating glass rod, the cavefish also exhibited LPC, with a consistent preference for prey located by their side, accompanied by the characteristic C-bend strike (Figure 2A–E). Both types of fish performed a forward strike when the glass rod was in front of their face (Figure 2F–J), although this was less frequently observed. On multiple occasions, an individual would repeatedly bite the glass rod within a period of less than a second in a feeding frenzy (Figure 2K–O). Some fish also engaged in multiple, independent LPC behaviors, interspersed with periods of swimming before returning to strike at the glass rod. During the 2.5 min observation period per individual, the maximum number of strikes seen was seven. This suggests that vibrations alone can trigger consecutive LPC behaviors,

despite the absence of stimuli, such as taste and smell, that are present when striking microcrustacean.

Is LPC the same as adult VAB?

LPC and VAB are both foraging behaviors initiated in

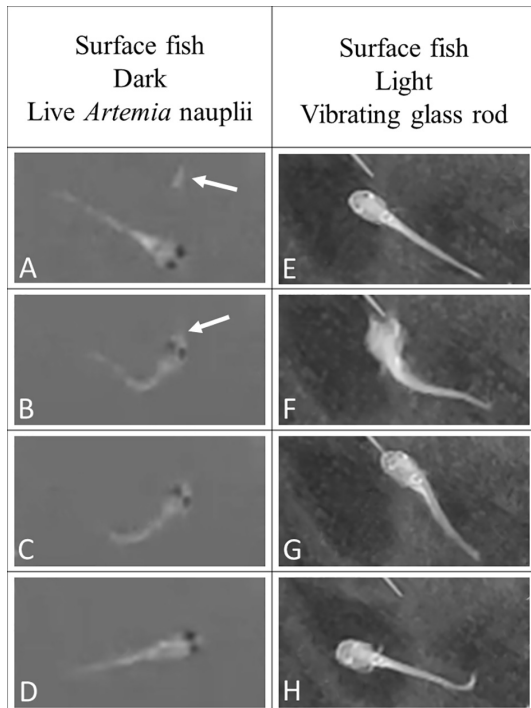


Figure 1 Vibrations singlehandedly elucidate LPC behavior
 A–D: Surface fish in the dark strike preferentially towards prey (arrow pointing at *Artemia* nauplii) on their side using a C-bend maneuver, in which the head initially turns towards adjacent prey. E–H: Upon presentation of a vibrating glass rod, surface fish display the same stereotyped strike and bite the glass rod (G). Even under illuminated conditions, when surface fish can likely see that the source of the vibrations is not a microcrustacean, vibrations still trigger LPC.

response to vibration stimuli. However, our results suggest that these behaviors may arise from at least partially distinct multimodal sensory processing mechanisms. VAB emerges in cavefish at approximately 3 months of age, coinciding with the increase in the number and size of orbital superficial neuromasts, which begins at about 2 months (Yoshizawa et al., 2010). VAB is evoked by vibration stimuli peaking at 35 Hz, which is linked to the mechanosensory function of the lateral line (Yoshizawa et al., 2010). In contrast, our results showed that cavefish larvae exhibited LPC at 16–21 dpf, some 2 months earlier than when VAB is first detected. To determine whether LPC shows a distinct frequency response peak compared to VAB, cavefish larvae were presented with a glass rod under 0, 5, 10, and 35 Hz vibration stimuli. As seen in Figure 3, LPC behavior reached a maximum at a vibration stimulus of 10 Hz, in contrast VAB, exhibits optimal responsiveness at 35 Hz (Yoshizawa et al., 2010).

Finally, we measured LPC in Pachón cavefish after neomycin treatment, which inhibits neuromast function without detectable effects on the inner ear (Yoshizawa et al., 2010). Results showed that lateral line functionality was significantly impaired in the tested fry. Their swimming became erratic, and they were unable to detect the walls or engage in the typical circular swimming behavior along the edges of the Petri dish. Despite these impairments, LPC behavior was still observed, with six of the nine treated specimens (66.6%) still striking at the glass rod vibrating at 10 Hz. This finding suggests that the treatment, which effectively eliminates VAB in adult *Astyanax*, did not exert a similar effect on LPC when confronted with a vibrating glass rod. Thus, LPC behavior involved in processing vibrations with a peak frequency of 10 Hz is likely mediated through mechanisms that differ, at least partially, from those observed in adult VAB.

Are there differences between surface and cave LPC?

Our results indicated that both Tinaja and Pachón fry exhibited a tendency to strike at prey located further away from their bodies compared to surface fish under dark conditions

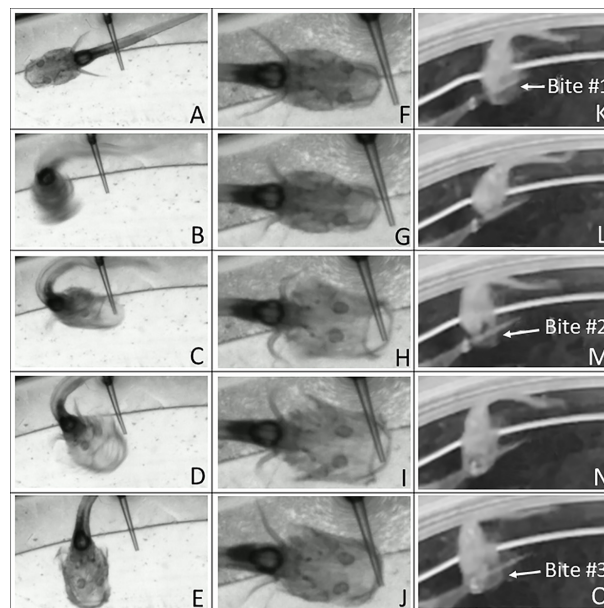


Figure 2 Vibrations from a glass rod trigger LPC in Pachón cavefish fry
 A–E: Stereotypical C-bend strike, where the fry preferentially strikes at a source of the vibrations on its side (A) by first bending its head (B), biting the glass rod (C), and expelling suctioned water through its gills (D, E). F–J: Forward strike. K–O: Vibrating glass rod can even generate “feeding frenzies”, whereby an individual rapidly and repeatedly bites the source of the vibrations.

(Figure 4A–C). This is consistent with the findings of Lloyd et al. (2018), showing that cavefish may have enhanced prey detection capabilities in darkness compared to surface fish. To account for potential developmental differences between cavefish and surface fish, we attempted to normalize the data. For example, cave embryos possess a slightly larger yolk, which is absorbed at a later developmental stage (Hinaux et al., 2011). Furthermore, longer fish could potentially strike at a greater distance. When strike distance was divided by body length, although both Pachón (average strike distance=0.28 body lengths±0.17 SD, n=61) and Tinaja (average strike distance=0.30±0.21, n=55) struck proportionally farther than surface fish (average strike distance=0.26±0.16, n=29), the differences were not

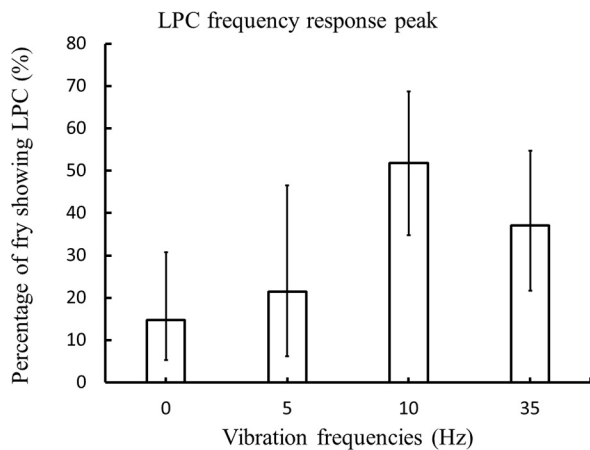


Figure 3 Relationship between LPC and vibration frequencies (Hz) in Pachón cave fry

Results suggest that striking is triggered by a stimulus centered at around 10 Hz. Confidence intervals were obtained through a binomial exact calculation using a 90% confidence level. *n* for 0, 10, and 35 Hz=27; *n* for 5 Hz=14.

significant (Pachón vs. Surface, $P=0.13$; Tinaja vs. surface, $P=0.08$).

When provided with live nauplii, the expression of LPC varied among the three populations. Surface fish displayed no significant preference for striking prey on either their right or left side, whereas cavefish expressed behavioral lateralization or side preference for striking. Among 29 surface fry, out of four successful strikes, an average of 2.14 ± 1.09 SD strikes were directed to the right (total 62 R: 54 L), which does not significantly deviate from the 1:1 ratio or expectation of two strikes to the right ($P=0.511$). In contrast, cavefish exhibited a clear preference for striking on one specific side. Among 61 Pachón cavefish fry, out of four successful strikes, an average of 2.43 ± 0.69 SD strikes were directed to the right (total 148 R: 96 L), deviating significantly from the 1:1 ratio ($P=0.001$). Among 55 Tinaja fry, out of four successful strikes, an average of 1.71 ± 1.05 SD strikes were directed to the right (total 114 R: 161 L, $P=0.050$). Thus, surface fry showed no side preference for LPC, whereas Pachón showed a preference for the right and Tinaja showed a preference for the left side (Figures 4D–F, 5). These results were replicated with a vibrating glass rod. Out of 72 available strike videos, a clear side preference was established. In Pachón fry, 52 (72.2%) strikes were performed when the glass rod was on their right side, while 20 (27.7%) strikes were performed on their left side ($P=0.0002$).

Mathematical model for laterality advantage in cavefish

A simple geometrical model for prey detection by fish was developed. This model can be used for many multisensory modalities but was applied here to clarify laterality of LPC in blind cavefish, with wave detection at around 10 Hz.

1. Definition of “range”: Let this model depend on the “range” at each side: i.e., r_r (right) and r_l (left), determined by the radii of two semicircles or semispheres for two- (2D) and three-dimensional (3D) cases, respectively. A graphical depiction of r_r and r_l is presented in Figure 6A.

For fish with no laterality $r_r=r_l$, but in the asymmetrical case,

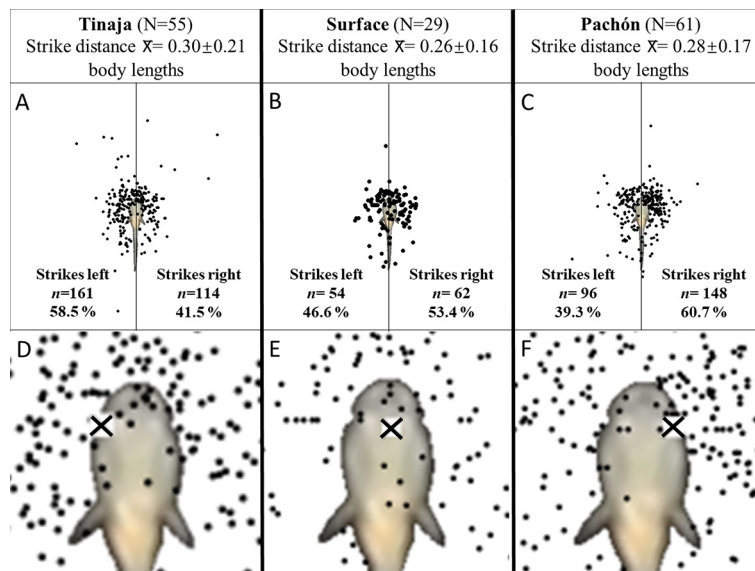


Figure 4 Position of prey before a successful strike

Dots show location of *Artemia* nauplii before strike in relation to position of the fry, represented by a sketch of the fish. A–C: Both Tinaja and Pachón cave fry strike slightly farther away from their body than surface fish, although differences were below statistical significance. D–F: Median position of prey prior to the strike (X) differed among populations, located in the center of the head in surface fish, towards the left, approximately below the eye, near where the left SO3 suborbital bone will develop in Tinaja cavefish, and towards the right side, also below the eye, near where the SO3 suborbital bone will develop in Pachón cavefish.

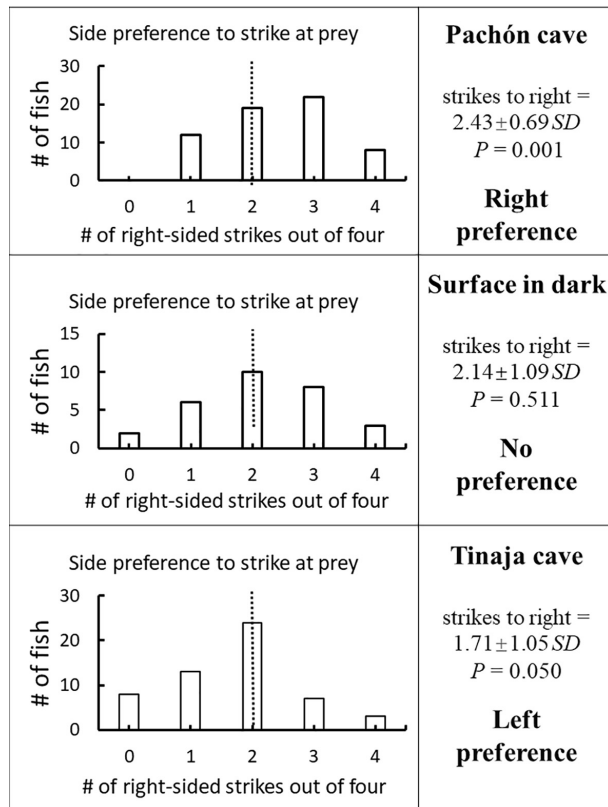


Figure 5 Laterality of LPC expression in cavefish

Each individual fish was evaluated based on four successful strikes directed at live nauplii, with the null hypothesis suggesting no side preference for striking, resulting in an equal distribution of two strikes to the right and two strikes to the left (indicated by the dashed line). While surface fish struck equally at either side of their body, Pachón cavefish preferred to strike right, while Tinaja cavefish preferred to strike left.

fish express laterality $r_r \neq r_l$. If there is a “strong” side, for instance, the left side, r_r may diminish relatively as more finite resources are devoted to extending the magnitude of r_l . The simplest model can be considered as:

$$r_r + r_l = 2r_0 \quad (1)$$

where r_r =right radius, r_l =left radius, and r_0 is a constant value. In the simplest model, the constant r_0 can be interpreted as the radius corresponding to a symmetrical fish with no laterality. A very general relationship can be written, as follows:

$$r_r + r_l = f(r_r, r_l) \quad (2)$$

where $f(r_r, r_l)$ represents a function of the ranges r_r and r_l .

2. Size of detected area or volume

Two-dimensional case: Physically, this case can be related to surface waves. Area A in which vibrating prey may be detected can be written as:

$$A = \frac{\pi}{2} (r_r^2 + r_l^2) \quad (3)$$

considering the simplest model ($r_r + r_l = 2r_0$), this yields:

$$A = \frac{\pi}{2} [r_r^2 + (2r_0 - r_r)^2] = \pi [(r_r - r_0)^2 + r_0^2] \quad (4)$$

Area A is a quadratic function of the range r_r . By letting A_d be the asymmetric fish detection area over the symmetric equivalent, then:

$$A_d = \frac{\text{asymmetric detection area}}{\text{symmetric detection area}} = \frac{A}{\pi r_0^2} = \left(1 - \frac{r_r}{r_0}\right)^2 + 1 \quad (5)$$

The ratio r_r/r_0 is the right-side range over the symmetrical one, which goes from 0 to 1 as we assume $r_r < r_0$. The A_d against r_r/r_0 plot is presented in Figure 6B, which shows the geometrical advantage of laterality ($A_d > 1$), where advantage increases as asymmetry grows.

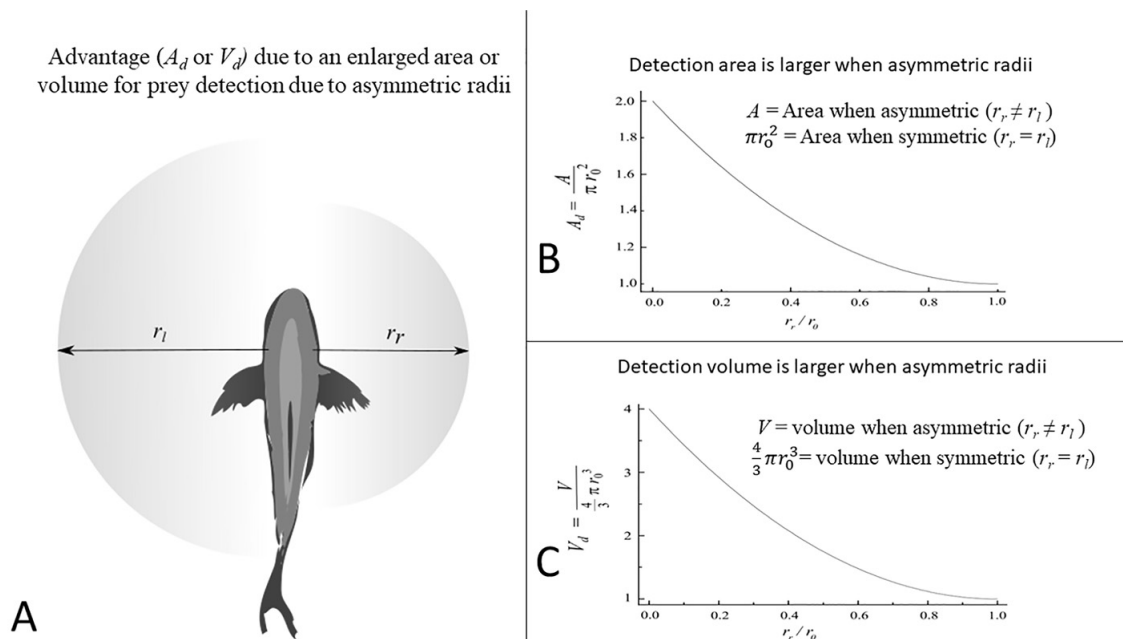


Figure 6 Total detection area or volume increases when radius on one side increases proportionally to a decrease on the other

A: Schematic of two detection semicircles dictated by their radii. When the radius on the left side is increased by a certain unit, and the right side is decreased by that same unit, the increase in area of the left semicircle is larger than the loss in the right semicircle. B: Advantage in the detection area of surface waves on a 2D plane when the radii are different on each side. C: Advantage is even larger when considering two 3D semispheres with different radii.

Three-dimensional case: Here, volume V in which a vibrating prey may be detected can be written as:

$$V = \frac{2}{3}\pi(r_r^3 + r_l^3) \quad (6)$$

considering the simplest model ($r_r+r_l=2r_0$), this yields:

$$V = \frac{2}{3}\pi[r_r^3 + (2r_0 - r_r)^3] = \frac{4}{3}\pi[r_0^3 + 3r_0(r_0 - r_r)^2] \quad (7)$$

By letting V_d be the asymmetric fish detection volume over the symmetric equivalent, then:

$$V_d = \frac{\text{asymmetric detection volume}}{\text{symmetric detection volume}} = \frac{V}{\frac{4}{3}\pi r_0^3} = 3\left(1 - \frac{r_r}{r_0}\right)^2 + 1 \quad (8)$$

The V_d against r_r/r_0 plot is presented in Figure 6C. While the profile is also parabolic, in the 3D case, the advantage of asymmetry over symmetry is more pronounced.

3. Food abundance

If food is over-abundant, an increase in detection volume or area will not be significantly advantageous because individuals, regardless of having an extensive or small range, will find enough food to survive and reproduce. Yet, if food is scarce, a larger range may be selected (Figure 7A, B). To introduce this parameter into our model simply, the following exponential function is considered, which represents the dependence of fish fitness on food abundance ρ and range ratio r_r/r_0 , for the 2D case:

$$a_{2D} = 1 - e^{-A_d\rho} = 1 - e^{-\left(1 - \frac{r_r}{r_0}\right)^2 + 1}\rho} \quad (9)$$

and for the 3D case:

$$a_{3D} = 1 - e^{-V_d\rho} = 1 - e^{-\left(3\left(1 - \frac{r_r}{r_0}\right)^2 + 1\right)\rho} \quad (10)$$

The functions a_{2D} and a_{3D} can be interpreted as survival

rates. If there is no food, survival is null, but as food abundance grows, survival increases. At first, survival grows linearly with food abundance ρ , but when food is so abundant that fish stop competing and all individuals can eat what they need, ρ can keep growing, but the survival rate will reach a constant value. The initial slope of the curve is greater for asymmetrical individuals expressing laterality as they can sense more area and/or volume than symmetrical individuals. Laterality provides an advantage when food is scarce. Figure 7C, D displays a_{2D} and a_{3D} as a function of food abundance for several values of r_r/r_0 .

The ratio between $a_{2D}(\rho, r_r/r_0)$ and $a_{2D}(\rho, r_r/r_0=1)$ can be interpreted as an indicator of advantage, represented by the function a_{2D} of both variables ρ and r_r/r_0 over the same function evaluated at $r_r/r_0=1$, which corresponds to the symmetrical case. This advantage is shown in Figure 7E-F for the 2D and 3D cases, respectively, where any value greater than 1 represents an actual advantage for laterality.

4. Disadvantage of laterality

Laterality in fish may be associated with specific asymmetries in their body or physiology, which may have various disadvantages. For example, cave-adapted fish have evolved pronounced cranial asymmetries, characterized by a “bend” in their skulls along the anteroposterior axis, most often biased towards the left (Powers et al., 2017). Such asymmetric body patterns may negatively affect swimming performance. For example, unequal lateral hydrodynamic resistance (drag) may be energetically costly and result in a propensity for swimming in circles when visual cues are absent (Figure 8A).

Additional asymmetries may also occur in the musculoskeletal system, energy allocation, and distribution of resources in the brain (Figure 8B) and sensory receptors. While these asymmetries may provide strength or precision in responses on one side, they may weaken the other side for

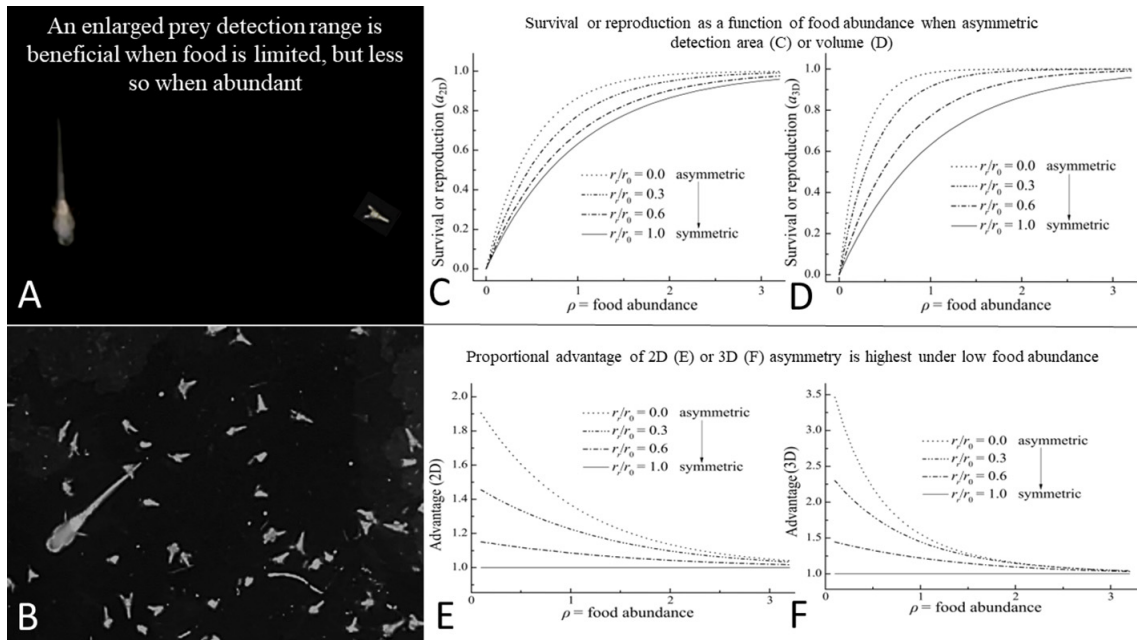


Figure 7 Correlation between advantages resulting from an expanded range due to laterality and food abundance

A, B: If food is over-abundant, an increase in detection volume or area will not be significantly advantageous because individuals, regardless of having an extensive or small range, will find enough food to survive and reproduce. C, D: Survival or reproduction as a function of food abundance for several 2D or 3D range values due to asymmetry. E, F: Advantages of an enlarged 2D or 3D range due to asymmetry depending on food abundance.

other tasks, such as escaping predators (Figure 8C) or responding to signals other than LPC. Fernandes et al. (2022a; 2022b) hypothesized that sharp turns consume more calories than typical swimming and further suggested that fish that use unilateral approaches may execute steeper turns more often than those that use bilateral approaches, resulting in energy loss.

To account for the aforementioned (and other) disadvantages that may be generated by laterality, a_{2D} and a_{3D} are modified by multiplying the original function by the factor $f = \frac{1}{2} \left[1 + \left(\frac{r_r}{r_o} \right) \right]$, then dividing the exponential argument by this same factor to yield:

$$b_{2D} = f \left(1 - e^{-\frac{A_d}{f} \rho} \right) = \frac{1}{2} \left(1 + \frac{r_r}{r_o} \right) \left[1 - e^{-\left[\frac{\left(1 - \frac{r_r}{r_o} \right)^2 + 1}{2 \left(1 + \frac{r_r}{r_o} \right)} \right] \rho} \right] \quad (11)$$

and

$$b_{3D} = f \left(1 - e^{-\frac{V_d}{f} \rho} \right) = \frac{1}{2} \left(1 + \frac{r_r}{r_o} \right) \left[1 - e^{-\left[\frac{3 \left(1 - \frac{r_r}{r_o} \right)^2 + 1}{2 \left(1 + \frac{r_r}{r_o} \right)} \right] \rho} \right] \quad (12)$$

so that the horizontal asymptotes in b_{2D} and b_{3D} are smaller if asymmetry is higher, but the initial slope is maintained. Figure 8D-E shows b_{2D} and b_{3D} as a function of food abundance ρ for several values of r_r/r_o . Under meager food conditions, laterality is favored, but as food becomes less scarce, different values of r_r/r_o favor no laterality. Just as a_{2D} and a_{3D} , the functions b_{2D} and b_{3D} can be interpreted as

survival rates. Again, the ratios $\frac{b_{2D}(\rho, \frac{r_r}{r_o})}{b_{2D}(\rho, \frac{r_r}{r_o} = 1)}$ and $\frac{b_{3D}(\rho, \frac{r_r}{r_o})}{b_{3D}(\rho, \frac{r_r}{r_o} = 1)}$

can be interpreted as an advantage, plotted in Figure 8F, G for the 2D and 3D cases, respectively. In conclusion, when high food availability is such that no competition occurs for food resources, asymmetrical individuals are disadvantaged. They do not gain from laterality, but incur the costs caused by their asymmetry. However, when food resources are low, such that the advantages of an enlarged range for detecting prey outweigh the losses caused by asymmetry, laterality is advantageous, as seen in Figure 8D–G.

5. Supporting data for proposed mathematical model

Based on our proposed mathematical model, populations with different evolutionary histories under different environments are also expected to vary in their expression of laterality. The Pachón and Tinaja caves have experienced different evolutionary histories, as observed by independent mutations in *Oca2* (Protas et al., 2006) and *Mc1r* (Gross et al., 2009). As predicted by the model, these two cavefish express different patterns of laterality. While the model was initially designed for LPC, it can be extended to other foraging behaviors using different sensory perceptions for organisms living in dark environments. For example, in the context of VAB, Espinasa et al. (2020) showed that adult Pachón cavefish preferentially explore a vibrating object with their right side, while Tinaja cavefish preferentially explore with their left side.

Based on our hypothetical model, it is expected that laterality expression will be enhanced in populations where food is a limiting factor, depending upon the balance between the advantages and disadvantages of asymmetry (Figure 8F, G). Espinasa et al. (2022) demonstrated that in pools with varying food quantities, highly responsive cavefish in VAB

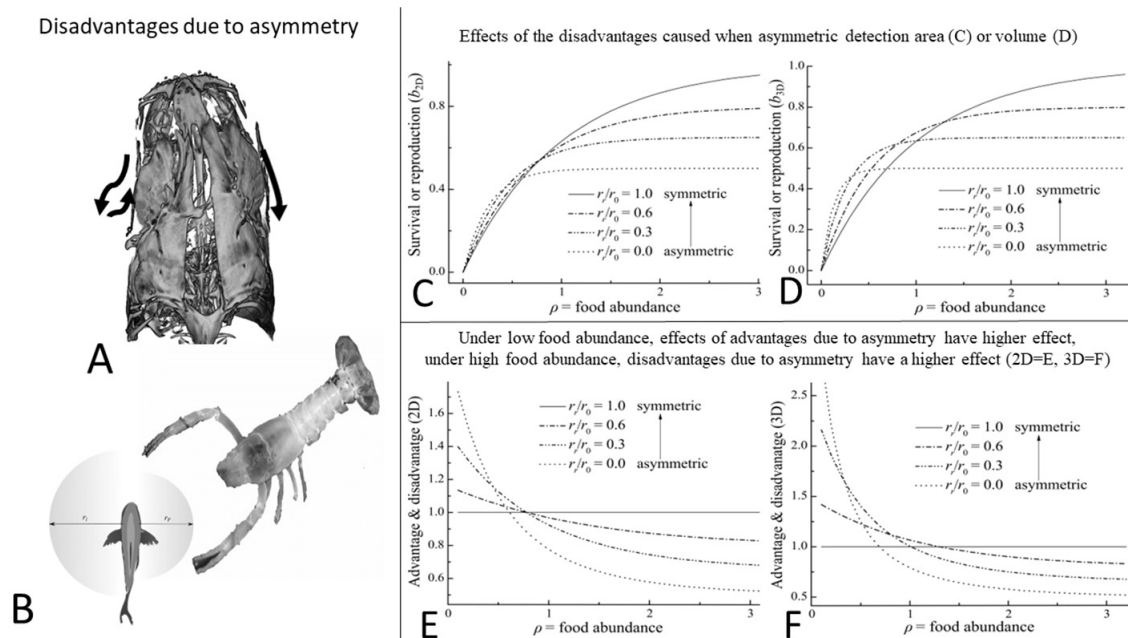


Figure 8 Disadvantages due to asymmetry vs. advantages conveyed by an enlarged range due to laterality

A, B: Asymmetry can reduce survivorship or reproduction in various ways. Fluid dynamics can create drag on one side, wasting energy to compensate for swimming (A). It can also require additional energy or neuronal network allocation in the brain, or decreased perception of predators on one side (B). C, D: Survival or reproduction may be lower when asymmetric, thus lowering the asymptote. E, F: When considering the advantages of an extended prey detection range resulting from laterality, food abundance, and the costs associated with asymmetry, the expression of laterality is likely to vary among populations based on their specific environmental conditions. Populations inhabiting food-poor environments may exhibit a preference for laterality, while those in food-rich environments may lean towards symmetry. A was adapted from Powers et al. (2017).

exhibit a higher proportion of left-side utilization compared to less responsive individuals. According to our results, nutritional state may also have a minor effect on the level of laterality. Pachón fry fed every day from 6–14 dpf ($n=39$) displayed an average of 2.17 ± 1.07 SD strikes (out of four successful strikes) to the right side, while siblings from the same clutch fed on days 6–10 dpf and tested at 14 dpf ($n=32$) after four days of fasting showed an increased preponderance for laterality (2.25 ± 1.18 strikes to the right out of four successful strikes). However, statistical differentiation between the two groups was not attempted due to the substantial sample size required (over 2 000) to detect an average difference of 0.08, considering variance in the sample.

The proposed model also indicated that the total detection area or volume increases when the radius on one side increases proportionally to a decrease on the other. Thus, it is expected that the distance at which prey is detected may vary between one side and the other. Plotting the median position of nauplii at the time of the strike revealed that in surface fish, it is located near the center of the head, while in cavefish, it is shifted to one side, specifically the right side in Pachón fry and the left side in Tinaja fry (Figure 4D, F). These findings align with the expected laterality preference exhibited by cavefish and lack of laterality exhibited by surface fish. Interestingly, despite Pachón and Tinaja having different preference sides, both cave populations displayed a consistent median placement near the base of the eyes when observed from a dorsal view, corresponding to the future development site of the SO3 suborbital bone and the location of the main neuromasts associated with adult VAB. In the case of Tinaja, a total of 126 and 94 recorded strikes were directed towards nauplii on the left and right sides, respectively. The model's assumption of a difference in detection radii between the two sides was supported by the observation that attacks towards the left occurred, on average, at a greater distance than those towards the right side (0.32 body lengths ± 0.21 vs. 0.29 ± 0.22 ; $P=0.04$).

DISCUSSION

LPC behavior is characterized by a fast-striking motion towards prey that takes place within tens of microseconds. LPC appears to be a plastic behavior that is modulated to integrate stimuli available most efficiently within a given environment. Under light conditions, with or without a functional lateral line, *Astyanax* surface fry primarily rely on vision, mainly striking prey directly in front of them, bending the most caudal region of their tail, similar to a J-turn (Lloyd et al., 2018). However, when confronted with the absence of visual stimuli under darkness, cavefish and surface fish rely on different sources of information to achieve successful LPC. They are attuned to activate strikes mostly at prey on their side, using a C-bend turn maneuver that involves turning the head towards adjacent prey (Lloyd et al., 2018). In zebrafish, distinct sensory inputs activate different neural circuits that result in C and J turns (Fajardo et al., 2013; Liu & Fetcho, 1999), supporting the notion that LPC is a plastic, multimodal sensory process.

In the dark, the LPC sensory mode of surface fry appears to rely almost exclusively on vibrations detected by the lateral line. When presented with non-moving, dead nauplii, or when treated with gentamicin or neomycin, striking disappears completely (Lloyd et al., 2018). In contrast, cavefish LPC can rely on other sources of information apart from those provided

by the lateral line. Notably, cavefish presented with non-moving, dead nauplii, or treated with neomycin, can still execute successful LPC (Lloyd et al., 2018).

Not only do cavefish rely on other yet-to-be-identified sensory modes, but they exhibit more efficient prey hunting. Our observations, as well as those of Lloyd et al. (2018), indicate that cavefish can strike at prey at farther distances relative to surface fish (Figure 4). This enhanced feeding behavior is supported in previous competition assays, where cavefish (17 dpf) are reported to consume more *Artemia* than surface fish under dark conditions (Espinasa et al., 2014). This enhancement is not related to the presence of eyes or training to hunt without visual cues, as eye ablation does not affect food consumption in surface fish (Espinasa et al., 2014). Thus, these skills are primarily modulated by processes independent of eye loss, e.g., Shh-independent processes, such as enhancement of superficial neuromast activity, as well as enhancement of mechanosensors, chemical sensors (nasal epithelium and tastebuds), or brain performance.

Our study showed that vibrations can singlehandedly elicit LPC in surface fish and cavefish (Figures 1, 2). At 16–21 dpf, larvae presented with a vibrating glass rod equivalent in size to normal prey of *Astyanax* fry elicited stereotypical striking using the C-bend turn maneuver. Even under light conditions, where surface fish can likely see that the source of the vibrations is not a stereotypical prey, such as a microcrustacean, a vibrating glass rod was sufficient to elicit striking behavior. Furthermore, the physical presence alone of an object of prey size (e.g., non-vibrating glass rod) was sufficient stimulus to elicit LPC in cavefish, although in smaller numbers. This suggests that cavefish use multisensory modes for LPC.

Both LPC and VAB are foraging behaviors triggered in response to vibration stimuli, with the former adapted for larvae and the latter adapted for adults. Espinasa et al. (2017) reported distinct feeding habits between post-larval and adult *Astyanax* cavefish. Furthermore, LPC and VAB also appear to arise, at least partially, from different sensory processing. VAB, which is first detected at 3 months in cavefish, is evoked by vibration stimuli peaking at 35 Hz, and is linked to the mechanosensory function of the lateral line (Yoshizawa et al., 2010). In contrast, cavefish larvae exhibit LPC shortly after birth, evoked by vibration stimuli peaking at 10 Hz. Notably, LPC remains actively evoked towards a vibrating rod even when the mechanosensory function of the lateral line has been compromised with neomycin.

Our findings, together with previous research (Lloyd et al., 2018), indicate an evolutionary shift in LPC towards dependence on non-visual cues in cavefish as an adaptation to their dark environments. Moreover, our results suggest that the evolution of LPC may have occurred via independent and parallel evolutionary processes in different cavefish populations. While both Pachón and Tinaja fry demonstrate enhanced LPC behavior, with successful strikes performed farther away from prey than those performed by surface fry, they are performed differently. Pachón fry significantly prefer to strike at their right side, while Tinaja fry show a preference for their left. In agreement, Espinasa et al. (2022) found that adult cavefish populations express behavioral lateralization or side preference when examining a vibrating object. Expression of VAB differs among the cave populations, with the Pachón cavefish showing preferential “handedness” on the right side, while the Tinaja and other cave populations (Sabinos, Molino, and Toro) show preferential exploration on their left side. Thus, adult VAB behavior in the field and LPC

behavior in the lab show the same preponderance for laterality and side preference in both cavefish populations.

Espinasa et al. (2022) hypothesized that during the evolution of foraging in darkness, laterality was enhanced through asymmetric sensitivity for different modes of stimuli or lateral swimming preference. Furthermore, they hypothesized that such an adaptive effect would favor asymmetry itself, rather than a specific side being specialized. This concept is supported by both VAB and LPC, which show different side preferences in different cave populations. However, why should asymmetric sensitivity or lateralization of behavior result in enhanced performance? To address this, we developed a mathematical model to explain laterality based on a balance between; 1) enlarged range due to asymmetry (behavioral or perceptive), 2) food abundance, and 3) disadvantages caused by laterality.

While our study concentrated on the detection of vibrations and LPC, our mathematical model can be applied to all sensory information gathered by sensory organs positioned on either side of the body, as they may play crucial roles in organizing brain and behavioral lateralization. For example, Cavelius et al. (2022) reported species-specific performances favoring one nostril over the other for odorant detection and active sampling, depending on odorants or contexts. These behavioral asymmetries may be attributed to peripheral anatomical or functional asymmetries in certain species. Cavelius et al. (2022) further reported instances of nostril asymmetry detection where an enlarged olfactory field, as predicted by our mathematical model, can have survival implications: e.g., fear odor emitted by other species (left) vs. fear odor emitted by conspecifics (right) in canids, or repulsive (left) vs. attractive stimulus (right) in rodents. Even in humans, detection of n-butanol exhibits different nostril sensitivity, with left- and right-handed subjects showing significant differences, i.e., left-handed subjects consistently showed greater sensitivity in the left nostril, while right-handed subjects showed a weak tendency towards greater sensitivity in the right nostril (Youngentob et al., 1982).

Fernandes et al. (2022a; 2022b) also proposed a mathematical model to explain laterality in adult cavefish when expressing VAB. In their model, they assume that fish performing rapid and short turns during food capture use significantly more calories than during typical swimming motions. Their model predicts that a unilateral sensing population that is not lateral in their behavior (i.e., uses bilateral approaches) has a higher chance of survival in a diet-poor environment. Thus, their model predicts that a bilateral sensing population of fish is selected in diet-rich environments, but unilateral or bilateral approaches are equally selected. In other words, their model predicts the opposite of our model in that, in a diet-poor environment, reduced behavioral laterality is expected.

What can explain the disagreement between our model and that of Fernandes et al. (2022a; 2022b)? One possible explanation is that different models are being studied. Fernandes et al. (2022a; 2022b) explored VAB in adult fish from Tinaja cave, while we explored LPC in 14-day-old fry from Pachón cave. Their results also appear to contradict prevailing understanding of cave ecology, with many studies suggesting that cave environments generally exhibit lower food availability due to the absence of autotrophic production (Aspiras et al., 2015). It seems contradictory that their mathematical model predicts an increased expression of

behavioral laterality in environments with abundant food, whereas both their study and ours demonstrate that cave populations exhibit higher levels of behavioral laterality compared to surface fish. Additionally, Fernandes et al. (2022a; 2022b) suggested that well-fed fish do not display behavioral laterality, as evidenced by their tests on 2-3-year-old fish fed daily from surface and cave populations (including Pachón, Tinaja, and Sabinos caves), as well as 1-year-old surface and Pachón cavefish populations, with only a single test involving 1-year-old Tinaja fish showing behavioral laterality.

The Tinaja specimens used in Fernandes et al. (2022a; 2022b) have also shown conflicting results in the past, perhaps due to the bottleneck effects of captivity. Yoshizawa (2016) described the Pachón, Sabinos, Piedra, and Toro cave populations as showing VAB, but not the surface and Tinaja cave populations. Espinasa et al. (2021) reported active expression of VAB in Tinaja cave, but very reduced VAB in the particular Tinaja pool from which the specimens used by Yoshizawa (2015) were originally collected. Fernandes et al. (2022a; 2022b) used the same Tinaja laboratory colony as Yoshizawa (2015), previously reported to lack apparent VAB, to demonstrate the presence of VAB laterality in 1-year-old fish. As such, caution should be exercised when interpreting results of this particular breeding colony.

As reported in Espinasa et al. (2021), VAB is a plastic trait in the blind Mexican tetra, displaying variations within subpopulations residing in the same cave and even among pools located in close proximity, responding to local environmental conditions. Fernandes et al. (2022a; 2022b) and our proposed mathematical model support the notion that the expression of laterality in VAB and/or LPC is dependent on food availability and the trade-offs associated with asymmetry. Future studies will corroborate and establish whether food-rich or food-poor environments are correlated with, or cause, enhanced expression of laterality in foraging and prey capture behavior.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

L.E. conducted experiments with live *Artemia* and glass rods, created the mathematical model, wrote the first manuscript, and provided funding. R.D. created the mathematical model and reviewed the manuscript. E.V. conducted experiments with glass rods and reviewed the manuscript. M.E. conducted experiments on the swim frequency of *Artemia* nauplii and reviewed the manuscript. All authors read and approved the final version of the manuscript.

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