Spooky interaction at a distance in cave and surface dwelling electric fishes

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ABSTRACT

Glass knifefish (*Eigenmannia*) are a group of nocturnal weakly electric fishes found throughout the Amazon basin. We made recordings of the electric fields of two populations of freely behaving *Eigenmannia* in their natural habitats: a troglobitic population of blind cavefish (*Eigenmannia vicentespelaea*) and a nearby epigean population (*Eigenmannia trilineata*). These recordings were made using a grid of electrodes to determine the movements of individual fish in relation to their electrosensory behaviors. Cavefish showed continuous evidence of territoriality whereas epigean fish modulated their social behavior in response to diurnal cues. Surprisingly, we routinely found nearby surface fish but not cavefish with sustained differences in electric field frequencies that were below 10 Hz. Such low-frequency differences in electric field frequencies should both cause detrimental sensory interference and trigger the jamming avoidance response. Both surface fish and cavefish showed significant interactions between their electric field frequencies and relative movements at unexpectedly large distances, of at least two meters, and at high differences in frequencies, often greater than 50 Hz.

Introduction

Gymnotiformes are a group of nocturnal fish characterized by a suite of adaptations which allow them to localize conspecifics[1] and capture prey[2] in complete darkness. These fishes produce a weak electric field, typically less than 2 V/cm, using an electric organ located along the sides of the animal and in its tail[3]. This electric field, known as the electric organ discharge or EOD, is detected using specialized electroreceptors embedded in the skin. These receptors encode modulations of the electric field generated through interactions with the electric fields of conspecifics and by nearby objects. This system provides a mechanism for communication among conspecifics and for the detection and characterization of nearby objects at night[4].

The nocturnal life histories of Gymnotiform species make them well suited for life in caves. Interestingly, a species of Gymnotiform fish, *Eigenmannia vicentespelaea*, has been discovered in a cave system in central Brazil[5–7]. These fish exhibit features commonly found in species adapted to life in caves, including reduced pigmentation and reduction and/or elimination of the eyes[8]. The population is estimated to be around 300 individuals[9].

Fish in this genus (*Eigenmannia*) produce quasi-sinusoidal electric signals that are maintained at frequencies between 200 and 700 Hz[3]. Individual*Eigenmannia* change their electric field frequencies in response to electrosensory signals produced by nearby conspecifics. In the Jamming Avoidance Response (JAR), individuals raise or lower their electric field frequency avoid frequency differences of less than about 10 Hz[3]. In envelope responses, fish change their electric field frequencies in relation to relative movement between individuals or avoid emergent low-frequency signals that can occur in groups of three or more fish[10].

To discover the potential consequences of adaptation for troglobitic life on electrosensory behavior, we compared the electric behavior and movement of the cavefish *Eigenmannia vicentespelea* to nearby epigean relatives, *Eigenmannia trilineata*, that live in the same watershed (Fig. 1A). We used a recently developed system for characterizing the electric behaviors and movements of weakly electric fishes in their natural habitats[11]. This system, which uses a grid of electrodes placed in the water, permits an estimation of the electric fields of each fish, and an analysis of their concurrent movement.

Results

General observations

Eigenmannia at both the epigean and cave sites were found in clear water streams with rock and sand substrate[6,7,9,12]. At the epigean site, there was a marked diurnal modulation of behavior. During the day, fish were found alone or in groups along the

banks of the Rio da Lapa, typically below or around boulders and rocks. We were unable to make recordings of the fish during the day, so a quantification of the distributions of fish was not possible. Nevertheless, the distribution of *Eigenmannia* appeared to be similar to that described previously at sites in Ecuador[13]. Unexpectedly, no other Gymnotiform species were detected in our short survey[14]. At night, *Eigenmannia* swam in open water in the center of the stream typically near the bottom.

Fish were observed foraging in sandy substrates. Foraging included hovering or slow forward or backward swimming punctuated by strikes into the substrate (**see Supplemental video S1**). These strikes involved tilting of the head and body downwards with a rapid forward lunge to drive the mouth into the sand a few millimeters. We observed groups of *Eigenmannia* simultaneously foraging with inter-fish distances on the order of 10s of centimeters. These distances suggest that fish experience significant ongoing electrosensory interference from conspecifics. At other locations, we visually observed single fish foraging while swimming along a trajectory rather than hovering or swimming around a particular location.

At the cave site, fish did not exhibit diurnal modulation of behavior. Cavefish were observed swimming along the banks of the stream and in small eddies and pools alone or in small groups of up to 10 individuals. Cavefish retreated to crevices and spaces within boulders and rocks when disturbed, forming temporary aggregates of individuals (**see Supplemental video S2**). Videos show eyeless cavefish orienting face-to-face during social interactions. Such movements are clear examples of movements that were controlled in relation to social electrosensory cues. The distinctive substrate foraging behavior routinely seen in the epigean fish was rarely observed in the cavefish.

Morphology

Eigenmannia vicentespelaea are known to be significantly larger than the nearby surface *Eigenmannia trilineata*[6]: mean length of the snout to the end of the anal fin base was reported to be 11.1 cm (sd=2.47) in cavefish and 8.45 cm (sd=2.67) in surface fish. Size is important as it likely has an impact on the strength of electric fields: larger fish typically can generate larger currents in their EODs.

Surface *Eigenmannia trilineata* had large eyes that were circumferential and of the same size whereas the cavefish *Eigenmannia vicentespelea* had eyes in various states of development, from microphthalmic to completely absent (Fig. 1B). Cavefish eyes were also sometimes present on only one side and at various stages of degeneration (observations from the field and from DiceCT). Preliminary review of scans of our four fish showed potential differences in the size of the electric organ (Fig. 1C), but additional material will be necessary for quantitative analysis of electric organ structure and physiology.

Electric fields

We used the grid recording system to estimate the EOD frequency and strength of each fish's electric field, and calculated their positions from the electrical data over time (Fig. 2A). The strength of electric fields (Ampere-meter, "Am") of cavefish were significantly greater than surface fish (T-test p= 0.01167, df=155.02, tstat = 2.55231) with mean strength of $9.66*10^{-4}$ Am) for cavefish and $6.5*10^{-4}$ Am for surface fish (Fig. 2B).

The distributions of EOD frequencies of the epigean and cavefish fish were significantly different (T-test p=0.0074, df=191, tstat=2.7090). Mean EOD frequencies were between 299.9 Hz and 435.6 Hz (N=110) whereas EOD frequencies of cavefish were between 230.0 Hz and 478.6 Hz (N=83) (Fig. 2A,C).

Each fish generally maintained a constant frequency, but chirps and other variations in frequency were observed (Fig. 2A). The variance of EOD frequency was significantly greater in cavefish than surface (T-test, p=7.9467e-07, df=191, tstat=5.1). The mean variance of EOD frequency in cavefish was 2.62 Hz and 0.78 Hz in surface fish. Please recall that there is diurnal modulation of behavior in surface fish, but we were did not collect data from the surface fish during the day. It is reasonable to expect that the variance of EOD frequencies in surface fish will differ during daylight hours.

Movement and territoriality

Cavefish showed continuous evidence of territoriality whereas epigean fish modulated their social behavior in response to diurnal cues. During daylight hours, epigean fish were found deep within crevices and other refugia along the sides of the Rio da Lapa, but emerged after dark for feeding and social behavior that occurred in open water throughout the stream. In contrast, cavefish were found swimming along the sides of streams and in pools and eddies with no apparent variation with respect to time of day.

Over periods of minutes, cavefish appeared to swim within in small regions or territories, on the order of 10s of centimeters in diameter. We divided the data into 5-minute duration epochs and fitted a minimum convex polygon to each fish's positions. Cavefish occupied slightly more compact areas over these 300 second duration epochs than surface fish (T-test, p=1.9266e-04, df=440, tstat=3.8). Further, there was significantly less overlap between convex polygons for pairs of fish (T-test, p¡1e-10, df=2219, tstat=13.4) for cavefish (mean 8.41%, std 9.10) than for surface fish (mean 13.89%, std 9.08).

There are relations between movement and social signalling. In surface fish, freely moving individuals produced chirps and other modulations of their EODs. The mean standard deviation of individual EOD frequencies for freely swimming fish in groups was 0.64 Hz (std=0.40, N=184). In contrast, fish that were placed in tubes had significantly lower variation in

their EOD frequencies (mean=0.40 Hz, std=0.36, N=29; T-test, p=0.0013, df=211, tstat=0.39). This is particularly interesting because the fish in tubes were surrounded by freely moving fish, and therefore exposed to conspecific electric fields that varied in amplitude. The dramatic reduction in the variation of their electric fields suggests that fish may modulate their electric signalling to conspecifics in relation to their own movement.

Further, we measured the EODs of three solitary cavefish. These fish also had reduced variability of their EOD frequencies (mean=0.24 Hz, std=0.09, N=9 300 second samples). This variability was not significantly different from the variability in EOD frequencies of surface fish in tubes (T-test, p=0.255). These data suggest that variability in EOD frequencies is driven not only by the fish's own movement, but rather by its interactions with conspecifics.

Differences in EOD frequencies

The JAR is a behavior that, in laboratory settings, reduces the likelihood that pair fish will have a difference in EOD frequency (dF) of less than 10 Hz[3]. Interestingly, we routinely found nearby fish with sustained differences in electric field frequencies that were below 10 Hz (Fig. 4A,B). In surface fish, there were 197 pair samples with mean dFs of less than 10 Hz, and of those, 96 were less than 5 Hz (Fig. 3C). In cavefish, there were 44 pair samples with mean dFs over samples that were less than 10 Hz, none of which were less than 5 Hz.

Despite these examples, the mean difference in EOD frequencies between cavefish and surface fish were significantly greater than 10 Hz. In cavefish the mean dF was 83.14 Hz (sd=50.01, N=1323) while the surface fish was 42.89 Hz (sd=28.93, N=1671). The distribution of dFs was significantly larger in cavefish than surface fish (p¡0.00001, df:2992, tstat:27.58).

Spooky Interaction at a distance

We examined the relations between relative movement and EOD frequency as a first step towards understanding the role of EODs in social dynamics. We measured the dFs of all pairs of fish and their simultaneous pairwise distances (Fig. 4). We found examples of fish that had strong positive and negative Pearson correlation values between distance and dF ('dF/distance correlation') both at distances of over 1.5 meters and for dFs of over 50 Hz (Fig. 4A). Indeed, dF/distance correlation coefficients above 0.7 were found in 20 epochs for pairs of cavefish that were over 1.5 meters apart and in two surface fish samples. Note that the grid for the surface fish was roughly a 1.5 meter square, and so these distances are at the limit of the test area. We found 12 epochs with dF/distance correlation coefficients above 0.7 at distances over 1 meter in the surface fish data.

We expected that nearby fish would have greater impacts on each other's EOD frequency than more distant fish due to the decrease in the strength of the EOD signals with increasing distance. We also expected that pairs of fish with low dFs would have stronger effects on each other's EOD frequency than fish with greater dFs, consistent with previous work on the jamming avoidance response[3]. We found that there was no effect of distance on dF/distance correlation (Pearson Correlation, p=0.28 for cavefish and p=0.89 for surface fish; Fig. 4B,C). Similarly, although we found a weak but significant correlation between dF and dF/distance correlation coefficient for cavefish (r=-0.16, p¡0.0001, N=1323), and we found no such correlation in surface fish (p=0.77, N=1671). These correlations demonstrate a strong role for envelope tracking behavior—movement-based envelope responses[10,15–17]—in the natural behavioral repertoire of *Eigemannia*.

We also examined the possibility that there might be differences between individual fish in their influence on the EOD frequencies of other fish in the group. For example, a larger sized fish with corresponding stronger EOD might preferentially affect the EOD frequencies of all surrounding fish. We calculated the mean dF/distance correlation for each pair of fish (Figure 4D, Y-axis values). We then calculated the mean dF/distance correlation of each fish on all other fish (Figure 4D, X-axis values).

Fish exhibited a range of mean influence on all other fish, from negative dF/distance correlations to positive dF/distance correlations. Most fish had mean negative dF/distance correlations with all other fish (Figure 4D, histograms). A few fish showed potential evidence of dominance. The left-most four cavefish (green, purple, blue, and dark-blue dots) each show a relatively tight cluster of negative dF/distance correlations with other fish below about -0.1. These fish had negative dF/distance with all of the nearby fish. Similarly, the right-most surface fish (purple) had positive dF/distance correlation with all but one of the nearby fish. Most fish, however, did not show consistent relations with all other fish: they showed a broader range of impacts, from strong negative to strong positive dF/distance correlations with other nearby individuals.

Discussion

We compared the electrical behavior and movement of a population of epigean *Eigenmannia* and a population of troglobitic *Eigemannia*. The cavefish were generally similar to the surface fish with some notable exceptions. Importantly, both the cavefish and surface fish showed significant interactions between movement and changes in dF, demonstrating that envelope responses are routine in freely moving *Eigenmannia*. Nevertheless, the cavefish have have no apparent diurnal modulation of behavior and exhibit anatomical changes that are routinely found in troglobitic fishes, including reduced or absent eyes and pigmentation.

0.1 Energetics

The EOD amplitudes of the troglobitic *Eigenmannia* were, on average, higher than the nearby surface fish. This may be in part due to size: a previous report[6] showed that the cavefish are generally larger than the surface fish. Nevertheless, the energetic cost of generating electric fields is high, consuming up to one quarter of an individuals energy budget[18,19]. The result suggests, therefore, that food resources are likely available and accessible to the cavefish. The loss of eyes and pigment in these cavefish, therefore, is likely not under strong selection for energetic costs, but rather neutral selection.

Indeed, Gymnotiform fishes throughout the Amazon basin have reduced size eyes. It is not uncommon to encounter *Eigenmannia* with a missing or damaged eye. Further, we routinely observe dense infestations of nematode parasites in the eyes of *Apteronotus*. These observations suggest that Gymnotiform fishes generally rely more heavily on active electric sensing than vision for survival and reproduction. This clade, and the troglobitic weakly electric cavefish *Eigenmannia vicentespelea*, represent a unique opportunity to study evolutionary changes related sensory perception and behavioral control.

0.2 EOD frequencies

The distributions of EOD frequencies for these species of *Eigenmannia* were unimodal, as has been previously observed[13]. Further, we did not identify any frequency-dependent signalling that might be correlated with sex. Sex differences in EOD frequencies are well known in *Apteronotus*[20] and *Sternopygus*[21], and there may be sex differences in EOD frequencies in *Eigenmannia*[22]. There was a difference in the distributions of EOD frequencies between the cavefish and surface fish, but the meaning of these differences remains unclear. The larger range of frequencies may be due to sustained interactions related to territoriality, where fish of adjacent territories increase their dFs over time.

Unexpectedly, we routinely observed sustained, low-frequency dFs in nearby surface fish. Low-frequency dFs potentially impair electrolocation in *Eigenmannia*, and can be avoided by performing a JAR[3] or moving further apart[13]. It seems unlikely that these fish were experiencing significant impairment of electrosensory function, as they were engaged in feeding on prey under the substrate in complete darkness. Nevertheless, elasmobranchs have been shown to detect and discriminate signals from substrate-bound prey using passive electroreception mediated by Ampullary receptors (CITATIONS).

0.3 Envelope responses

Both surface and cavefish exhibited interactions between their distance and EOD frequencies at large distances of over 1.5 meters and dFs of over 50 Hz. Both surface fish and cavefish showed significant interactions between their electric field frequencies and relative movements at unexpectedly large distances, of at least 1.5 meters, and at high differences in frequencies, often greater than 50 Hz.

0.4 Territoriality

Territoriality is a form of space-related dominance[23]. The most prominent function of having a territory is to provide the holder with an secured supply of resources. It is unclear what are the resources distributions in a cave that have influenced the territoriality behavior in *Eigenmannia vicentespelaea*.

Methods

These observational studies were reviewed and approved by the Rutgers Institutional Animal Care and Use Committee and follow guidelines established by the National Research Council and the Society for Neuroscience.

Study sites

The study sites were located in Terra Ronca State Park (46° 10'–46° 30' S, 13° 30'–13° 50' W), in the Upper Tocantins river basin, state of Goiás, central Brazil (Figure 1A). We measured the electric behavior of the cavefish *Eigenmannia vicentespelaea* in the São Vicente II cave (13° 58'37" S, 46° 40'04" W) in October of 2016. The electric behavior of the epigean species, *Eigenmannia trilineata*, was measured in the Rio da Lapa at the mouth of the Terra Ronca cave (13° 38'44" S; 46° 38' 08" W) in April of 2016. These streams have moderate water currents, clear water, and the substrate is formed of sand, rocks, and boulders.

Anatomy

Four formalin fixed specimens were provided by Dr. Bichuette from her collection. Specimens were submerged in 11.25 Lugol's iodine (I2KI) solution for up to 36 hours prior to scanning. Stained specimens were removed from Lugol's solution, rinsed in water to remove excess stain and sealed in rubber sleeves to prevent dehydration. Samples were then loaded into 50 mL polypropylene centrifuge tubes for scanning.

Stained and unstained specimens were scanned at the Core Imaging Facility of the American Museum of Natural History (New York, NY), using a 2010 GE Phoenix v—tome—x s 240CT high-resolution microfocus computed tomography system

(General Electric, Fairfield, CT, USA). Diffusable iodine based contrast enhanced computer tomography (DiceCT) permits visualization of soft tissue details of the head and the body. Scans were made at 125 kV, with an exposure time of 60 seconds. Voxel sizes were 20-25.9μm. Volume reconstruction of raw X-ray images were achieved using a GE Phoenix datos—x.

Recordings of electric fields

Eigenmannia were initially identified and located using hand-held single-electrode probes (Figure 1B). These probes translate the electric fields of the fish into an audio signal. Further, the water was sufficiently clear to see and photograph the animals (GoPro 3) when they were swimming in open water (**see Supplemental video S1**). Behavioral recordings were made using a recently developed system[11] composed of an array of amplified electrodes with 50 cm spacing. For measurements of the epigean fish, an array of 8 electrodes was placed along the edges of the Rio da Lapa stream after sundown when the fish were active. In the São Vicente II cave, an array of 16 electrodes was placed in eddies and side pools along the primary stream. Unfortunately, we were unable to use the larger grid at the surface site due to a concurrent religious festival. As a result, the XY range of the surface grid is about 50 cm diameter smaller than the cave grid. In all other respects, the measurements from each grid are identical.

We used an algorithm[11] (code available at). In short, each fish was identified by its fundamental EOD frequency. An estimate of the strength of the electric field for each fish was then calculated. Fish were considered to be ideal current dipoles—a source-sink pair of equal but time varying strength $I(t)$, separated by a small distance d. The electric current dipole moment for the fish is defined as $p = Id$ which has the units of Ampere-metre or "Am." The distribution of power at each EOD frequency across the grid of electrodes was used to estimate the position and pose of the fish. In these recordings, which were made in shallow water of no more than 40 cm depth with a planar array of electrodes, the position estimates were restricted to the XY-plane.

Continuous recording sessions using the grid were made both at the cave site (N=14) and surface site (N=5) with durations of 600 seconds to over 1200 seconds. Because fish could not be tracked between recording sessions, it is likely that some individual fish were measured across sessions.

Analysis of position and EOD frequency data

The position and EOD frequency data were analyzed, unless otherwise described, over 300 second epochs with 50% overlap. All analyses were conducted using Matlab (Mathworks, Nantick MA). The mean EOD frequency, mean EOD amplitude, and the standard deviation of EOD frequency was calculated for each fish. We estimated the XY region of movement for each fish for each epoch using a minimum convex polygon fitted to its estimated positions. We then calculated the pairwise overlap between each convex polygon. Euclidean distances between pairs of fish were also computed.

We tried several approaches for assessing relations between pairs of EOD frequencies, distances between pairs of fish, and their relative movement. These included transfer entropy, cross correlation, and Pearson correlation. Here we report values obtained using Pearson correlation between instantaneous distance between pairs of fish (distance) and instantaneous difference in EOD frequency (dF). Pearson correlations were measured over 300 second epochs with 150 second overlap between epochs. These "dF/distance correlations" ranged from -0.93 to 0.90. These dF/distance correlations were used because they best matched our impressions of the strengths of relations between dF and distance by visual inspection.

We calculated the correlation between mean distance and dF/distance correlation. For instance, a reasonable prediction is that the correlation between dF/distance might differ between fish that are close together versus far apart. Finally, we calculated the correlation between mean dF and dF/distance correlation. Similarly, a reasonable prediction is that the correlation between dF/distance might differ between fish with small versus large mean dFs.

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Author contributions statement

DS conceived and organized the experiments. ESF, MEB, and DS travelled to the study sites and collected the data. ESF and NJC conceived the analytic approach that was implemented by MM and RJ. ESF, NA, MM, and RJ analyzed the electrical data. DS collected an analyzed the microCT data. All authors have reviewed the manuscript.

Additional information

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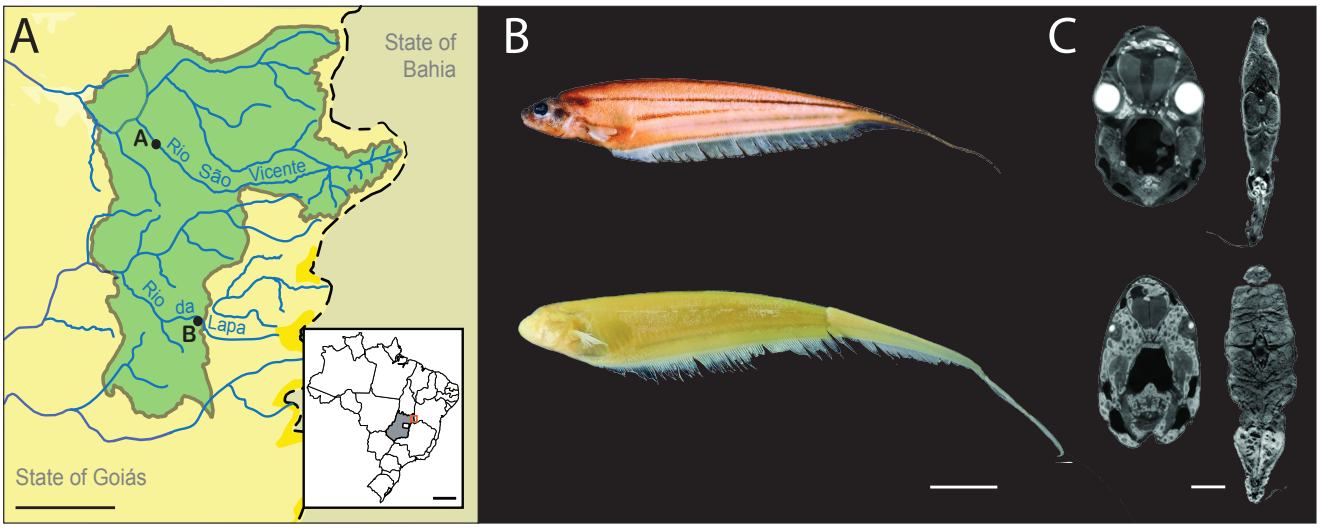

Figure 1. Surface and cave *Eigenmannia*. A) Study sites are in a clear water system in the Rio da Lapa karst region in Goiás, Brazil. Cave *Eigenmannia* are found in the São Vicente II cave (a) and the surface *Eigenmannia* site was in the entrance of the Rio da Lapa cave (b). B) Surface *Eigenmannia* have well-developed eyes and distinctive markings whereas cave *Eigenmannia* have poorly developed or missing eyes and lack pigmented features. C) CT imaging reveals a potential difference in the relative size of electric organs: large, bright cells in the caudoventral coronal sections appear relatively larger in the cave versus surface species.

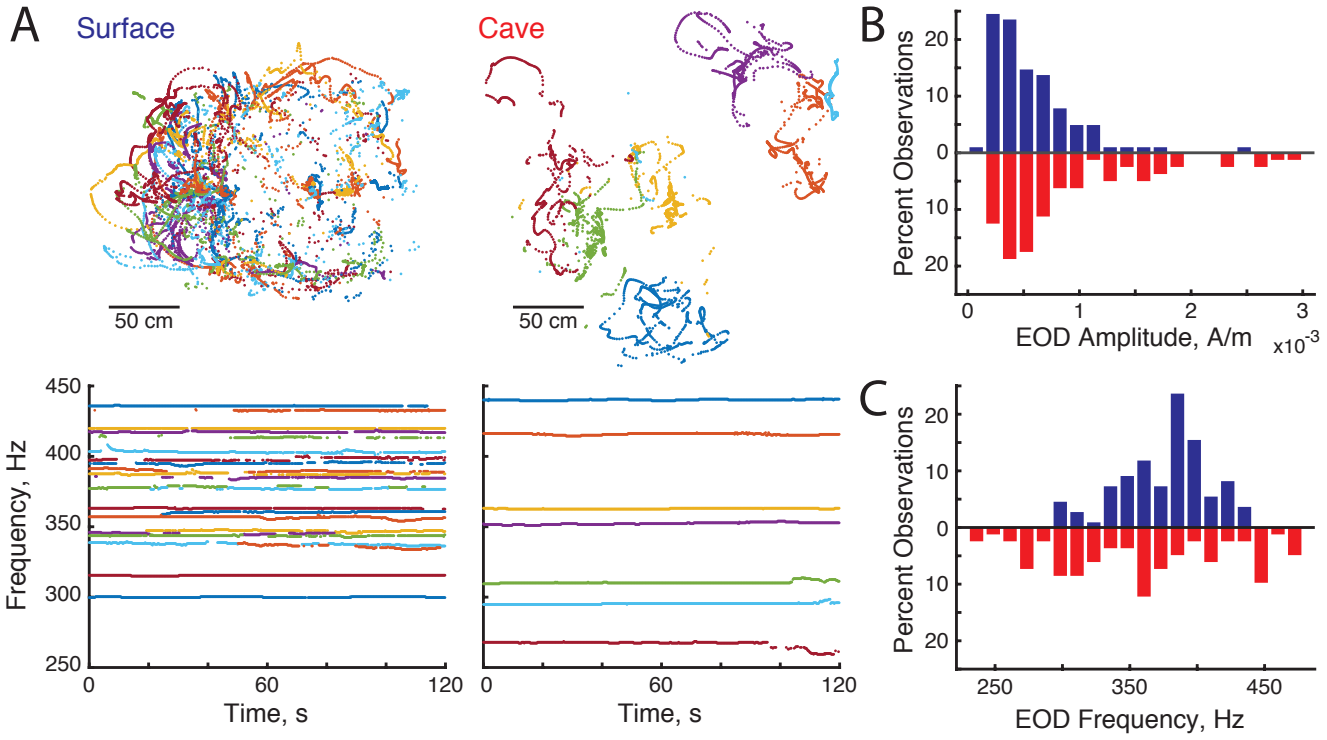

Figure 2. Electrical and locomotor behavior of *Eigenmannia*. A) Positions (top) and EOD frequencies (bottom) of surface and cave *Eigenmannia* over a period of two minutes. B) Histograms of EOD amplitudes: surface (blue) and cave (red) EOD amplitudes are significantly different. C) Cavefish have a wider range of EOD frequencies than surface fish.

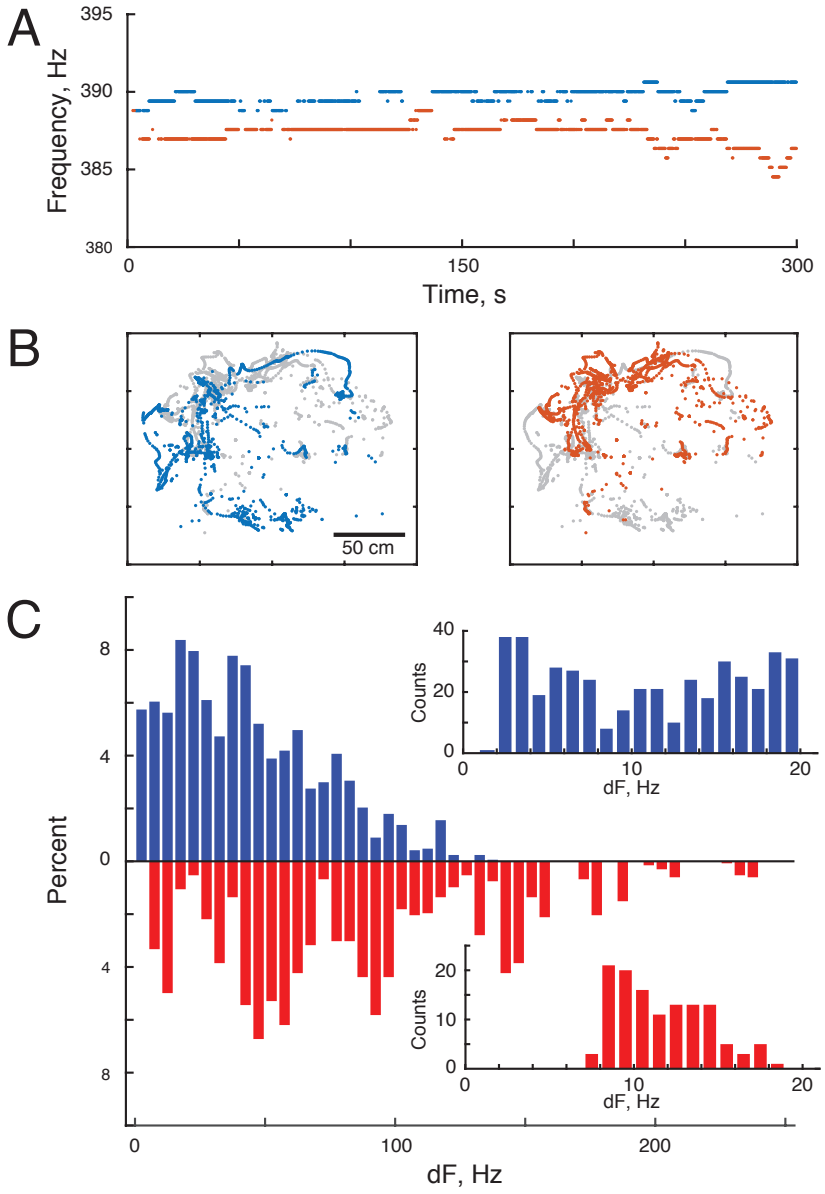

Figure 3. Differences in EOD frequency. A) EOD frequencies of two surface fish with less than 10 Hz dF over a period of greater than 300 seconds. B) The positions of the two individuals shown in A largely overlapped while maintaining the low dF between the fish. C) Histogram of the distribution of pairwise dFs of surface fish (blue) and cavefish (red). Insets highlight the low-frequency dF range, below 20 Hz, for both surface fish and cavefish. Low-frequency dFs (below about 10 Hz) are rare in cavefish but common in surface fish.

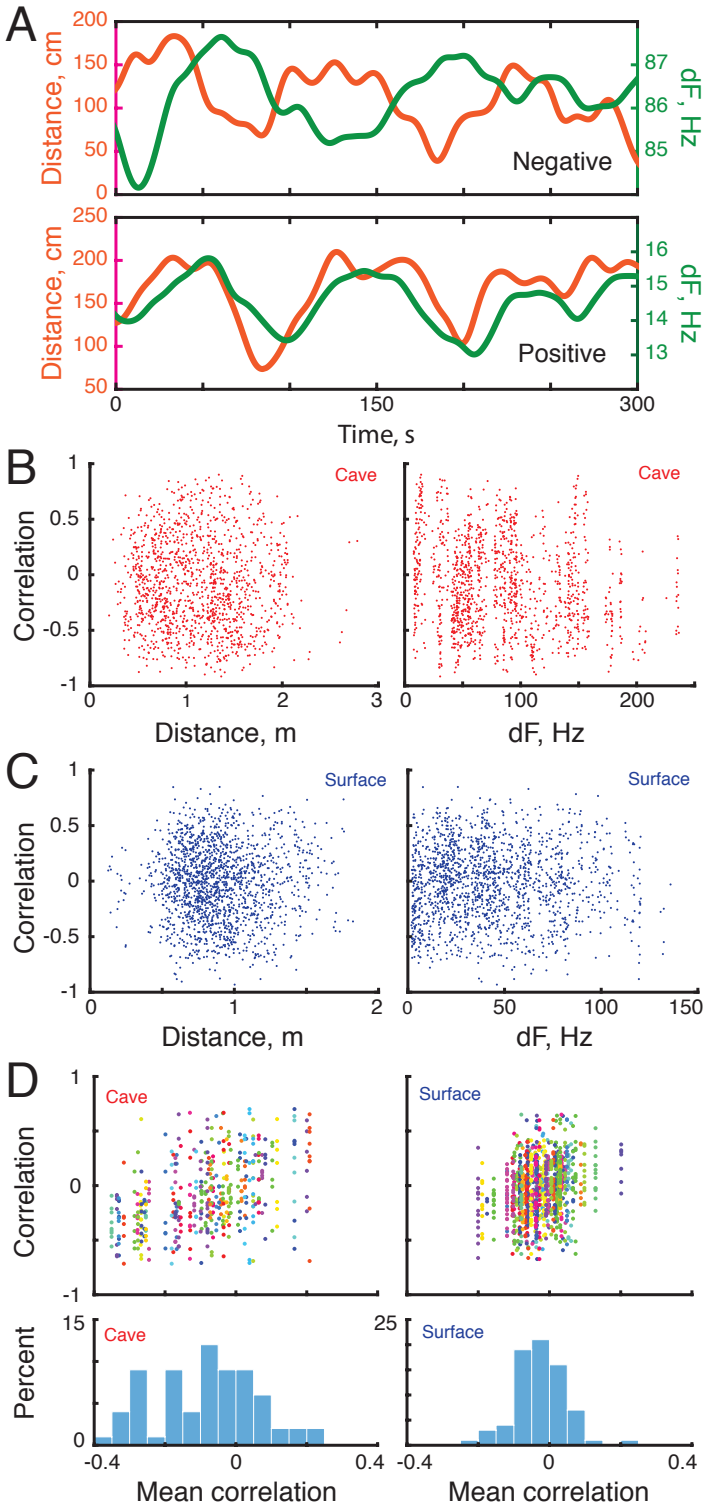

Figure 4. Correlations in EOD frequency and movement. A) Distance (magenta) and dF (green) of a pair of *Eigenmannia* over a period of 5 minutes. Top shows example of strong negative correlation and bottom is positive correlation between distance and dF. B) Pairwise correlations between dF and distance in cavefish plotted versus distance (left) and dF (right). C) Same as in B for surface fish. D) Pairwise correlations between distance and dF across individuals. Each color is an individual fish. The Y-value of each dot is the mean dF/distance correlation between that fish and a unique other fish. X-axis values are the mean pairwise correlations between a fish and all other fish. Histogram shows distribution of mean correlations over the population we sampled.