

RESEARCH ARTICLE

Differences in electrosensory anatomy and social behavior in an area of sympatry between two species of mormyrid electric fishes

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ABSTRACT

Sensory systems play a key role in social behavior by mediating the detection and analysis of communication signals. In mormyrid fishes, electric signals are processed within a dedicated sensory pathway, providing a unique opportunity to relate sensory biology to social behavior. Evolutionary changes within this pathway led to new perceptual abilities that have been linked to increased rates of signal evolution and species diversification in a lineage called 'clade A'. Previous field observations suggest that clade-A species tend to be solitary and territorial, whereas non-clade-A species tend to be clustered in high densities suggestive of schooling or shoaling. To explore behavioral differences between species in these lineages in greater detail, I studied population densities, social interactions, and electric signaling in two mormyrid species, *Gnathonemus victoriae* (clade A) and *Petrocephalus degeni* (non-clade A), from Lwamunda Swamp, Uganda. *Petrocephalus degeni* was found at higher population densities, but intraspecific diversity in electric signal waveform was greater in *G. victoriae*. In the laboratory, *G. victoriae* exhibited strong shelter-seeking behavior and competition for shelter, whereas *P. degeni* were more likely to abandon shelter in the presence of conspecifics as well as electric mimics of signaling conspecifics. In other words, *P. degeni* exhibited social affiliation whereas *G. victoriae* exhibited social competition. Further, *P. degeni* showed correlated electric signaling behavior whereas *G. victoriae* showed anti-correlated signaling behavior. These findings extend previous reports of social spacing, territoriality, and habitat preference among mormyrid species, suggesting that evolutionary divergence in electrosensory processing relates to differences in social behavior.

KEY WORDS: Electoreception, Animal communication, Sensory processing, Sensory ecology, Brain evolution, Social behavior

INTRODUCTION

Communication signals play a key role in mate choice and thereby mediate pre-mating reproductive isolation. Evolutionary divergence in signals can promote species diversity by directly fueling divergence between incipient species or reinforcing existing species boundaries (Boughman, 2002; Hoskin and Higgie, 2010; Panhuis et al., 2001; Ritchie, 2007; West-Eberhard, 1983). Sensory systems play an important role in this process because they determine the relative detectability and discriminability of signal variants (Endler and Basolo, 1998; Guilford and Dawkins, 1991; Rowe, 2013). For example, sensory adaptation to local environments can alter receiver perception, causing divergent selection on signals that leads to reproductive isolation, a

phenomenon known as sensory drive (Boughman, 2002; Seehausen et al., 2008). Evolutionary change in sensory systems can also lead to an expansion of perceptual abilities and thereby increase the potential for signal diversification and speciation (Carlson, 2012; Ryan, 1986).

African mormyrid fishes are a well-suited model system for relating evolutionary change in sensory systems to species differences in communication (Carlson and Gallant, 2013). Mormyrids communicate by generating a species-specific electric organ discharge (EOD). Sexual selection acting on EODs has likely been a driver of mormyrid species radiations (Arnegard et al., 2010; Carlson and Arnegard, 2011; Feulner et al., 2009b), and evolutionary change in the electrosensory system is associated with increased rates of EOD evolution and species diversification (Carlson et al., 2011). Although the EOD is used for both communication and active sensing, these two functions are served by separate sensory pathways (Bell, 1989). Thus, sensory divergence can be linked to social communication without other behavioral functions confounding the interpretation.

Electric communication signals are detected by electroreceptors on the surface of the skin called knollenorgans (Bennett, 1965). In most species within the subfamily Petrocephalinae, these receptors occur in three clusters on the head called rosettes (Carlson et al., 2011; Harder, 1968a,b). Evolution of the behavioral ability to detect EOD waveform variation was associated with an expansion of the spatial distribution of knollenorgans across the body surface, along with an increase in the size and complexity of a midbrain region that processes sensory input from these receptors (Carlson et al., 2011). Thus, while species in one lineage, clade A, use the EOD waveform for species, sex, and possibly individual recognition, most petrocephaline species lack this capacity. Clade A is unique in having this perceptual ability along with evolutionarily novel electric signal production mechanisms (Carlson and Arnegard, 2011; Carlson et al., 2011; Sullivan et al., 2000). The combination of these two innovations is associated with >10-fold higher rates of EOD evolution and threefold to fivefold higher rates of species diversification in clade A (Carlson et al., 2011).

This dramatic example of sensory divergence, combined with the dedicated role of this pathway in the processing of communication signals, raises the question: do species that differ in electrosensory anatomy also differ in social behavior? Field observations suggest that clade-A species tend to be solitary and territorial, seeking shelter amongst benthic vegetation and detritus (Chapman et al., 1996; Friedman and Hopkins, 1996; Hopkins, 1980, 1981; Hopkins and Bass, 1981; Nichols and Griscom, 1917). Laboratory studies have also described territoriality, agonistic behavior, and social dominance hierarchies in several clade-A species in captivity (Bauer, 1972; Bauer and Kramer, 1974; Bell et al., 1974; Carlson et al., 2000; Crockett, 1986; Kramer, 1976a,b,c, 1979; Kramer and Bauer, 1975, 1976; Terleph, 2004; Terleph and Moller, 2003). By contrast, species in the subfamily Petrocephalinae are often found in

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Received 2 July 2015; Accepted 23 October 2015

high densities in the wild, frequently in open water, suggestive of schooling or shoaling (Chapman et al., 1996; Hopkins, 1980, 1981; Lavoué, 2012; Lavoué et al., 2008, 2004, 2010; Nichols and Griscom, 1917). Although the social behavior of petrocephalines has been less studied in the laboratory, petrocephaline species have been observed to school or shoal in captivity (Baker et al., 2015). In addition, the rosette knollenorgans of petrocephalines are maximally sensitive to the EOD interval patterns produced by these large groups (Baker et al., 2015), unlike the knollenorgan receptors of clade-A species, which are tuned to the power spectrum of the individual, species-specific EOD (Arnegard et al., 2006; Baker et al., 2015; Hopkins, 1981; Lyons-Warren et al., 2012). These various lines of evidence suggest that evolutionary divergence in the knollenorgan electrosensory pathway is related to differences in social behavior. As a first step in exploring these evolutionary differences, I sought to examine the behavior of two species, one from each clade, in greater detail than has been done previously. To minimize possible confounds of habitat differences and species interaction effects, I worked in Lwamunda Swamp, Uganda, where just one clade-A species (*Gnathonemus victoriae*) and one petrocephaline species (*Petrocephalus degeni*) occur in sympatry.

MATERIALS AND METHODS

Electroreceptor and brain anatomy

Fish were euthanized in MS-222 (300 mg l⁻¹ tricaine methanesulfonate). Specimens were then placed in 10% phosphate-buffered formalin for ~2 weeks, followed by long-term storage in 70% ethanol. I analyzed the skin and brain of one preserved specimen each of *G. victoriae* and *P. degeni* to determine how knollenorgan electroreceptor distributions and the gross anatomy of the midbrain exterolateral nucleus (EL) compared with previous descriptions of clade-A and non-clade-A species, respectively. As described in detail previously (Carlson et al., 2011), the locations of knollenorgan electroreceptors on the surface of the skin were mapped by removing the outer layer of skin in pieces and staining each piece in 0.05% Toluidine Blue. The morphology of knollenorgans allow them to be easily distinguished from mormyromasts and ampullary organs, the other types of electroreceptors in mormyrids. The brains of fixed specimens were removed from the skull and then photographed. The cerebellum was then removed to improve visualization of the underlying midbrain, and the brain was photographed again. The EL is visible as a postero-lateral protrusion of the torus semicircularis (Bell and Szabo, 1986). The anterior and posterior regions of the EL (ELa and ELp, respectively) can be distinguished as the former has a distinctive white, glossy appearance owing to extensive myelination (Lyons-Warren et al., 2013a,b). Line drawings of gross midbrain anatomy were made by tracing the photographs.

Study system

Lake Nabugabo is a small lake located ~4 km west of the northwest edge of Lake Victoria in Uganda (Chapman et al., 1996). Lwamunda Swamp surrounds the lake, and *Gnathonemus victoriae* and *Petrocephalus degeni* are the only mormyrid species known to occur in the swamp (Chapman et al., 1996; Chapman and Hulén, 2001). Following Greenwood (1966), the latter has been referred to as *P. catostoma*, but is here referred to as *P. degeni* based on a recent revision and reinstatement (Kramer et al., 2012).

Lwamunda Swamp contains several permanent lagoons and small, intermittent streams (Chapman et al., 1996). I focused on two lagoons located northeast of Lake Nabugabo: Petrol Lagoon (0°19'6"S, 31°56'48"E) and Snake Lagoon (0°19'4"S, 31°56'27"E). Both lagoons give rise to small streams that enter isolated patches of forest. Both species were found in these lagoons at relatively high densities compared with the surrounding wetlands and other nearby lagoons. Field work was performed between 13 June and 7 July 2014, shortly after the onset of the dry season.

Mormyrids were located using Ag-AgCl electrodes placed on the end of a stick and connected to a portable audio amplifier (Radioshack 277-1008). To capture mormyrids for laboratory study, a seine net was used to encircle

fish and limit their movement along the stream, and dip nets were used to capture fish while using the electrodes for localization. Live fish were transported to the Nabugabo Research Station, where they were housed indoors in a mixture of rainwater and Lake Nabugabo water in single-species group aquaria containing PVC tubes for shelter.

EOD recordings, playback experiments, and behavioral assays were performed indoors at the field station. All animal procedures were in accordance with the guidelines established by the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee at Washington University in St Louis.

Transect and *in situ* recordings

On 24 June 2014, I measured the width and depth of Petrol Lagoon and estimated the relative vegetative cover at 1 m intervals along a transect that extended from the lagoon to just inside the forested region. Vegetative cover was classified as '0' (no vegetation at the water's edge), 'minimal' (sparse vegetation along the bank), 'partial' (continuous vegetation along the bank), or 'full' (continuous vegetation along both banks that extended above the water and met in the middle for full coverage).

To obtain relative estimates of population densities, Ag-AgCl electrodes were connected to a battery-powered AC/DC amplifier with a built-in speaker (BMA-200, CWE Incorporated; amplifier gain=100×, bandwidth=1 Hz–50 kHz, audio gain=maximum). EODs from *G. victoriae* were audibly distinguished from EODs of *P. degeni* based on their higher amplitude and lower spectral content. The reliability of this method for species identification was confirmed by both specimen capture and analysis of digitized *in situ* recordings. Individual mormyrids generate EODs at intervals ranging from ~10 ms to several hundred milliseconds (Carlson, 2002; Hopkins, 1986; Kramer, 1990). More than one individual in a recording is readily apparent as EOD intervals below this range (Terleph, 2004). Further, EODs from different individuals in a recording can be distinguished based on differences in amplitude if individual fish differ in their proximity to the recording electrode (Arnegard and Carlson, 2005).

I made recordings on both sides of the lagoon at 1 m intervals along the transect. To minimize disturbance, I made recordings by gently submerging the electrode from the water's edge, without entering the water. At each sampling point, I classified the density of both species separately as '0' (no individuals) if no EODs were detected; 'low' if EODs from a single individual were detected; 'medium' if EODs from multiple fish were detected, but it was possible to distinguish the EODs of different individuals by moving the electrode slightly and detecting increases and decreases in the amplitudes of different EODs; and 'high' if it was not possible to distinguish the signals of individual fish based on amplitude variation. Continuous recordings from the amplifier were obtained at a sampling rate of 40 kHz using a USB-powered portable data acquisition device connected to a laptop (USB-1608GX-2AO, Measurement Computing). *In situ* recordings were made on eight different days between 14 June and 5 July 2014, shortly after the onset of the dry season.

EOD recording and analysis

Shortly after transport to the laboratory, individual fish were placed in a plastic container filled with water from the collection locality (conductivity=20–50 μS cm⁻¹, temperature=19–23°C, pH=4–5). Recordings were made using Ag/AgCl electrodes connected to a differential amplifier (BMA-200; amplifier gain=10×, bandwidth=1 Hz–50 kHz). The position of the fish with respect to the positive and negative poles was monitored using a digital oscilloscope (Instek GDS-122) to identify the head-positive portions of the EOD. EODs were digitized at 200 kHz using a USB-powered portable data acquisition device connected to a laptop (USB-1608GX-2AO) and saved to disk using custom MATLAB software. EODs were saved with the head-positive portions as positive values, by convention. I recorded at least 10 EODs per individual.

An example EOD was randomly selected from each individual for presentation and for performing fast Fourier transform in MATLAB. I analyzed EOD variation following Carlson et al. (2011). First, I randomly selected two EODs from each individual, and cross-correlated them (intra-individual). Then, I randomly selected a single EOD from each

individual and performed pairwise cross-correlations between the EODs of all individuals (inter-individual). Finally, I took the maximum of the absolute values of cross-correlation coefficients from each cross-correlation to generate a measure of EOD similarity ranging from 0 (no similarity) to 1 (identical EOD waveforms). I used the maximum coefficients from all inter-individual comparisons to generate a matrix of EOD similarities, and then I applied multidimensional scaling to this matrix using Kruskal's normalized stress1 criterion in MATLAB to generate a two-dimensional space describing EOD variation (stress=0.0347).

Shelter assay

A 30×21 cm aquarium was filled to a depth of 6–7 cm. The aquarium contained a grey PVC tube for shelter (diameter ~5 cm, length ~13 cm) placed along the center of one long edge of the aquarium. All assays were performed during daylight hours (between 08:00 and 18:00 h), with indirect sunlight provided by windows so as to maximize the motivation to seek shelter. The tank was also illuminated by four infrared LEDs (Sima SL-100IR). An infrared-sensitive camera (Quick 1.3MP WebCam with night vision) and video recording software (Debut Video Capture, NCH Software) were used to record each trial (AVI files, MJPEG video compression).

After starting the video recording, fish were added to the tank in groups of 1, 2, 5, or 10 individuals and then videorecorded for ~11 min, at which point the trial ended and the fish were returned to their home aquarium. The goal here was not to allow for acclimation to the test aquarium, but to instead measure shelter-seeking behavior just after the stress of capture and transport to a new environment. Each video was analyzed off-line to determine the number of fish inside the shelter throughout the recording, with all movements into and out of the shelter recorded to the nearest second. A fish was scored as inside the shelter if any portion of its body was inside the shelter. The time weighted average number of fish in the shelter (N_{TWA}) throughout the recording was determined as:

$$N_{TWA} = \frac{\sum_{i=0}^n it_i}{\sum_{i=0}^n t_i}$$

where i represents the number of fish in the shelter, t_i represents the amount of time throughout the recording in which i fish were in the shelter, and n represents the total number of fish in the trial.

Electric communication

Two fish of the same species were each placed inside separate 10×10×10 cm plastic cubes containing 64 holes of 5 mm diameter on each face (AZOO Little Aquarium Fish House). These containers limited fish movement and prevented overt interaction while allowing for electric communication. The cubes were placed at opposite corners in a 30×21 cm aquarium filled to a depth of 11–12 cm, with a recording electrode placed along the inside of each long edge of the aquarium. The recording electrodes were connected to the left and right channels of a digital audio recorder (M-Audio MicroTrack II). After a 5-min acclimation period, I recorded 5-min segments from each pair of fish (uncompressed WAV files, 16 bits, 44.1 kHz sampling rate). Data from the two channels were analyzed in MATLAB using differences in amplitude between the two channels to sort and timestamp the EODs produced by the two fish (see Arnegard and Carlson, 2005). EOD rates over time were estimated by convolving EOD times of occurrence with a Gaussian kernel having a standard deviation of 125 ms (Carlson and Hopkins, 2004). Cross-correlations of the resulting EOD rates were performed with 1 ms resolution.

EOD playback experiments

Individual fish were placed in a 30×21 cm aquarium filled to a depth of 6–7 cm. The aquarium contained a recording electrode and a grey PVC tube for shelter placed along the center of one long edge of the aquarium, and four stimulus electrodes in different locations. All playback experiments were performed after sunset (between 20:00 and 02:00 h), in near-complete darkness (only indirect light from a dimly lit laptop computer screen), so as to maximize the likelihood of subjects exiting the shelter in response to stimulation.

The tank was illuminated with four infrared LEDs. An infrared-sensitive camera and video recording software were used to videotape each playback. The recording electrode was connected to an amplifier (BMA-200; amplifier gain=10×, bandwidth=1 Hz–50 kHz). The audio track of the video consisted of the amplified signal from the recording electrode on one channel, and a copy of the stimulus sent to the tank on the other channel. This made it possible to sync the stimulus and video, and to separate the stimulus from the subject's EODs on the recording electrode.

Stimuli consisted of a conspecific EOD presented with conspecific interval patterns for 60 s. Each EOD and interval sequence were randomly selected from a library of conspecific EODs and interval sequences. Stimuli were generated in MATLAB and then delivered at 97.6 kHz with a peak-to-peak source voltage of 1 V using a mobile processor (Tucker-Davis Technologies RM1) and analog stimulus isolation unit (A-M Systems Model 2200).

After placing a fish in the aquarium, I allowed for a 10-min acclimation period before starting the playbacks. Each trial consisted of one playback from each of the four electrodes presented in a random sequence. Each video consisted of ~2 min of recording: ~30 s of pre-stimulus baseline, followed by the 60-s stimulus, followed by ~30 s of post-stimulus. Each video was analyzed off-line to determine the percentage of time the fish spent in the shelter during the pre-stimulus and stimulus periods, as well as the percentage of time the fish spent within one body length of each of the four stimulus electrodes during the stimulus period. A fish was scored as inside the shelter if any portion of its body was inside the shelter.

Statistics

Alpha was set at 0.05 for all statistical tests. All independent variables consisting of multiple measurements from each individual were treated as repeated measures in ANOVAs (e.g. responses to stimulation from the four different electrodes during playback experiments).

RESULTS

Gnathonemus victoriae had a broader distribution of electroreceptors and a larger, more complex midbrain electrosensory region than *Petrocephalus degeni*

The distribution of knollenorgan electroreceptors and the gross anatomy of the midbrain extero-lateral nucleus (EL) of *G. victoriae* and *P. degeni* were similar to the general patterns previously described for clade-A and non-clade-A species, respectively (Carlson et al., 2011). In *G. victoriae*, knollenorgans were distributed across the head, back, and underbelly (Fig. 1A). In *P. degeni*, knollenorgans were found in three rosettes on each side of the head (Fig. 1B). In *G. victoriae*, the EL consisted of distinct anterior and posterior regions (ELa and ELp, respectively; Fig. 1A) that were not apparent in the relatively smaller EL of *P. degeni* (Fig. 1B).

Intraspecific EOD variation was greater among *G. victoriae* compared with *P. degeni*

I recorded EOD waveforms from 50 *P. degeni* and 15 *G. victoriae* after capture (Fig. 2A). The EODs of *P. degeni* consisted of a large head-positive peak (P1), followed by a large head-negative peak (P2), and finally a small head-positive peak (P3). The EODs of *G. victoriae* consisted of a small head-negative peak (P0), followed by a large head-positive peak (P1), and then a large head-negative peak (P2). In two individuals, this was followed by a fourth small head-positive peak (P3). The EODs of *G. victoriae* had lower peak power frequencies than the EODs of *P. degeni* (Fig. 2A,B).

As shown previously in all mormyrid species studied, individual fish had constant EOD waveforms, reflected in cross-correlation coefficients >0.99 for all intra-individual EOD comparisons (Fig. 2C). By contrast, EOD waveform varied significantly more between individuals among both *P. degeni* (Mann–Whitney U -test: $Z_{50,1225}=10.4$, $P<1e^{-6}$) and *G. victoriae* ($Z_{15,105}=6.2$, $P<1e^{-6}$).

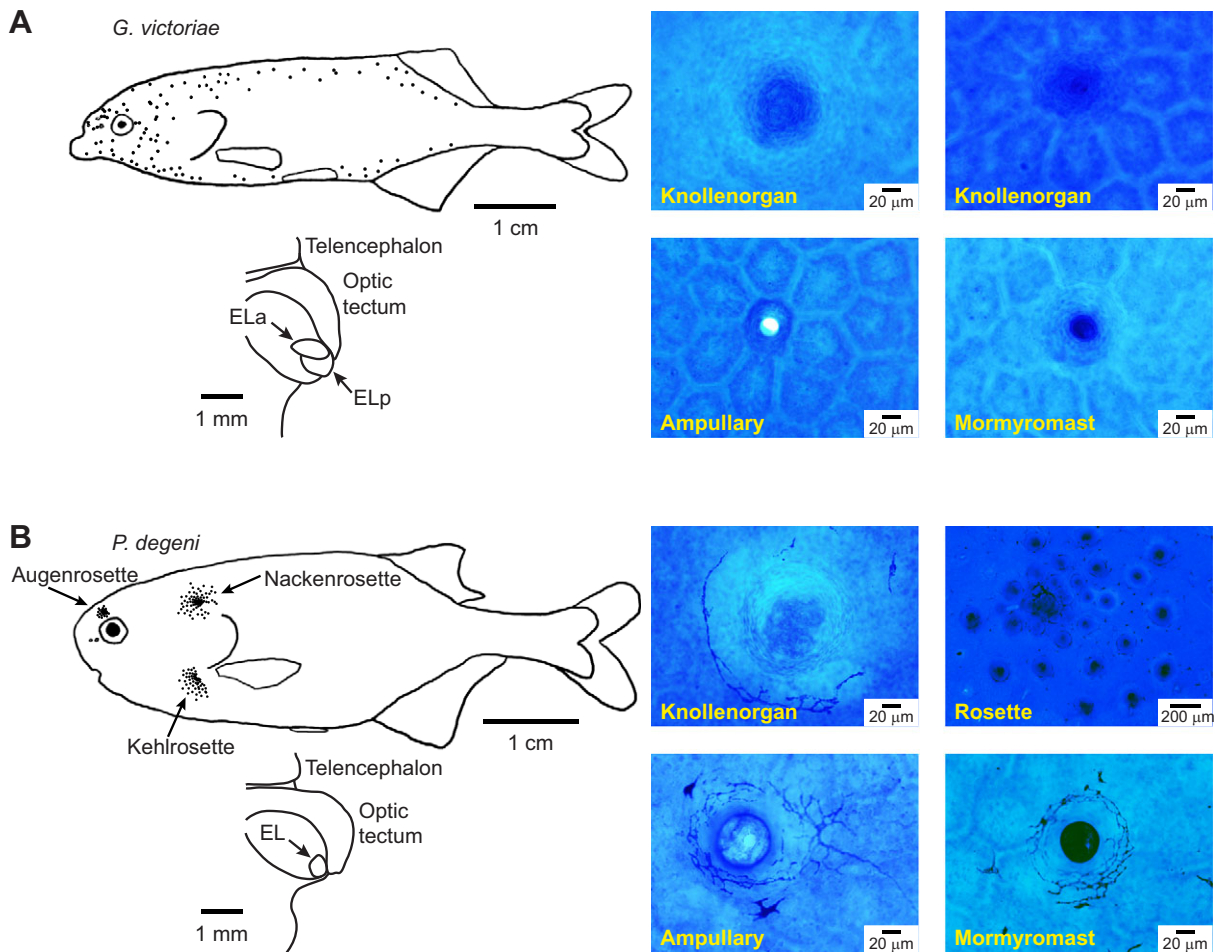


Fig. 1. *Gnathonemus victoriae* and *Petrocephalus degeni* differed in electrosensory anatomy. (A) Locations of knollenorgans along the skin surface mapped in a *G. victoriae*, with a line drawing illustrating midbrain anatomy. Knollenorgans were distributed across the head, back, and belly. *Gnathonemus victoriae* also had an enlarged extrolateral nucleus (EL) with distinct anterior and posterior divisions (ELa and ELp, respectively). Examples of Toluidine-Blue-stained skin used to map knollenorgan locations illustrate the different appearances of knollenorgans, mormyromasts, and ampullary electroreceptors. (B) Locations of knollenorgans along the skin surface mapped in a *P. degeni*, with a line drawing illustrating midbrain anatomy. Knollenorgans occurred in three rosettes on the head (Augen, eye; Nacken, neck; and Kehl, throat; after Harder, 1968b). *Petrocephalus degeni* also had a relatively small EL lacking distinct anterior and posterior divisions. Examples of Toluidine-Blue-stained skin as in A.

Further, there was significantly greater inter-individual variation among the EODs of *G. victoriae* compared with *P. degeni* ($Z_{1225,105}=12.1$, $P<1e^{-15}$).

The EODs of the two species were completely separable within multivariate signal space (Fig. 2D). Further, the EODs of *G. victoriae* occupied a greater region of this space, again reflecting greater individual variation among EODs in *G. victoriae*. I found no evidence for sex differences in EOD waveform, which are usually only apparent during the rainy breeding season (Carlson and Arnegard, 2011; Hopkins, 1986).

Petrocephalus degeni* was found at higher population densities than *G. victoriae

To estimate relative population densities, I made *in situ* electrical recordings in Petrol Lagoon, where the greatest numbers of both species were found. A small stream flowed in a south–north direction from the swamp proper to form a lagoon that gave way to a stream that penetrated the surrounding grassland and then entered a forested area (Fig. 3A). A small dirt road crossed the stream, which was used mainly by grazing cattle and their herdsman.

Across 8 days of sampling, *G. victoriae* was found at medium density in just one location on one day (Fig. 3). In all other instances

in which I detected *G. victoriae*, only one individual was detected at a given sampling point (i.e. low density, Fig. 3). By contrast, *P. degeni* were frequently found at medium and high density (Fig. 3). The overall numbers of both species tended to increase over time (Fig. 3C), likely reflecting the emergence of fish from the flooded forest as the water level steadily dropped after the onset of the dry season. Both species showed an apparent preference for vegetative cover (Fig. 3).

Fewer individuals were detected in the nearby Snake Lagoon, but their relative abundance fit a similar pattern. Just two *G. victoriae* were found in Snake Lagoon, both on 2 July. By contrast, 22 *P. degeni* were found in Snake Lagoon, five on 28 June, five on 2 July, and 12 on 5 July.

Gnathonemus victoriae* exhibited greater shelter-seeking behavior and greater competition for shelter compared with *P. degeni

Previous laboratory studies on mormyrids have documented strong motivation to seek shelter, especially during the day (Carlson et al., 2000; Moller et al., 1989; Rojas and Moller, 2002; Terleph and Moller, 2003). This behavior has been used to score relative dominance, as a dominant individual will maintain sole access to the

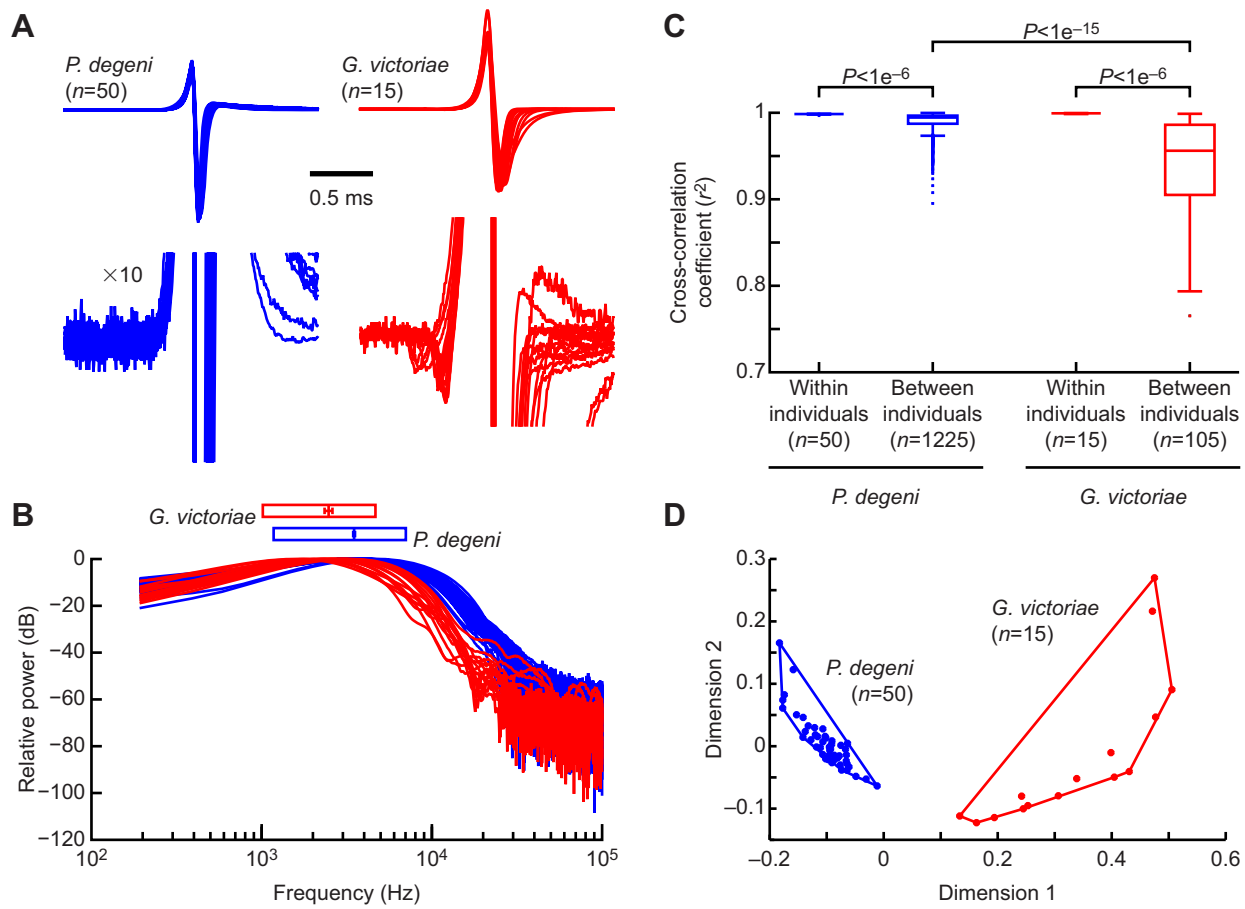


Fig. 2. Electric organ discharge (EOD) waveforms were more variable among *Gnathonemus victoriae* than *Petrocephalus degeni*. (A) EOD waveforms of *P. degeni* and *G. victoriae* captured in Lwamunda Swamp. The EODs are amplitude-normalized and plotted head-positive up, with the EODs from different individuals superimposed and aligned to the head-positive peak. The same EODs are plotted on an expanded vertical scale below. (B) Frequency power spectra of the same EODs estimated using Welch's averaged, modified periodogram method. Each trace is normalized to the maximum power. Bars above the traces show the average range of frequencies within 3 dB of the peak power frequency, along with the mean peak power frequency (\pm s.e.m.). (C) Box plots of maximum cross-correlation coefficients for all pair-wise EOD waveform comparisons. Within-individual comparisons were made by randomly selecting two EODs from the same individual; between-individual comparisons were made by randomly selecting an EOD from each of the two different individuals. Statistical comparisons were performed with Mann–Whitney *U*-tests. (D) Minimum polygons enclosing the EOD waveforms collected from *G. victoriae* and *P. degeni* plotted in bivariate signal space. An EOD was randomly selected from each individual of both species, followed by pair-wise cross-correlation between all these EODs. The maximum coefficients from all pair-wise cross-correlations were used to generate coordinates for each EOD using multidimensional scaling.

shelter by aggressively excluding a subordinate (Carlson et al., 2000; Terleph and Moller, 2003). Here, I used a similar assay not to score relative dominance, but to quantify shelter-seeking behavior and competition for shelter as a function of fish number.

Isolated *G. victoriae* tended to enter the shelter shortly after the start of the trial and then remain in the shelter throughout the trial, whereas isolated *P. degeni* tended to spend less time in the shelter and exhibited more movement into and out of the shelter (Fig. 4C,D). The density dependence of sheltering also differed between species. When groups of two *G. victoriae* were added to the tank, they tended to fight over the shelter. This was reflected in an average of approximately one fish occupying the shelter at any given moment, as in the trials with one individual, but greater numbers of movements into and out of the shelter compared with the trials with one individual (Fig. 4C,D). Increasing the number of *G. victoriae* led to an increase in the average number of fish in the shelter at any given moment as well as an increasing number of movements into and out of the shelter (Fig. 4C,D). At these higher densities, the number of fish in the shelter tended to peak just after the start of the trial and then decrease to a steady plateau (Fig. 4C). However, this

plateau did not reflect a lack of competition, but instead an equilibrium between fish entering and exiting the shelter, evident as steady increases in the cumulative number of movements into and out of the shelter (Fig. 4D).

Comparing isolated fish and groups of two fish in *P. degeni* revealed almost no difference in either the number of fish in the shelter or the number of movements into and out of the shelter (Fig. 4C,D). At greater densities of *P. degeni*, both the number of fish in the shelter and the number of movements into and out of the shelter tended to increase, but less so than in *G. victoriae* (Fig. 4C,D). Notably, in groups of 10 *P. degeni*, the average number of fish in the shelter peaked shortly after the start of the trial, but decreased rapidly to <1 (Fig. 4C), concurrent with a decrease in the rate of movements into and out of the shelter over time (Fig. 4D).

These species differences were readily apparent when these data were summarized as the time-weighted average number of fish in the shelter (Fig. 4E) and the average rate of movements into and out of the shelter (Fig. 4F). *Petrocephalus degeni* spent significantly less time in the shelter than *G. victoriae* (two-way ANOVA: $F_{1,56}=25.1$, $P<1e^{-5}$), and there was a significant interaction effect

between group size and species, reflecting differences in density dependence ($F_{3,56}=8.2, P<1e^{-3}$). There was also a significant interaction effect between group size and species with respect to the average rate of movements into and out of the shelter (two-way ANOVA: $F_{3,56}=3.2, P<0.05$).

The behavior of isolated fish can be used to generate predictions for group behavior under the assumption that all fish in a trial are independent actors (i.e. no interactions between fish). Under this assumption, the expected group behavior is simply a product of the behavior of isolated fish and the number of fish in the trial. In both

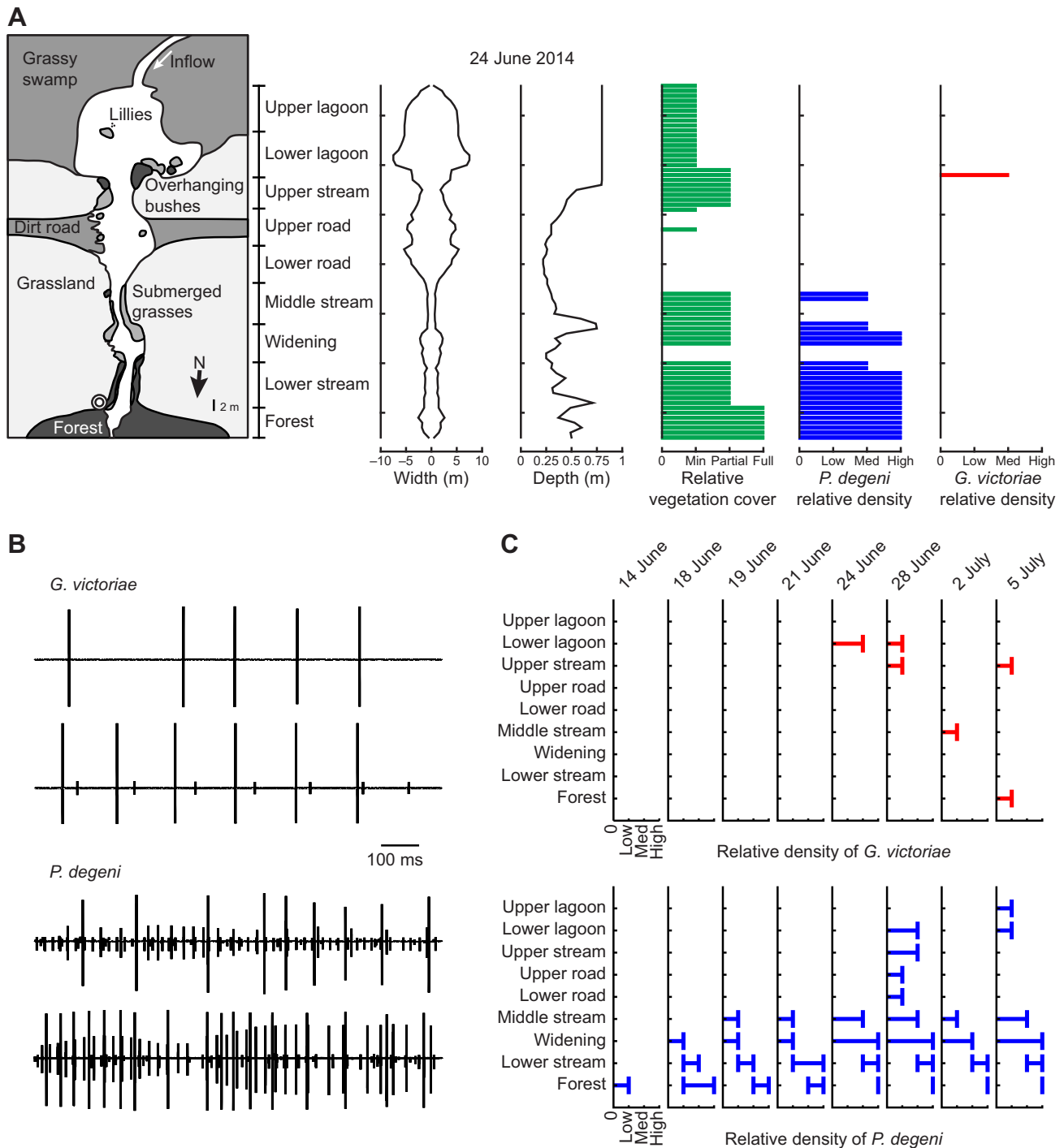


Fig. 3. *Petrocephalus degeni* was much more abundant than *Gnathonemus victoriae* in Petrol Lagoon. (A) Sketch of Petrol Lagoon on 24 June 2014. The lagoon was divided into nine different regions. The bulls eye marks 0°19'6" S, 31°56'48" E. The width and depth of the lagoon, vegetative cover, and densities of both species were measured at 1 m intervals along a transect. (B) Examples of *in situ* recordings of electric signals in Petrol Lagoon. *Gnathonemus victoriae* were typically encountered in isolation, evident as EODs with a single amplitude (top trace). In one extreme case (bottom trace), two individual *G. victoriae* were detected at the same location, evident as EODs with two distinct amplitudes. *Petrocephalus degeni* were often encountered in much larger densities, with ≥ 3 distinct EOD amplitudes apparent in the recordings (top and bottom traces). (C) The ranges of densities of both species within the nine different regions of the lagoon on eight different days following the onset of the dry season. *Petrocephalus degeni* was found at higher densities than *G. victoriae*, and the densities of both species tended to increase as the dry season progressed.

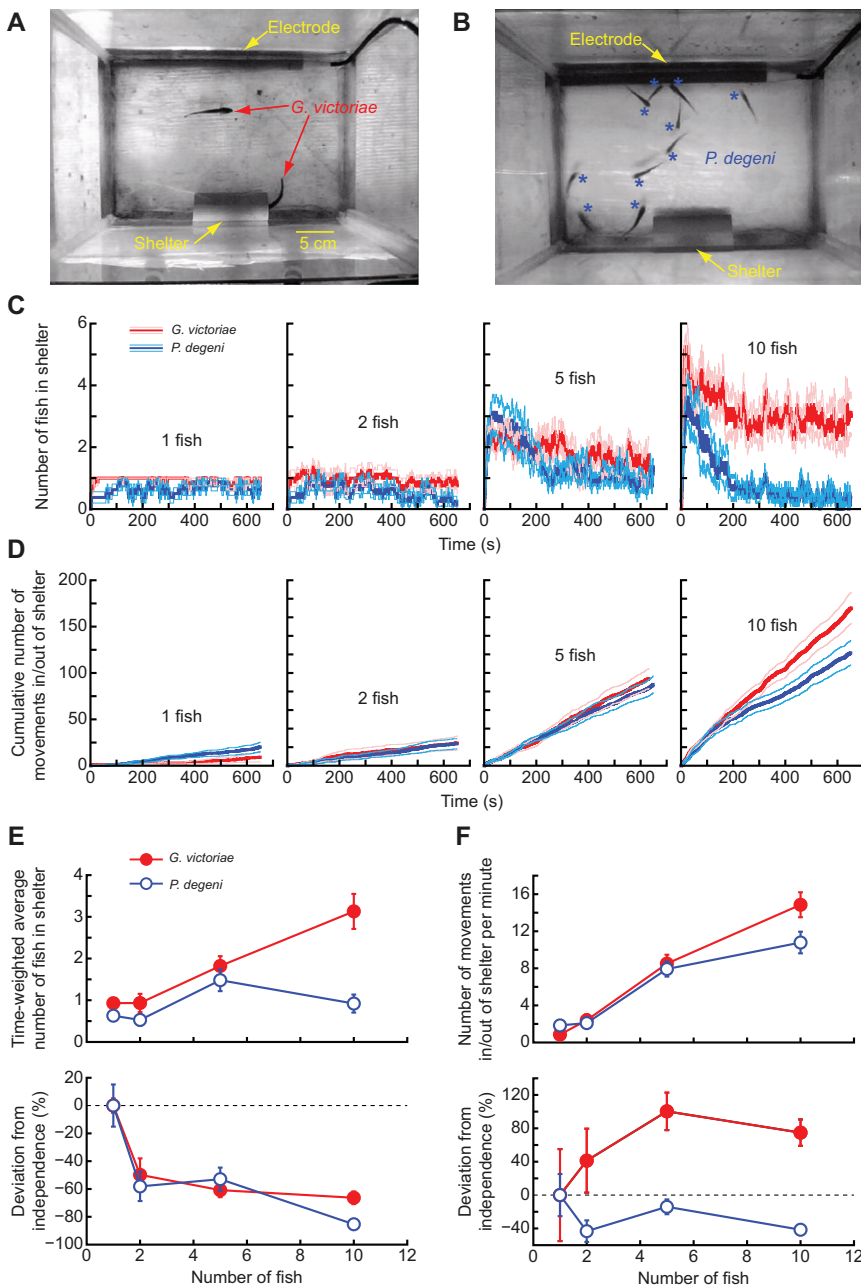


Fig. 4. *Gnathonemus victoriana* exhibited greater shelter-seeking behavior and greater competition for shelter than *Petrocephalus degeni*. (A) Single frame from the video recording of a trial with two *G. victoriana* (red arrows). (B) Single frame from the video recording of a trial with 10 *P. degeni* (asterisks). (C) Mean (\pm s.e.m.) number of fish inside the shelter over time for trials with 1, 2, 5, and 10 fish ($n=8$ trials for all fish numbers and both species). (D) Mean (\pm s.e.m.) cumulative number of movements into and out of the shelter over time for trials with 1, 2, 5, and 10 fish. (E) Top panel: time-weighted average number of fish inside the shelter throughout the trials as a function of fish number (mean \pm s.e.m.). Bottom panel: degree to which the time-weighted average number of fish in the shelter deviates from the assumption of independence between fish (i.e. the mean time-weighted average for one fish multiplied by the number of fish). (F) Top panel: rate of movement into and out of the shelter as a function of fish number (mean \pm s.e.m.). Bottom panel: degree to which the rate of movement into and out of the shelter deviates from the assumption of independence between fish (i.e. the mean rate of movement for one fish multiplied by the number of fish).

species, the time-weighted average number of fish in the shelter was less than predicted for all group sizes (Fig. 4E). Thus, fish in groups were less likely to shelter than isolated fish. This could reflect either competitive exclusion from the shelter or reduced shelter-seeking behavior. In *G. victoriana*, the number of movements into and out of the shelter was greater than predicted for all group sizes (Fig. 4F), consistent with increased competition for the shelter. By contrast, the number of movements into and out of the shelter was less than predicted for all group sizes in *P. degeni* (Fig. 4F), consistent with decreased shelter-seeking behavior. Alternatively, fewer movements into and out of the shelter could reflect extreme competitive exclusion, in which one fish dominates the shelter and completely prevents other fish from even entering the shelter. However, the average number of fish in the shelter for trials with 10 *P. degeni* was <1 for all time points after ~ 3 min from the start of the trials (Fig. 4C), which is inconsistent with this interpretation.

Electric signaling was anti-correlated between *G. victoriana* individuals, but correlated between *P. degeni* individuals

Recordings of electrical interactions between pairs of fish revealed distinct patterns of signaling behavior in the two species (Fig. 5A–C). *Petrocephalus degeni* generated EODs at significantly higher rates than *G. victoriana* ($t_{118}=4.4$, $P<1e^{-5}$). This was due at least in part to two distinct behaviors: *G. victoriana* generated pauses in EOD output (defined as an interval >500 ms) significantly more frequently than *P. degeni* (Fig. 5B,D), whereas *P. degeni* generated EOD bursts (defined as bouts of intervals <30 ms) significantly more frequently than *G. victoriana* (Fig. 5C,D). Pauses in *G. victoriana* tended to occur close in time to when the other fish in the pair accelerated its EOD rate (Fig. 5B), resulting in a strong negative correlation between EOD rates (Fig. 5E). By contrast, burst rates in *P. degeni* were correlated among communicating pairs ($r^2=0.52$, $t_{26}=5.3$, $P<1e^{-4}$; Fig. 5F).

In response to playback of conspecific signals, *P. degeni* was more likely to approach a stimulus than *G. victoriae*, and *P. degeni* responded with increased bursting whereas *G. victoriae* responded with pauses

To further evaluate the role of electric signaling in the social behavior of *P. degeni* and *G. victoriae*, I delivered conspecific electrical stimuli to individual fish when they were sheltering

(Fig. 6A). The percentage of time spent outside the shelter increased significantly during stimulation compared with the pre-stimulus period for both *P. degeni* (repeated-measures ANOVA: $F_{1,8}=18.2$, $P<0.01$) and *G. victoriae* ($F_{1,8}=8.9$, $P<0.05$). Compared with *G. victoriae*, *P. degeni* were significantly more likely to leave the shelter during playback (two-way repeated-measures ANOVA: $F_{1,16}=7.7$, $P<0.05$; Fig. 6B), and they were

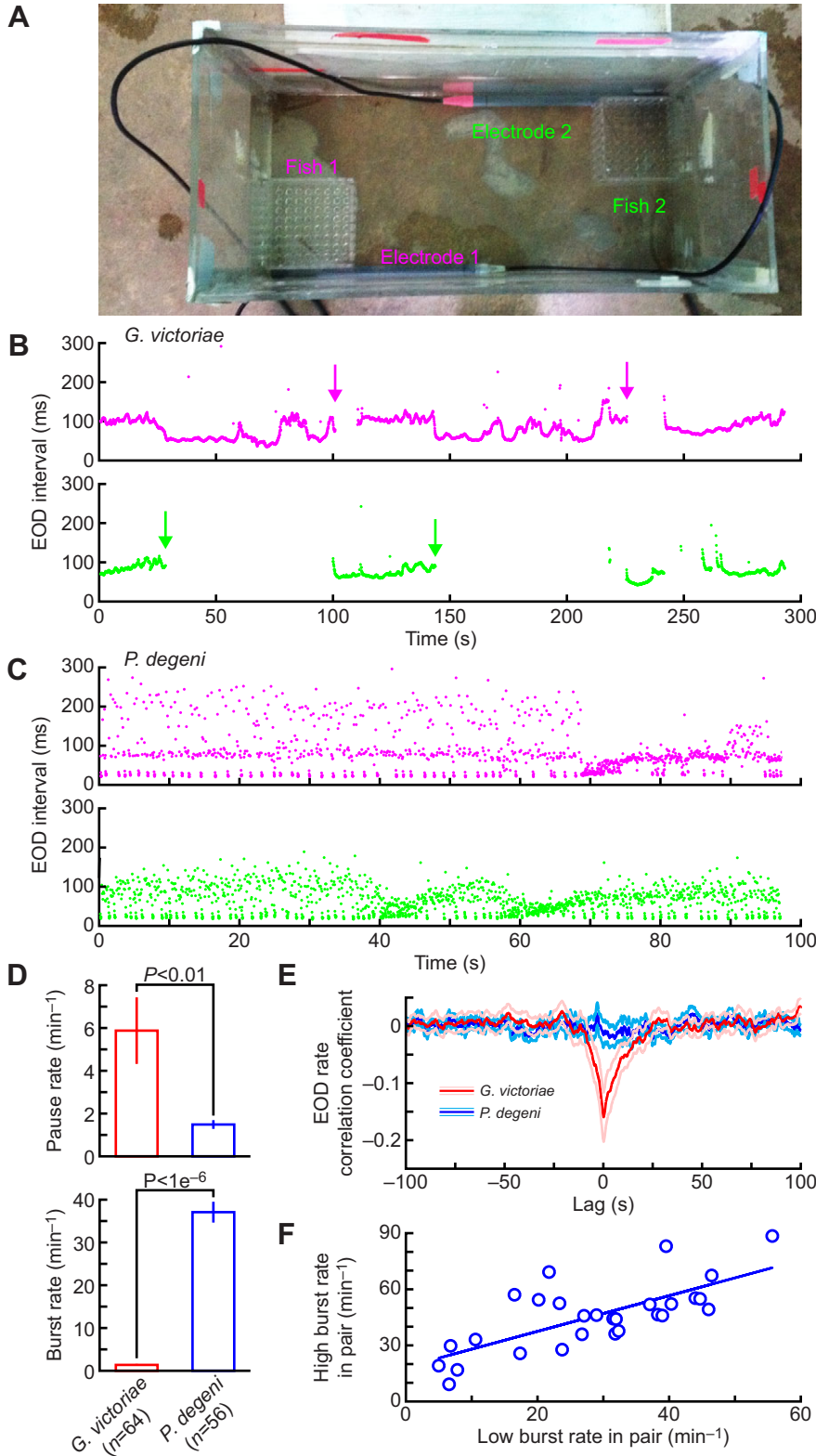
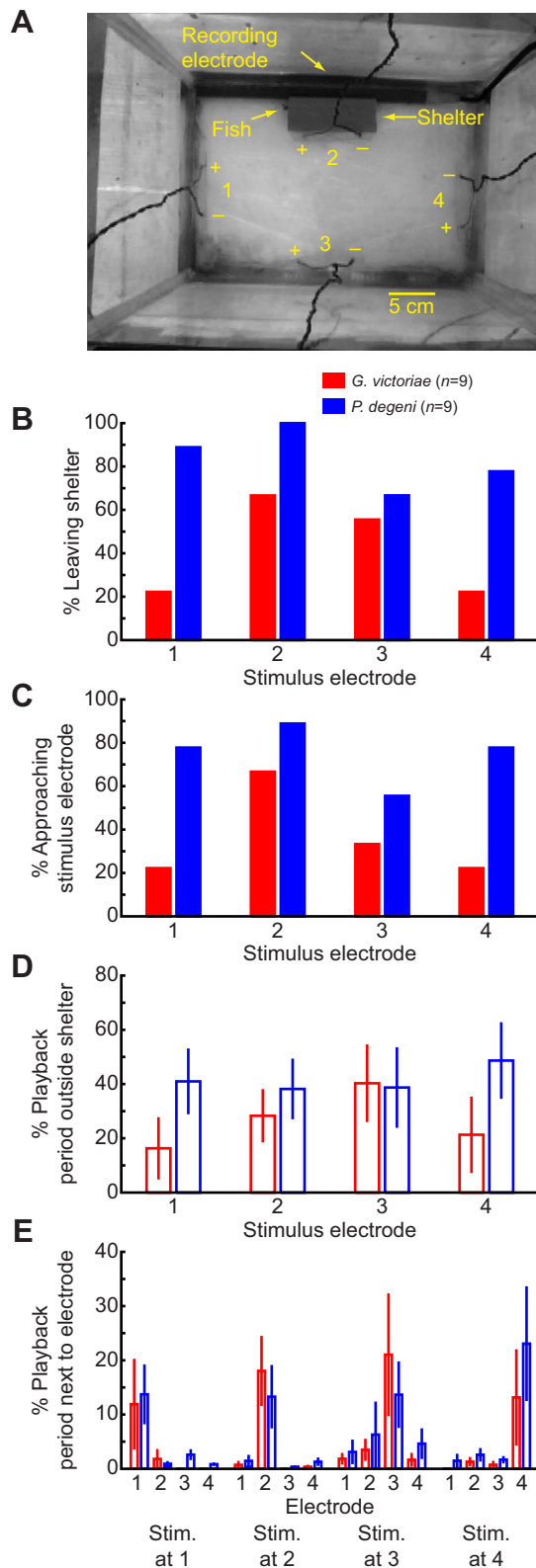


Fig. 5. *Gnathonemus victoriae* exhibited anti-correlated electric signaling behavior, whereas *Petrocephalus degeni* exhibited correlated electric signaling behavior. (A) Photograph illustrating the experimental setup. (B) An example of electric signaling between two *G. victoriae*. Note the occurrence of long pauses in EOD output (onset indicated by arrows), which tend to occur close in time to when the other fish increases its EOD rate. (C) An example of electric signaling between two *P. degeni*. Note the repetitive bursting behavior, evident as transient decreases in EOD interval. (D) *Gnathonemus victoriae* generated significantly more pauses than *P. degeni*, whereas *P. degeni* generated significantly more bursts than *G. victoriae* (statistical comparisons based on *t*-tests). (E) Cross-correlation of EOD rates from all 32 paired recordings in *G. victoriae* and all 28 paired recordings in *P. degeni*. (F) The burst rate of the fish that generated more bursts in a pair plotted against the burst rate of the fish that generated fewer bursts in that pair for all 28 paired recordings in *P. degeni*. Burst rates were significantly correlated across pairs ($r^2=0.52$, $t_{26}=5.3$, $P<1e^{-4}$).



significantly more likely to approach the electrode delivering the stimulus ($F_{1,16}=4.8$, $P<0.05$; Fig. 6C). The percentage of fish that left the shelter varied significantly depending on which electrode was delivering the stimulus ($F_{3,48}=3.5$, $P<0.05$), as did the percentage of fish that approached the stimulus electrode ($F_{3,48}=3.7$, $P<0.05$), with the greatest number of fish responding

Fig. 6. *Petrocephalus degeni* were more likely to show positive electro taxis to playback of conspecific electric signals than *Gnathonemus victoriae*. (A) Single frame from a video recording illustrating the experimental setup consisting of a shelter, recording electrode, and four stimulus electrodes arbitrarily numbered 1–4, with the location of the anode (+) and cathode (–) indicated for each. (B) Percentage of fish that left the shelter during a 60-s playback from a given electrode. (C) Percentage of fish that approached a given stimulus electrode during the 60-s playback period. An approach was defined as the fish coming within one body length of the electrode delivering the stimulus. (D) Mean (±s.e.m.) percentage of time during the 60-s playback period that fish were outside the shelter. (E) Mean (±s.e.m.) percentage of time during the 60-s playback period that fish were within one body length of the various electrodes.

when the stimulus was delivered from the electrode closest to the shelter (electrode 2; Fig. 6B,C).

However, across all subjects, there were no significant species differences in the proportion of time spent outside the shelter during playback (two-way repeated-measures ANOVA: $F_{1,16}=1.1$, $P>0.3$; Fig. 6D). In addition, although time spent in proximity to the four different electrodes varied depending on which electrode was delivering the stimulus (three-way repeated-measures ANOVA, interaction between stimulus electrode and response: $F_{9,144}=7.0$, $P<1e^{-6}$; Fig. 6E), there was no species difference in the amount of time spent next to the various electrodes ($F_{1,16}=0.1$, $P>0.7$). These findings suggest that *P. degeni* are more likely to approach an electric stimulus than *G. victoriae*, but when *G. victoriae* do approach a stimulus, they spend more time inspecting it. Indeed, considering only those fish that approached the stimulus electrode during playback revealed that *G. victoriae* spent significantly more time near stimulus electrodes than did *P. degeni* (mean of $44.4\pm 6.7\%$ vs $22.1\pm 4.4\%$ of the playback period across all four stimulus electrodes; Mann–Whitney U -test: $Z_{13,26}=2.9$, $P<0.01$).

I also monitored the patterns of electric signaling in test subjects. *Gnathonemus victoriae* often responded to the onset of a stimulus with long pauses (Fig. 7A), similar to those observed during electric communication (Fig. 5B). To quantify pause duration, I determined the maximum EOD intervals during the pre-stimulus and playback periods (Fig. 7B). The pauses were longest during playback from electrode 2, the electrode closest to the shelter (two-way repeated-measures ANOVA, interaction effect: $F_{3,24}=3.3$, $P<0.05$). By contrast, *P. degeni* responded to playback of electric signals by increasing the rate of bursting (Fig. 7C,D). Burst rate increased significantly during stimulation compared with the pre-stimulus period (two-way repeated-measures ANOVA: $F_{1,8}=12.6$, $P<0.01$), independent of which electrode was delivering the stimulus (interaction effect: $F_{3,24}=1.0$, $P>0.4$).

DISCUSSION

I found that the two mormyrid species in Lwamunda Swamp, *G. victoriae* (clade A) and *P. degeni* (non-clade A), exhibit anatomical differences in their knollenorgan electrosensory systems (Fig. 1) that are similar to the general patterns previously described for other species in their respective clades (Carlson et al., 2011; Harder, 1968a,b; Lavoué et al., 2004, 2010). The broad distribution of knollenorgans across the body surface and enlarged midbrain ELa/ELp that characterize clade A are associated with the ability to detect EOD waveform variation, which is lacking in petrocephalines having knollenorgan rosettes and a reduced EL (Carlson et al., 2011). In this light, the observation of greater intraspecific EOD variation in *G. victoriae* compared with *P. degeni* is particularly interesting (Fig. 2).

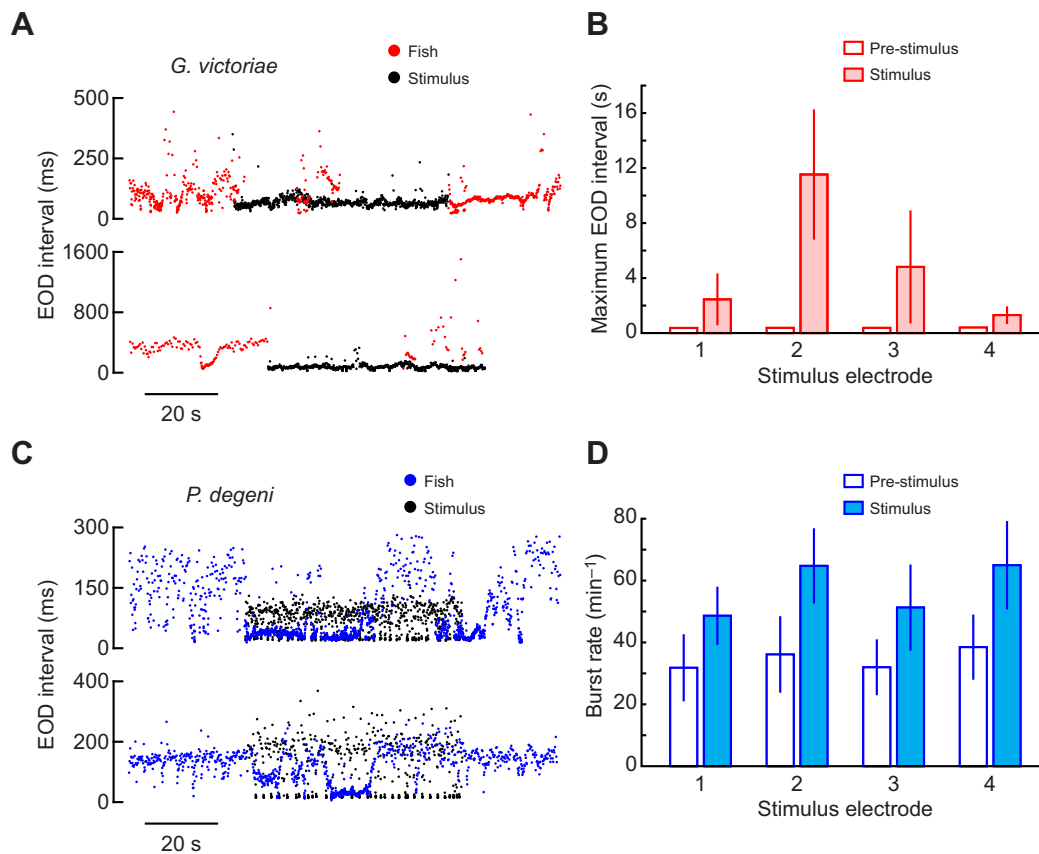


Fig. 7. *Gnathonemus victoriae* responded to playback of conspecific electric signals with pauses, whereas *Petrocephalus degeni* responded to playback of conspecific electric signals with increased bursting. (A) Two examples of electric signaling during playback from stimulus electrode 2 (see Fig. 6A) in *G. victoriae*. (B) Maximum EOD interval (mean±s.e.m.) in *G. victoriae* before and during a 60-s playback from a given electrode. Stimulation elicited long pauses, evident as an increase in maximum EOD interval. (C) Two examples of electric signaling during playback from stimulus electrode 2 (see Fig. 6A) in *P. degeni*. (D) Burst rate (mean±s.e.m.) in *P. degeni* before and during a 60-s playback from a given electrode.

Differences in the degree of intraspecific variation could be due to random genetic drift (Gallant et al., 2011), or it could function in relative dominance (Carlson et al., 2000; Terleph and Moller, 2003) or individual recognition (Graff and Kramer, 1992). Regardless of its adaptive significance, the ability of clade-A species to sense EOD waveform variation suggests that *G. victoriae* can detect these individual differences. Further, previous work has linked this perceptual ability to greater EOD diversity among clade-A species (Carlson et al., 2011). Thus, the findings of the present study link a macroevolutionary pattern (differences in interspecific EOD diversity between clades) to a microevolutionary pattern (differences in intraspecific EOD variation between sympatric species within these clades).

Previous field observations suggest that clade-A and petrocephaline species differ in habitat preference. Whereas clade-A species are typically found within dense vegetation and detritus, petrocephalines are often found in open water (Chapman et al., 1996; Friedman and Hopkins, 1996; Hopkins, 1980, 1981; Hopkins and Bass, 1981; Lavoué, 2012; Lavoué et al., 2008, 2004, 2010; Nichols and Griscom, 1917). Consistent with these observations, *G. victoriae* exhibited greater shelter-seeking behavior compared with *P. degeni* (Fig. 4). Further, petrocephalines generally have larger eyes, larger visual brain regions, and increased visual acuity compared with clade-A species (Stevens et al., 2013), consistent with a more open-water lifestyle. The roots, vegetation, and detritus in which clade-A species form territories result in reduced ambient light and numerous visual obstacles, which may be related to greater

reliance on the electrosense during social behavior. Microhabitat partitioning has likely played an important role in a rapid radiation within the clade-A genus *Campylomormyrus* (Feulner et al., 2007, 2008). The apparent difference in habitat preference between petrocephalines and clade-A species raises the possibility that spatial niche differentiation may also have been a factor in the early diversification of mormyrid lineages.

Within Lwamunda Swamp, *G. victoriae* were found at lower population densities than *P. degeni* (Fig. 3). Previous field studies have similarly shown that, in clade-A species, individuals tend to be spread out (Friedman and Hopkins, 1996; Hopkins, 1980), whereas petrocephalines are often found in high densities suggestive of schooling or shoaling (Chapman et al., 1996; Hopkins, 1980, 1981; Lavoué, 2012; Lavoué et al., 2008, 2004, 2010; Nichols and Griscom, 1917). Further, solitary individuals of clade-A species are repeatedly encountered at the same location over multiple days, which is suggestive of territoriality (Friedman and Hopkins, 1996). This interpretation is supported by laboratory studies of clade-A species, which have consistently documented agonistic interactions, establishment and defense of individual territories, and strict dominance hierarchies (Bauer, 1972; Bauer and Kramer, 1974; Bell et al., 1974; Carlson et al., 2000; Crockett, 1986; Kramer, 1976a,b,c, 1979; Kramer and Bauer, 1975, 1976; Terleph, 2004; Terleph and Moller, 2003).

Petrocephaline behavior has not been studied as thoroughly, but laboratory observations have documented territoriality similar to that seen in clade-A species (Lamml and Kramer, 2008; Scheffel

and Kramer, 2000). However, these studies were performed in tanks housing relatively small numbers of conspecifics. Fishes that shoal or school typically do so in captivity only when housed in sufficiently large groups, and can exhibit elevated aggression or stress in smaller groups (Magurran and Pitcher, 1983, 1987; Pavlov and Kasumyan, 2000; Saxby et al., 2010). Indeed, my unpublished observations of *Petrocephalus soudanensis* housed in the laboratory revealed highly coordinated schooling behavior in groups of ≥ 10 individuals, but extreme aggression in groups of two to five individuals. *Petrocephalus tenuicauda* and *Petrocephalus microphthalmus* form open-water shoals and schools, respectively, when housed in aquaria with ≥ 10 individuals (Baker et al., 2015).

The present study expands upon these previous observations by directly comparing the social behavior of a clade-A species and a petrocephaline species occurring in sympatry. Previous studies have documented strong shelter-seeking behavior in mormyrids (Carlson et al., 2000; Moller et al., 1989; Rojas and Moller, 2002; Terleph and Moller, 2003). I used this behavior to quantify species differences in sociality by determining how sheltering was influenced by fish density. As the density of fish increased, *G. victoriae* exhibited increased competition for shelter, whereas *P. degeni* showed decreased shelter-seeking behavior (Fig. 4). This suggests a greater degree of social affiliation in *P. degeni*, with motivation to seek shelter being outweighed by attraction to conspecifics. Indeed, in response to playback of conspecific signals, *P. degeni* were significantly more likely than *G. victoriae* to exit the shelter and approach the playback electrode (Fig. 6). Other studies have similarly found that species with a propensity for shoaling are more likely to seek shelter or structurally complex habitats when there are few or no conspecifics nearby (Huijbers et al., 2011; Magurran and Pitcher, 1983, 1987). Both sheltering and group living can reduce predation risk (Almany, 2004; Magurran, 1990; Pavlov and Kasumyan, 2000; Pitcher and Parrish, 1993; Shulman, 1985), and numerous predators are found in Lwamunda Swamp, including piscivorous birds, fish, snakes, and aquatic insects (Chapman et al., 1996; Randle and Chapman, 2004).

Given the evidence that *G. victoriae* exhibits greater shelter-seeking behavior and social competition, the observed intraspecific differences in their behavioral responses to playback is particularly interesting. Although *G. victoriae* were less likely to leave the shelter and approach electric stimuli overall, those fish that did approach spent approximately twice as much time inspecting stimuli as did *P. degeni*. Thus, individual *G. victoriae* fell at one of two extremes: either they did not leave the shelter, or they spent a substantial amount of time investigating stimuli. This difference is suggestive of shy versus bold personalities, respectively, as described in the behavioral syndromes literature (Sih et al., 2004). Boldness has been shown to correlate with relative dominance in some fish species (Colléter and Brown, 2011; Dahlbom et al., 2011; Sundström et al., 2004), raising the possibility that intraspecific variation in the responses of *G. victoriae* to playback represents the behavior of submissive versus dominant individuals. However, this remains speculative: although behavioral syndromes have been described in several fish species (Conrad et al., 2011), they have not yet been studied in mormyrids.

Gnathonemus victoriae and *P. degeni* also differed in their patterns of signaling behavior, both during electrical interactions between pairs of fish (Fig. 5) and in response to playback of conspecific signals (Fig. 7). *Gnathonemus victoriae* responded to playback with long pauses (Fig. 7A,B). They also generated long pauses during electric signaling (Fig. 5B,D), particularly when the

other fish in the pair increased its discharge rate (Fig. 5E). Long cessations of electric signaling have been observed previously in mormyrids, and the findings of several studies that looked at correlations between social behavior and signaling suggest they function as a submissive appeasement signal, or as a way to hide from potential competitors or aggressors (Bell et al., 1974; Bratton and Kramer, 1989; Carlson, 2002; Hopkins, 1986; Kramer, 1974; Kramer and Bauer, 1976; Moller, 1970, 1995; Moller and Bauer, 1973; Moller et al., 1989; Scheffel and Kramer, 2000; Wong and Hopkins, 2007). This interpretation fits with the strong shelter-seeking behavior and competition for shelter observed in *G. victoriae*, as silencing electric signaling upon detecting a conspecific would help a fish avoid disclosing its location and thereby evade a potential agonistic encounter and competitor for its shelter.

By contrast, *P. degeni* tended to generate EOD bursts (Fig. 5C,D). They responded to playback with increased bursting (Fig. 7C,D), and burst rates were correlated during electrical interactions (Fig. 5F). A variety of burst types, with diverse communicative functions, have been described in several mormyrid species (reviewed in Carlson, 2002; Hopkins, 1986). In pack-hunting *Mormyrops anguilloides*, synchronized bursting between pack members has been interpreted as a contact signal (Arnegard and Carlson, 2005). Given the results indicating affiliative behavior in *P. degeni*, the observation of correlated bursting is consistent with a similar interpretation. However, further study is needed to conclusively determine the function of these particular bursts in social behavior.

By studying two species in sympatry, confounding environmental differences that may influence social behavior are minimized. Further, the low degree of mormyrid diversity in Lwamunda Swamp offers the important advantage of minimizing potential confounds of interspecific interactions such as reproductive character displacement on electric signals and behavior (Crampton et al., 2011; Howard, 1993). The two clades studied exhibit major differences in the perception of electric signals and this is associated with differences in the rate of signal evolution (Carlson et al., 2011). Thus, high sympatric mormyrid diversity could have very different effects on EOD waveform variation and social behavior on species in the two clades (Hoskin and Higgie, 2010).

However, it is not clear whether the differences observed between these populations generalize to their species as a whole. Further, it is not possible to generalize differences between just two species to their entire respective clades. Mormyrids are phylogenetically and phenotypically diverse, including at least 175 clade-A species and 30 petrocephaline species (Carlson and Arnegard, 2011). Further, mormyrids are distributed throughout sub-Saharan Africa and the Nile River Basin (Hopkins, 1986; Moller, 1995), a range of incredible ecological diversity. Finally, the social behavior of only a handful of mormyrid species has been studied (for reviews, see Carlson, 2002; Hopkins, 1986; Kramer, 1990; Moller, 1995). Thus, it is not yet possible to determine conclusively whether the behavioral differences observed in the present study are representative of broader patterns that link evolutionary divergence in electrosensory anatomy to lineage-specific differences in social behavior.

Compared with gregarious species, a solitary, territorial lifestyle places a premium on locating individual conspecifics and determining their species, sex, and individual identity, as this is crucial for identifying potential competitors and mates, distinguishing neighbors from intruders, and maintaining stable dominance hierarchies. Indeed,

EOD waveform variation has been implicated in species recognition, mate choice, relative dominance, and individual recognition in several clade-A species (Arnegard et al., 2006, 2010; Carlson et al., 2000; Feulner et al., 2009a; Graff and Kramer, 1992; Hopkins, 1980, 1981; Hopkins and Bass, 1981; Machnik and Kramer, 2008; Terleph and Moller, 2003). By contrast, petrocephalines with knollenorgan rosettes and a reduced midbrain EL cannot detect EOD waveform variation. Thus, one possible hypothesis to explain evolutionary divergence in the knollenorgan electrosensory system is that a shift in behavior towards greater territoriality, increased social spacing, and structurally more complex microhabitats near the origins of clade A selected for increased reliance on electric signaling and thereby drove evolutionary change in the electrosensory system. Alternatively, changes in the electrosensory system may have arisen first, resulting in new sensory abilities that drove differences in social behavior. Although phylogenetic patterns of divergence in the knollenorgan electrosensory system have been clearly mapped out (Carlson et al., 2011), there is not yet enough comparative data to characterize the history of evolutionary change in mormyrid social behavior. Behavioral, ecological, and genomics studies in a wide range of mormyrid species are needed to evaluate these hypotheses and identify the ultimate causes of evolutionary change in the electrosensory system and its consequences for the perception of communication signals.

Acknowledgements

I thank Lauren Chapman and Dennis Twinomugisha for logistical support with field work, Tyler Moulton for his help catching fish and obtaining transect measurements, and the team of field assistants and fishers at Lake Nabugabo for their assistance. Nancy Fang, Ji-Yun Suh and Anan Lu helped analyze videos from the shelter assays and playback experiments. Alejandro Vélez provided helpful feedback on an early draft.

Competing interests

The author declares no competing or financial interests.

Author contributions

B.A.C. performed all experiments and analyses. B.A.C. wrote and edited the paper.

Funding

This study was supported by the National Science Foundation [NSF IOS-1255396 to B.A.C.].

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