RESEARCH ARTICLE

Eye convergence is evoked during larval prey capture (LPC) without visual stimulus and in blind cavefish

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Abstract

In zebrafish larvae, the first response when detecting prey is an oculomotor behavior; eye convergence. Eye convergence increases the overlap between the visual fields of the left and right eyes to prepare for tracking prey. A high vergence angle is maintained throughout the prey-tracking and capture swim phases, enhancing binocular depth. Since the discovery of eye convergence, hundreds of articles reporting on this behavior in zebrafish have been published. In this study, we found that the larvae of blind tetra cavefish, *Astyanax mexicanus*, despite being adapted to the absence of visual stimuli due to the lack of light in the cave, have retained the oculomotor behavior of eye convergence in their vestigial eyes. In *Astyanax*, eye convergence responses can be triggered singlehandedly by vibrations elicited with a glass rod at frequencies similar to those generated by its prey (10–35 Hz). The blind cave tetra offers an intriguing combination of regression of the eye structure, while retaining several of the physiological functions and actions performed in the eye, including light-entrained retinomotor rhythms and eye convergence.

Keywords

Astyanax, behavior, binocular vision, eye convergence, larval prey capture, ocular vergence, troglobite

Introduction

Darwin (1872) recognized descent with modifications during evolution by observing remnant structures, such as the reduced wings of flightless birds, the hind limb remnants of pythons, and the degenerate eyes of blind cave organisms. A vestigial structure is part of an organism that has diminished in size during its evolution because the

function it served decreased in importance or became unnecessary (Bergman 2000). The regressive phenotypes of cave animals puzzled Darwin, who famously remarked, "As it is difficult to imagine that eyes, though useless, could in any way be injurious to animals living in darkness, I attribute their loss solely to disuse."

Often, natural selection cannot eliminate vestigial structures because they have retained some essential function. For example, the human embryo has gills slits like a fish. Why are the gill slits retained? During development, these gills become various structures essential for survival, such as the ear cavities, middle ear bones, muscles for chewing, the lower jaw, and certain parts of the neck including the thymus and thyroid (Manley and Capecchi 1998). Likewise, vestigial structures can acquire new functions. For instance, male pythons have little, claw-like structures derived from the hindlimbs of their four-legged ancestry. These vestigial structures now aid with courtship (Bejder and Hall 2002).

Cave animals are excellent models which can provide insight into the general principles of regressive evolution. Many organisms in caves retain features that can no longer serve their ancestral purpose. For example, the Cholevid beetle, *Ptomaphagus hirtus*, common in Mammoth Cave, Kentucky, has tiny vestigial eyes that retain light perception and have a complete circadian clock gene network (Friedrich et al. 2011). Likewise, the cave amphipod, *Stygobromus allegheniensis*, from the Ice caves in N.Y., follows circadian rhythms albeit modified (Espinasa et al. 2016). In the family Gonyleptidae, cave-dwelling opilionid species possess elongated appendages but retain some of the pigmented layer and the eye's lens (Pérez and Kury 2002).

Studies of regressed structures generally assume them to be nonfunctional (Fong et al. 1995). However, and in following with Darwin's statement that something useless may not necessarily be in any way injurious to animals, Espinasa and Jeffery (2006) questioned whether the loss of the physiological function and the cessation of the actions performed by an organ is a prerequisite for the structural degeneration of an organ. The blind cave tetra, *Astyanax mexicanus* offers an intriguing combination of regression of the structure of the eyes, while retaining some of the physiological functions and actions performed in the eye (Espinasa and Jeffery 2006); In teleosts living in surface habitats, during the day the cells in the retinal pigmented epithelium (RPE) disperse their pigmented granules, shielding photoreceptors from excessive light (King-Smith et al. 1996). At night, the situation is reversed and exposed photoreceptor can catch the maximum amount of available photons. Light and an endogenous circadian rhythm regulates the movement of cones and rods (Burnside 2001). In the blind tetra, fry retain the capacity to exhibit light-entrained retinomotor rhythms that move their retinal pigmented epithelium (Espinasa and Jeffery 2006). These retinomotor rhythms would appear to have no function in blind cavefish. Movements of an unpigmented RPE would not reduce the extent of bleaching of photoreceptors, which are essentially absent in cavefish. Likewise, maintenance of retinomotor activity would not be expected to optimize visual capabilities in cavefish, which do not experience visual stimuli in the cave, nor a daily circadian rhythm is expected in a habitat that does not experience light differences between day and night.

Adult *Astyanax* cavefish have minute optic capsules buried deep beneath the integument, which are not responsive to visual stimuli (Voneida and Sligar 1976; Voneida and Fish 1984). Surface fish have large eyes (Fig. 1A, B). Despite the absence of functional eyes in adult cavefish, eye primordia, including the lens vesicle, the optic cup, most of the retina, and the RPE are initially formed in embryos (Yamamoto and Jeffery 2000). This is consistent with both surface and cavefish fry showing strong adverse reactions when exposed to intense light. This reaction persists for a few days after birth. For a short period, a normal-appearing retina, including ganglion, bipolar, horizontal, and amacrine cells, is present (Jeffery et al. 2000). The first sign of eye degeneration occurs at 1.5 days postfertilization (dpf) when the lens begins to undergo apoptosis (Jeffery and Martasian 1998; Yamamoto and Jeffery 2000), and by 2 dpf retinal degeneration can also be detected (Langecker et al. 1993). Subsequently, eye growth arrests Retinal and RPE organization is gradually disrupted. By 10 dpf, only a few cells containing rhodopsin mRNA are found in the cavefish retina at this stage (Strickler and Jeffery 2009). By two weeks (Fig. 1D and F), cones are essentially absent in Pachón cavefish (Espinasa and Jeffery 2006). Eventually, the degenerating eye sinks into the orbit and is covered by an integument.
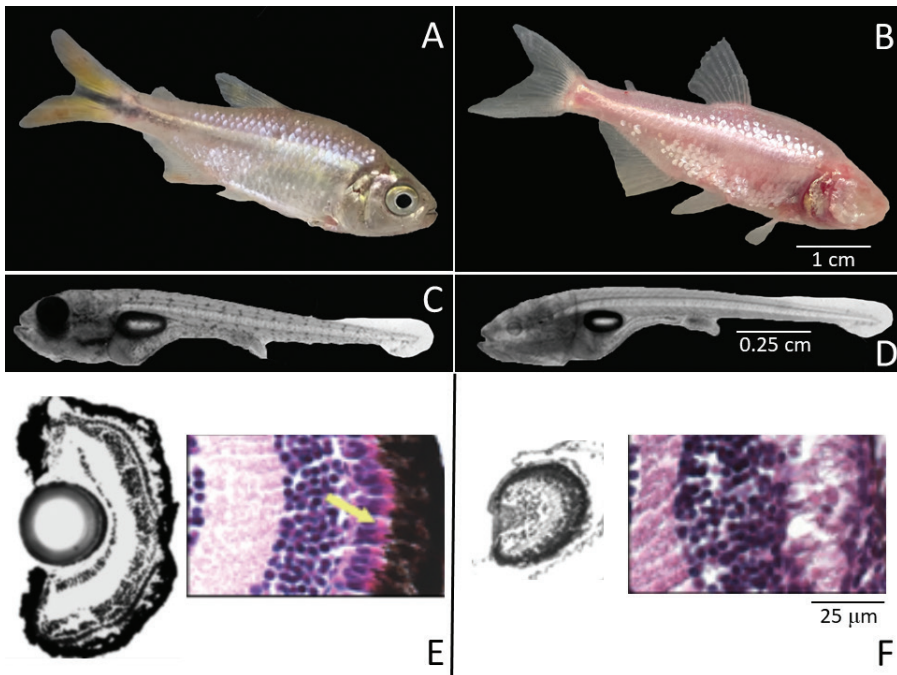
Figure 1. *Astyanax mexicanus* has two morphs; a surface, eyed morph (**A**) and a blind, cave morph (**B**) whose nonfunctional optic capsules are buried deep beneath the integument. At birth, cavefish have eyes and respond to light, but soon after, the eye degenerates. At 16–21 dpf, both the surface (**C**) and the cavefish larvae (**D**) have eyes. However, while the eye capsule and retina of the surface larvae (**E**) are well suited for vision, the eye capsule of the cavefish (**F**) has degenerated, the lens has undergone apoptosis, the outer nuclear layer of the retina is not completely differentiated, and there are essentially no photoreceptors. Cavefish larvae may detect light and darkness at this stage, but they lack central visual acuity and are thus blind to form perception. The scale in the right column is the same for the left column.

For this study, we concentrated on another physiological function and actions performed by the eyes of fish; eye convergence during Larval Prey Capture (LPC) behavior. LPC is characterized by a fast-striking motion toward the prey within tens of milliseconds. Serial time-lapse images of single prey capture events have revealed that in *Astyanax* (Espinasa et al. in press), when visual stimuli are available, they strike mostly at a target directly in front of them, bending the most caudal region of their tail (J turn). Conversely, surface fish under dark conditions and cavefish in both light and dark conditions strike mostly at prey on their side, using a C-bend turn. In zebrafish, distinct sensory inputs activate different neural circuits that result in C and J turns (Liu and Fetcho 1999; Fajardo et al. 2013). The most significant difference between the two morphs of *Astyanax* is that strike distance is significantly greater in cavefish compared to surface fish, suggesting cavefish may have improved their ability to detect prey in the dark (Lloyd et al. 2018). The expression of LPC in fry, and the Vibration Attraction Behavior (VAB) for surface fish is symmetric, with fish striking or examining prey equally on both sides. For cavefish fry or adults in the field, it is different among cave populations, with Pachón's "handedness" preferentially striking or examining with the right side of their head while the Tinaja, Sabinos, Molino and Toro cave populations preferentially using their left side (Espinasa et al. 2022; Espinasa et al. in press). These authors proposed that if there is an adaptive effect for asymmetric sensitivity, it selects for asymmetry itself. Not necessarily for the side being specialized.

Analysis of zebrafish conducting LPC while hunting paramecia uncovered a novel oculomotor behavior, eye convergence, which constitutes the first response of larvae to their prey (Bianco et al. 2011). In the study, prey or visual stimuli such as moving dots causes converging eye movements and a J-turn of the tail. These became the defining characteristics of a zebrafish's natural hunt. Eye convergence will likely increase the overlap between the visual fields of the left and right eyes to prepare for tracking prey. A high vergence angle is maintained throughout the prey-tracking and capture swim phases, enhancing binocular depth (Bianco et al. 2011). Since Bianco's et al. discovery of eye convergence, hundreds of articles reporting on this behavior have been published, many of them to understand neural networks.

This study aims to establish if fry from cavefish, despite being adapted to living in an environment characterized by perpetual darkness, have retained eye convergence when conducting LPC.

Methods

Fish rearing and maintenance

For this study we used the recordings of LPC used by Espinasa et al. (In press). For that study, animal husbandry was carried out as previously described (Borowsky 2008). Most work was conducted at the German Sumbre laboratory at the Institut de Biologie de l'ENS (IBENS), CNRS, France. All experiments performed at German Sumbre

laboratory were approved by Le Comité d'Éthique pour l'Expérimentation Animale Charles Darwin (APAFIS#27495-2020100614519712 v14). Some specimens originated from Sylvie Rétaux at the Paris-Saclay Institute of Neuroscience, CNRS and University Paris-Saclay, France laboratory. Sylvie Rétaux's authorization for the use of *Astyanax mexicanus* in research is 91–116. The animal facility of the Institute received authorization B91272108 from the Veterinary Services of Essonne, France, in 2021. Fish were housed at 21 °C ± 1 °C. Lights were kept on a 14:10 h light-dark cycle. All fry used for experiments were fed on live *Artemia* nauplii starting on the 6th dpf. This study used two populations: Pachón cave's, and a surface population derived from the Choy River. Descriptions of the cave and the surface locality can be found in Elliott (2018).

Artemia preparation

Approximately 24 hours before behavioral experiments, Brine shrimp cysts (*Artemia salina*) 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 a temperature of 28 °C, with constant aeration. Immediately prior to testing, *Artemia* were rinsed with fresh water and placed into recording chambers. Only newly hatched *Artemia* nauplii, of the 1st instar stage, were used in behavioral experiments to ensure consistency of vibrational stimuli.

Recording of larval prey capture (LPC) behavior

As mentioned before, recordings of LPC were the same as the ones used by Espinasa et al. (In press). To get enhanced amplification and finer resolution, some new recordings were done with a Baumer camera attached to a microscope, plus some new recording with an iPhone 12 Mini, iOS version 15.5, attached to a tripod. These recordings were 1080p HD.

For recordings of LPC behavior on 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 minutes before the experiment began. 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's PC-10 Dual-Stage Glass Micropipette Puller. Borosilicate glass capillaries were heated and pulled to get fine needles, like those used for cell injection. The tip of the glass rod had a diameter of ~0.15 mm, about half the size of an *Artemia* nauplii. The vibration stimulus was generated using the ~0.15 mm diameter glass rod attached to an audio speaker (8ohm 0.1W 38 mm speaker) that produced 10 Hz with a TTI TG210 2MHz Function Generator. The peak-to-peak voltage was set to 21V. The axis of the vibration was in the horizontal plane. Individual fish were placed in a 9 cm diameter petri dish or a 3.5 cm diameter petri dish with water to a depth of ~3 mm. Fry acclimated in the experimental room for at least 2 hours. They were then transferred gently to the Petri dishes, where they further acclimated for 2 minutes before introducing the glass rod. The age of the fry tested was 16–21 dpf (Fig. 1C, D).

## Quantification of eye convergence during LPC behavior

An analysis frame by frame of the recording was done starting 2 seconds before the initiation of movement toward the prey or vibrating glass rod until 2 seconds after LPC ended. Eye vergence angles were measured before, during, and after responses to the stimuli by drawing two lines along the width of each eye until the line from one eye converged with the line drawn from the other (Figs 2C and 3C). This allowed for predicting changes in the binocular visual field in response to the stimuli (Figs 2C vs. 2E and 3C vs. 3E).

A line was also drawn perpendicular to the eye's width, passing through the center of the pupil, in the direction of the center of that eye's visual field (Fig. 4A). This allowed for showing of the changes in the direction at which an eye is pointing in response to the stimuli (Figs 4A vs. 2D).

Results

Do surface *Astyanax* display eye convergence with non-visual stimuli?

Larval prey capture (LPC) behavior is characterized by a fast-striking motion toward the prey within tens of microseconds. Our first experiment tested if surface *Astyanax*
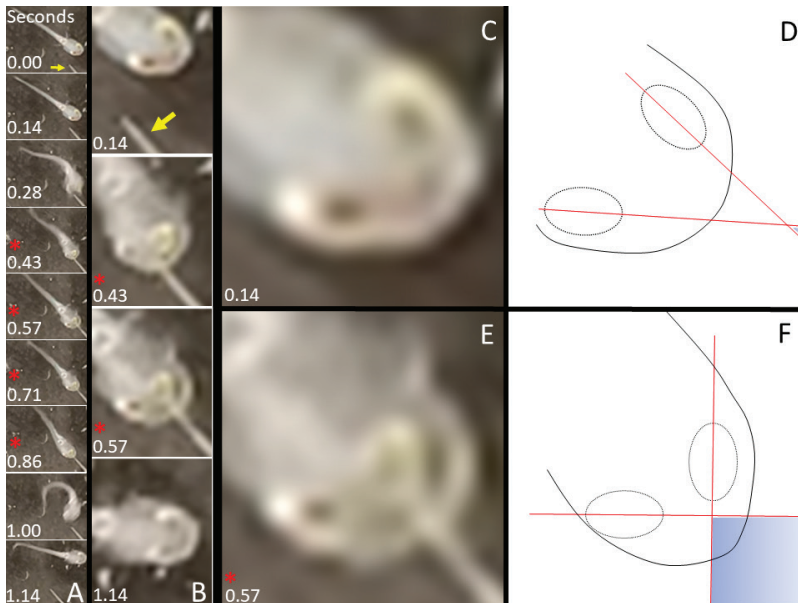

Figure 2. Eye vergence in surface fish stimulated by a vibrating (10 Hz) glass rod (**A, B**). Larval prey capture (LPC) behavior is characterized by a fast-striking motion toward the vibrating glass rod (yellow arrow) within tens of microseconds. Red asterisks highlight instances when the eyes converged. Higher magnification to highlight the changes in eye position during a strike (**C–F**). Freely swimming larvae have eyes pointing sub-perpendicular to their body in which the binocular overlap (blue) region of their visual space is minimal (**C, D**). During LPC, the mean eye vergence angle changes, largely expanding the binocular area of visual space (**E, F**).
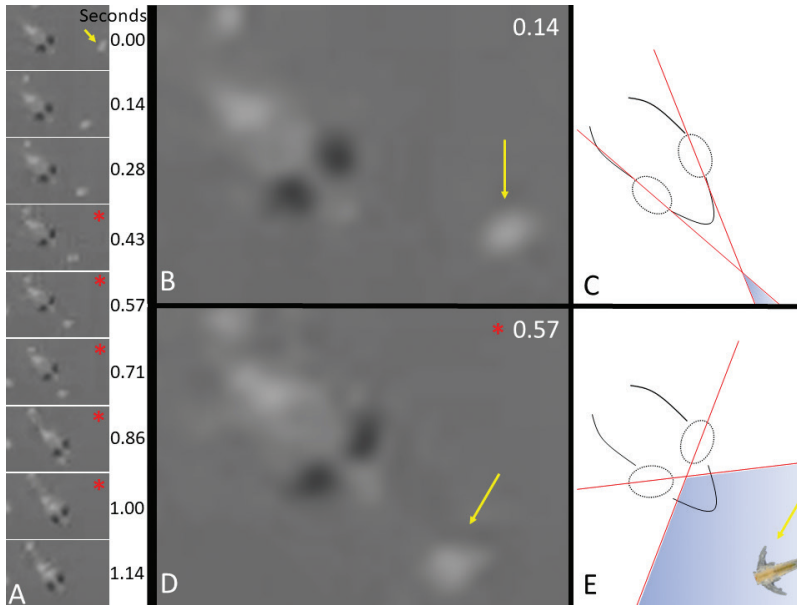
Figure 3. Eye vergence in surface fish while in the dark (**A**). Higher magnification to emphasize that despite being in the dark and without visual stimuli, the eyes change position (**B, D**) which, if illuminated, would have largely expanded the binocular proportion of visual space shown in blue (**C, E**). Notice that eyes converged when prey is detected at a distance (**A:0.43** and **D**), followed by a strike (**A:0.86**). Soon after, eyes return to normal position (**A:1.14**). Yellow arrows highlight the prey and red asterisks highlight instances when the eye converged.
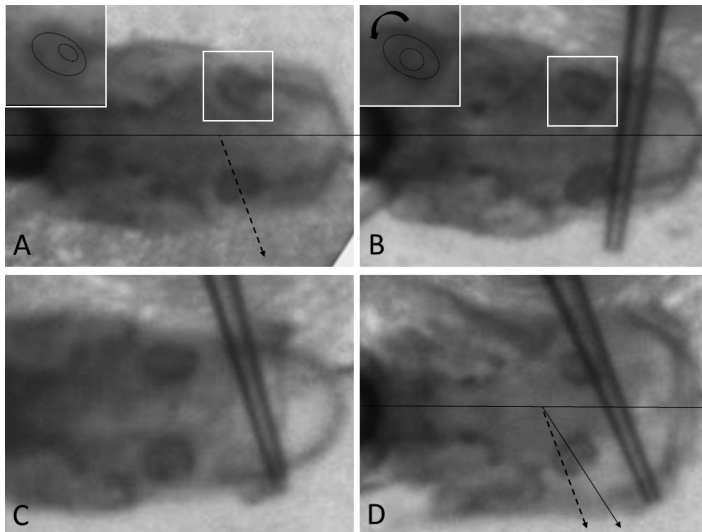
Figure 4. Blind cavefish *Astyanax* larvae have ocular vergence during LPC in response to vibrations from a glass rod, which elicits a strike behavior. Freely swimming larvae have eyes pointing sub-perpendicular to their body (**A**). When the source of a vibration stimulus is over the head, eyes turn upward (**B**). This was followed by a strike in which the cavefish larvae bit the glass rod (**C**). Eyes vergence remains for a few moments after a strike (**D**). Dotted arrows highlight eye angle before vergence to show the change of eye position.

larvae have ocular convergence during LPC when presented with a source of vibrations under light conditions that are not the stereotypical image of prey, such as a microcrustacean. For this, we used a vibrating glass rod at a frequency of 10 Hz. Ten Hz is a frequency similar to the one generated by *Artemia* nauplii that preferentially trigger successful strikes by *Astyanax* larvae (Espinasa et al. in press). We observed that, during striking episodes, larval surface fish converged their eyes (Fig. 2). Furthermore, the eyes were maintained at a high vergence angle throughout the LPC until soon after the release of the glass rod. Surface larval *Astyanax* seem to engage in a binocular viewing mode when hunting. Scale drawings showed predicted changes in the binocular visual field of larval *Astyanax* due to eye convergence (Fig. 2D versus 2F). Freely swimming larvae have eyes pointing sub-perpendicular to their body. As such, the region of binocular overlap (blue) of their visual space is minimal (Fig. 2D). During LPC, the mean vergence angle changed, advancing the binocular visual field to close to the front of the mid-point of the eyes, and largely expanding the binocular proportion of visual space.

Our second experiment tested if surface *Astyanax* larvae have ocular convergence during LPC with no visual stimuli. For this, we recorded LPC with an infrared LED light source. In trials, vergence of the eyes responded to the non-visual stimulus (Fig. 3). Both eyes could orient in the direction of the stimulus (Fig. 3A; 0.43–0.71 seconds), before the strike motion (Fig. 3A; 0.71–0.86 seconds). The first element of the behavioral response to the moving prey is a nasally-directed rotation of the eyes. C-bend turn movements or J-turns of the tail commence tens of a second later. Ocular convergence ended soon after the capture of the prey (Fig. 3A; 1.14 seconds). Despite being in the dark, convergent eye movements appeared to represent the first behavioral element in the hunting routine. Convergent eye movements can occur at the onset of the distinctive series of prey-tracking maneuvers in which larval fish reduce the distance and angular deviation between themselves and their prey. In surface *Astyanax* larvae, non-visual stimuli appear to activate LPC concurrently with ocular convergence. Of note, eye vergence started when the source of the stimuli was still far from the body. Direct contact with the body by the prey was not needed, and ~10 Hz vibrations, sound, smell, and/or other stimuli can activate the ocular vergence at a distance.

Do "blind" cave *Astyanax* display eye convergence?

As the introduction mentions, *Astyanax* cavefish and surface fish are initially born with equivalent eye structures, and both respond actively to light stimuli. While up to adulthood, cavefish may have some type of detection and response to light, cavefish larvae become effectively blind to patterns other than shadows early on. As reviewed in the introduction, in the 16–21 dpf cavefish larvae used for this study there is an overall degeneration of the eye capsule and almost complete regression of the outer nuclear layer that contains the cell bodies of the photoreceptor cells (Fig. 1E, F). It is assumed that while the "blind" cavefish larvae used for this study may detect light and darkness, they completely lack central visual acuity for form perception.

Our third experiment tested if blind cavefish *Astyanax* larvae have ocular vergence during LPC when presented exclusively with vibrations in the range generated by their prey. For this, we used a vibrating glass rod at a frequency of 10 Hz. Recordings for experiments showed that freely swimming cavefish larvae have eyes in a lateral-oriented position, but when a stimulus is above them, their eyes move upward, as reflected by the position of the pupil (Fig. 4A, B). Likewise, when the stimulus is in front of them, ocular convergence occurs with the center of the eye positioning forward (Fig. 4D). Despite their apparent blindness, the eye tracked the respective position of the source of vibrations with respect to the body of the fish (Fig. 5A, B). When the glass rod was presented to the fish, but with the vibrations off, we detected no eye vergence nor eye tracking when the fish swam by the side of the glass rod.

Scale drawings showed that, just as in surface fish, freely swimming larvae have eyes pointing sub-perpendicular to their body, with the region of binocular overlap (blue) being minimal (Fig. 5C). During LPC, mean vergence angle also changes in the cavefish, advancing the binocular field to close to the front of the mid-point of the eyes (Fig. 5D). In the case of the cavefish larvae used in this experiment, changes in the binocular field serve little function as visual space may be irrelevant due to the degree of blindness by this stage.
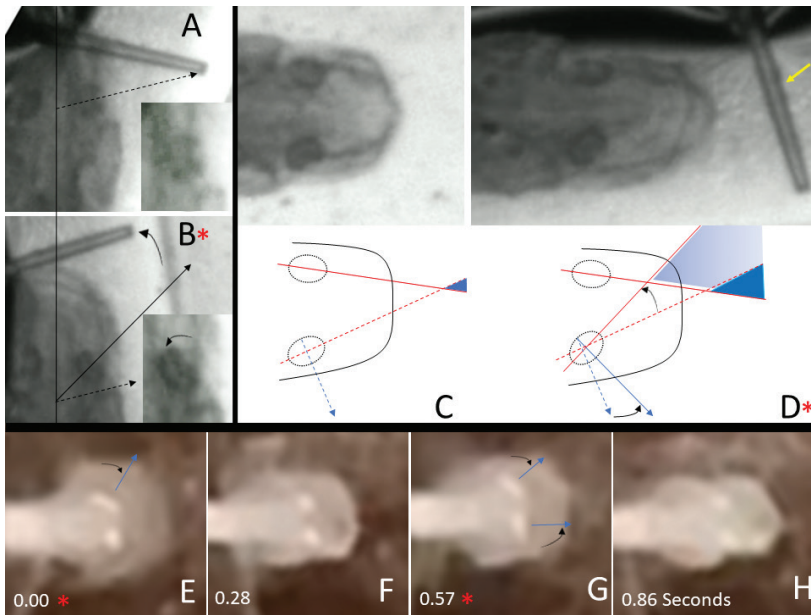
Figure 5. Despite being blind, the eyes of cavefish larvae tracked the position of the source of vibrations (**A, B**). During LPC, the mean vergence angle changes in the cavefish, advancing the binocular field to close to the front of the mid-point of the eyes (**C, D**). Each eye can have its own and different angle of vergence (**E–H**). Depending on the position of the source of the vibrating stimulus, a single eye may move forward, while the other remains laterally pointing (**E**). At another position of the stimulus, both eyes may converge forward (**G**). Soon after, eyes return to normal position (**F, H**). Eye vergence may represent a vestigial behavioral character, left-over in the evolution of *Astyanax* cavefish, since changes in the binocular field may be irrelevant due to the blindness of larvae by this stage of development and because of living in the dark with no visual stimuli.

Each eye can have its own vergence. Depending on the position of the source of the vibrating stimulus, a single eye may move forward while the other remains laterally pointing (Fig. 5C, D and 5E, F). At another position, both eyes may converge forward (Fig. 5G, H). In blind cavefish *Astyanax* larvae, it appears that non-visual stimuli activate LPC concurrently with ocular convergence. Direct contact with the body by the source of vibrations is unnecessary, and vibrations at a frequency of ~10 Hz activate the ocular vergence at a distance.

Discussion

Astyanax cavefish have been reported to be blind and lack physiological response to light in the tectum (Voneida and Fish 1984), but Lloyd et al. (2022) found that the cavefish tectum showed some, although severely reduced, Ca2+ responses to the presented visual stimuli. Nonetheless, at least in the case of the Pachón population, the retina does not respond to light and the light responsiveness observed in the optic tectum of cavefish must stem from a non-visual source such as the pineal gland or deep-brain internal photoreceptors (Lloyd et al. 2022).

Our behavioral data suggest that surface *Astyanax* fish may have several prey-capture specific motor programs. One may start with a visual stimulus that activates ocular convergence for enhanced binocular processing of visual information. This is followed by the appropriate J-turns of the tail, or C-bend turns movement for a strike towards the prey. Another motor program may start with non-visual stimuli, such as a 10 Hz vibration. This may activate in synchrony the J- or C- turns while positioning the eyes in convergence. The advantage of this synchronous activation is that under light conditions when the fish has positioned itself for the final strike motion based on vibration information, binocular optimization can then occur. This is supported by observations in zebrafish (Bianco et al. 2011); eye convergence during hunting behavior in larval zebrafish is not to direct gaze toward the prey, because the eyes adopted a symmetrical converged configuration independent of prey location. Rather, it is more likely that eye convergence acts to increase the binocular overlap between the visual fields of the left and right eyes in preparation for prey tracking. If visual information does not occur such as at night, in *Astyanax* surface fish, the strike is activated only with the available data, and eye vergence becomes irrelevant. This is also supported in zebrafish (Patterson et al. 2013), where while in the dark, paramecia trigger ocular vergence. A difference between zebrafish and *Astyanax*, both surface and cave, is that while in zebrafish the strike is activated only when the prey enters in contact with the anterior extremities of fish larvae, in *Astyanax* prey is detected at a distance (Espinasa et al. in press). *Astyanax* may rely more on other non-visual sensory modes that result in more efficient prey hunting in the dark than Zebrafish. *Astyanax* cavefish can strike at prey at farther distances than *Astyanax* surface fish (Lloyd et al. 2018: Espinasa et al. in press). The increased distance of the strike may

contribute to the enhanced feeding of cavefish, as seen in a competition assay in 17 dpf fry, where cavefish consumed more *Artemia* than surface fish under dark conditions (Espinasa et al. 2014).

For cavefish, the second motor program may be at work. LPC may start with a non-visual stimulus, such as a 10 Hz vibration, smell, or sound. This may activate in synchrony the C- or J- turns while positioning the eyes in convergence. In the case of the blind cavefish, the vergence of the eyes may currently serve no function while in the dark environment of the cave and be a left-over of evolution, where natural selection or other evolutionary forces have not regressed this behavior. The activity of premotor neurons producing eye convergence commands is assumed to have been a fundamental component of the activity pattern underlying all behavioral responses to prey-like stimuli in the ancestral surface fish that gave rise to the cavefish. Just as it may be in the existing surface *Astyanax* fish. This activity has not regressed at the same pace as structural eye degeneration.

Espinasa and Jeffery (2006) showed that loss of physiological function does not necessarily precede or occur at the same pace as structural degeneration during regressive evolution in the cavefish eye. They showed that the capacity to exhibit light-entrained retinomotor rhythms has been conserved in the degenerating embryonic eyes of *Astyanax* cavefish populations. The results indicate that loss of circadian retinal function does not precede and is, therefore, not required for eye degeneration in blind cavefish. In *Astyanax* surface fish during the day, the retinal pigment epithelium (RPE) extends to shield the rod photoreceptor outer segments, reducing the extent of bleaching. During the night, it retracts to expose the photoreceptors, allowing them to catch the maximum number of photons. These retinomotor rhythms would appear to have no function in blind cavefish. Therefore, movements of unpigmented RPE granules (e.g., Pachón cavefish) would not be needed to reduce the extent of bleaching of photoreceptors, which are absent in cavefish. Likewise, maintenance of retinomotor activity would not be expected to optimize visual capabilities in cavefish, which do not respond to visual stimuli in the laboratory (Voneida and Sligar 1976; Voneida and Fish 1984). Therefore, they considered retinomotor rhythms a vestigial physiological character in *Astyanax* cavefish.

Retinomotor rhythms and ocular vergence may have been preserved fortuitously in the degenerating cavefish eye evolution. The persistence of the retinomotor movements in response to a circadian rhythm, and the eye vergence during LPC, suggest that both are controlled by genetic and physiologic signals independent of degenerating cavefish eye. The expression of sonic hedgehog and tiggy-winkle hedgehog genes is enhanced along the anterior midline of cavefish embryos (Yamamoto et al. 2004). Consequently, the increase in hedgehog signaling causes eye degeneration by triggering lens apoptosis. The signals that regulate eye degeneration are independent and nonconflicting with those that control retinomotor movements and eye vergence.

Conclusions

The blind tetra, *Astyanax mexicanus*, despite being adapted to the absence of visual stimuli due to the lack of light in the cave, have retained the oculomotor behavior of eye convergence in their vestigial eyes as a response to prey stimuli. In *Astyanax*, eye convergence responses can be triggered singlehandedly by vibrations elicited with a glass rod at frequencies similar to those generated by its prey (10–35 Hz).

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