

RESEARCH ARTICLE

The captivating effect of electric organ discharges: species, sex and orientation are embedded in every single received image

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ABSTRACT

Some fish communicate using pulsatile, stereotyped electric organ discharges (EODs) that exhibit species- and sex-specific time courses. To ensure reproductive success, they must be able to discriminate conspecifics from sympatric species in the muddy waters they inhabit. We have previously shown that fish in both *Gymnotus* and *Brachyhypopomus* genera use the electric field lines as a tracking guide to approach conspecifics (electrotaxis). Here, we show that the social species *Brachyhypopomus gauderio* uses electrotaxis to arrive abreast a conspecific, coming from behind. Stimulus image analysis shows that, even in a uniform field, every single EOD causes an image in which the gradient and the local field time courses contain enough information to allow the fish to evaluate the conspecific sex, and to find the path to reach it. Using a forced-choice test, we show that sexually mature individuals orient themselves along a uniform field in the direction encoded by the time course characteristic of the opposite sex. This indicates that these fish use the stimulus image profile as a spatial guidance clue to find a mate. Embedding species, sex and orientation cues is a particular example of how species can encode multiple messages in the same self-generated communication signal carrier, allowing for other signal parameters (e.g. EOD timing) to carry additional, often circumstantial, messages. This ‘multiple messages’ EOD embedding approach expressed in this species is likely to be a common and successful strategy that is widespread across evolutionary lineages and among varied signaling modalities.

KEY WORDS: Sex recognition, Image processing, Electrotaxis, Electric fish

INTRODUCTION

Sex recognition is a critical component of sexual selection, mate choice and species maintenance. This requires communication channels among individuals of the same species via use of both a common signal carrier and a common code to interpret these signals.

The weakly electric fish *Brachyhypopomus gauderio* employs a communication system based on the emission of a series of electric organ discharges (EODs) with a species- and sex-specific time course that exhibits power spectral density distributed over a wide frequency range that overlaps with that of sympatric species (Rodríguez-Cattaneo et al., 2008, 2013; Waddell et al., 2016;

Waddell and Caputi, 2020a). They also use these signals to locate conspecifics via electrotaxis (Hopkins, 2005; Waddell and Caputi, 2020b). Signal manipulation indicates that fish are able to discriminate between species using the time course of the signal emitted by an attracting electric source (Waddell and Caputi, 2020b). The Davis–Schluger–Hopkins behavioral model of electrotaxis proposes that fish maximize the current flowing along their body by adapting the fish shape to the field lines (Davis and Hopkins, 1988; Hopkins et al., 1997; Schluger and Hopkins, 1987; Shieh et al., 1996). While early experiments were performed by playing single sinewave cycles, and did not show differences between the source poles (Hopkins, 2005), later research compared fish behavior using signals that resembled the species-specific EODs and suggested that while the aggressive *Gymnotus carapo* follows the signals approaching the head of an emitting fish (‘head-heading’ swimming; Scudamore and McGregor, 1993), and the social *B. gauderio* follows signals approaching the tail (‘tail-heading’ swimming; Curtis and Stoddard, 2003). This suggests that pulse Gymnotiformes evaluate the time course of the emitter signal to determine species and orient swimming movements during conspecific approach (Waddell and Caputi, 2020a,b). Previous studies have also implicated the time course of the EOD as a signal for sex recognition (Gavassa et al., 2013). Here, we tested these two hypotheses.

To evaluate the effective time course cue for signal identification using the innate behavior of electrotaxis, we had to evaluate two non-exclusive mechanisms compatible with the Davis–Schluger–Hopkins model (Davis and Hopkins, 1988; Hopkins et al., 1997; Schluger and Hopkins, 1987; Shieh et al., 1996). First, fish may use the direction of the external field of every single pulse to control the side-to-side difference in the main muscular tonus which determines both body bending and the traveling wave of the caudal fin, which determines velocity and rostrocaudal direction. Second, the fish may additionally have a ‘field hunting’ strategy in which the motor control results from comparison of the present electric image with a stored one. Electric image analyses have shown that the spatial profile of the signal on the receiver’s skin depends on the funneling effect of the fish’s highly conductive body, which elicits a head–body and a side-to-side image gradient (Aguilera et al., 2001; Caputi and Budelli, 1995; Castello et al., 2000) when the fish is aligned with or transverse to the field direction, respectively. Thus, it can be predicted that even in a constant uniform field, with zero gradient, the fish is able to track the field lines based only on the effect of the field on its own body.

Here, we show evidence confirming single image sufficiency for field direction evaluation, and implemented a forced-choice decision making test using stimulation with a uniform field, to test and show that differences in conspecific-generated electric images including the time course of local signals and the spatial profile are sufficient for sex recognition and motivate fish to swim along the field lines towards the conspecific’s tail.

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MATERIALS AND METHODS

Ten fish of a single species, *Brachyhypopomus gauderio* (Giora and Malabarba, 2009), were used in this study. Fish were captured using dip nets in the Rio Negro (32°49'9.58"S, 56°30'31.15"W) Uruguay, under permission of the Ministerio de Agricultura y Pesca. All experiments were performed under the regulations of animal care and use committees of the Instituto de Investigaciones Biológicas Clemente Estable (protocol no. 001/03/2011). None of the procedures caused harm to the specimens, nor did they involve surgical procedures or chemical agents, and all specimens were returned to captivity after completion of trials. All experiments were carried out within 6 weeks of capture, while fish were still in reproductive status. We chose the five most clearly sexually dimorphic individuals of both sexes, e.g. males with long, thick tails (Giora and Malabarba, 2009), and gravid females. Individuals ranged from 12.1 to 20.4 cm for males (mean 16.0 cm, median 14.5 cm), and 9.2 to 16.8 cm for females (mean 14.2 cm, median 14.3 cm). During the captivity period, fish were kept in individual tanks with conditions matching those we found in the wild (water conductivity of 70 $\mu\text{S cm}^{-1}$, 25°C water temperature) and were fed *ad libitum* with insect larvae. The light cycle was natural, provided by large windows, and matched light levels found in wild conditions.

The aim of the first series of experiments was to evaluate whether fish of a given sex prefer to approach a conspecific of the same or other sex either heading toward the head or heading toward the tail, and so we used the same setup as in a previous article (Waddell and Caputi, 2020b). A given EOD signal was played between two electrodes separated by 15 cm and placed alongside the short side of the arena (1.5×1.0×0.07 m) to elicit electrotaxis. Fish were placed in a shelter, positioned perpendicular to the stimulating dipole, parallel to the long side of the tank, at the center of the arena, and after an adaptive period of 10–15 min the door of the shelter was opened. Male and female characteristic signals were the average head-to-tail EODs of 5 sexually dimorphic individuals of each sex (EODs were recorded by placing the fish in the middle of a large tank with an electrode at each longitudinal end of the tank). Stimuli were normalized by root mean square (rms) voltage to ensure the two

stimuli had the same amount of energy (Fig. 1) and played back via the audio port of a personal computer and isolated from ground (A-M Systems analog stimulus isolator model 2200). These stimuli consisted of a series of EODs quantized at 16 bits and sampled at 44,100 Hz, following a natural timing pattern obtained from a resting male *B. gauderio* recorded over an hour. Six trials were carried out per fish, 3 for each waveform, and were video-monitored with a web camera (Motorola G4, 13-MP CMOS, f/2.0) placed 1 m above the bottom of the tank.

The second and third series of experiments were performed in a frosted glass wall Y-maze filled with aquarium water to evaluate (i) whether a single electric image has enough information and is effective for eliciting the electrotaxis behavior and (ii) sex preference in forced-choice experiments. The stem ended on the center of a transversal connector which also connected with the branches at a straight angle (Movie 1). Distinct sexual stimuli were played between a common electrode made from a loop of bare nichrome wire affixed at the end of the central stem and at each end of the decision branches, and covered by a 3 cm sponge. The stem and branches of the maze had a constant section (5×5 cm) and were the same length (40 cm) to constrain the current flow in a common section's corridor. The rationale behind this maze shape was to generate a uniform field in the absence of fish. Therefore, when a difference of potential was applied between the end of a branch and the end of the stem, the electric field and current density at each section were constant all along the trajectory with the exception of the decision point where the geometry varied. While the fish presence causes a distortion of the field which in turn is translated into the electric image seen by the fish, this distortion – and thus the detected image – is only dependent on the alignment of the fish axis to the branch direction and is independent of fish position along the branch. Thus, the progress of the fish along the track cannot be attributed to a search for a larger signal gradient. This technique allowed us to rule out the necessity of the 'field hunting' mechanism, and test whether single image processing is sufficient for field direction evaluation. Whereas in the common stem, male and female longitudinal electric fields stimulated the fish, only one of these signals stimulated the fish when it was inside a branch. At the opening of the transverse connectors, the field had a nearly orthogonal direction to that in the central stem and branches.

In the second series of experiments, the field was experimentally confirmed to be uniform in the absence of a fish, and after placing a fish at the middle of the stem, the highly conductive body deformed the field and elicited a transcutaneous image. To check the uniformity of the applied stimulus field, we recorded it at different points along the stem and branches to confirm the constancy of the waveform and amplitude. To evaluate the electric image, transcutaneous stimuli along the head and body were evaluated by recording the applied stimulus electric field adjacent to the skin when the fish was in the stem, the branches and the decision point. Sexual stimuli were played between the end of one of the Y-maze branches and the end of the stem as trains of typical EODs repeated at 30 Hz. Male and female trains were separated by a short silence and lasted 5 s each. Received signals were recorded between two electrodes separated by 3 mm, connected to the input of a differential amplifier (A-M Systems microelectrode amplifier model 1800, gain ×1000, bandwidth 10 Hz to 10 kHz), and its output was digitized at 50 kHz, 12 bits (Axon Instruments, Digidata 1322A). The orientation of the electrode pair was adjusted until the largest juxta-cutaneous field was recorded. With the fish at the middle of the stem branch, the local stimulation fields at 16 equally spaced positions starting at the snout and ending on the tip of the tail

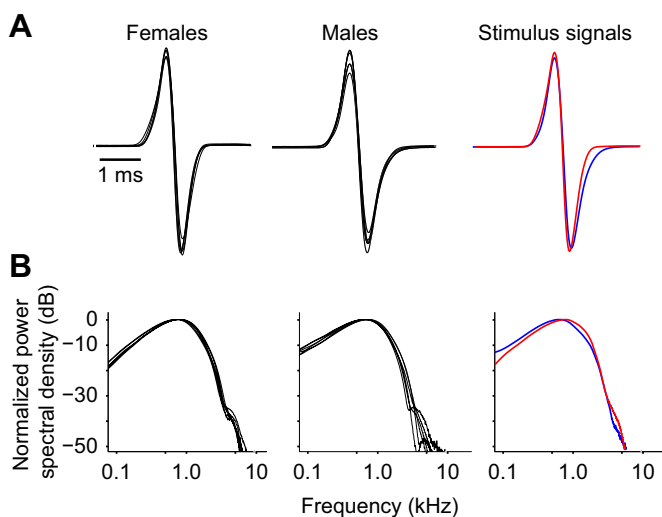


Fig. 1. Head to tail electric organ discharge (EOD) of *Brachyhypopomus gauderio*. (A) Time course of the root mean squared normalized waveform for females and males, and the superimposed averages (stimulus signals: male, blue; female, red). (B) Power spectral density (normalized to the peak) for females and males, and the superimposed averages (male, blue; female, red).

of a fish of 15 cm length were recorded. With the fish head at the decision point, we recorded the signals at symmetrical points of the head at either side.

In the third series of experiments, we applied this uniform stimulus field technique to study decision preference between sexes. Each of the 10 fish used in the study were involved in two series of 11 trials in which trains of sexually characteristic signals were played between each end of the decision branches and the stem. Male and female typical signals were emitted with a natural sequence of intervals using a current source driven by a computer (see above). The male and female signals described above were simultaneously applied between one electrode placed at the tip of each branch and a common electrode at the bottom of the common stem of the Y-maze. Sex stimuli applied to each branch were randomly determined from trial to trial. In one of the series, the stimuli had the same polarity as the self-emitted one (i.e. 'tail-heading'); in the other series, they had the opposite polarity ('head-heading'). Each trial started with the fish placed with its tail 5–10 cm from the end of the common stem heading to the decision point. After an adaptation period, the stimuli were generated through the stereo-audio port of the computer and simultaneously played through two similar isolation units (A-M Systems analog stimulus isolator model 2200). A potentiometer interfaced the ports and isolation units to enable the two signals to be finely equalized at 10 mV cm^{-1} all along each track. At the decision point, each signal had its maximum on opposite sides of the fish's head and rostral body. We evaluated decisions by observing fish behavior up to 1 h after stimulus onset.

In order to evaluate whether fish choice departed from a random-null hypothesis distribution, we counted the number of trials in which each fish swam down either the female or male stimulus branches, and calculated an individual preference score as the difference between male and female signal choices over all trials. We then calculated the probability of getting the observed distribution under the assumption of equal probability for each of the two options. We additionally tested whether the median score departed from zero for each sex group and the difference in score value between groups, using non-parametric tests.

RESULTS

Brachyhypopomus gauderio prefers tracking the field lines that converge on a conspecific's tail

In the first experimental series, we evaluated whether fish of a given sex prefer to approach a conspecific of the same or the other sex either heading toward the head or heading toward the tail. Male and female fish were exposed to electric fields characteristic of either sex in an arena of $1 \times 1.5 \text{ m}$, filled with $70 \mu\text{S cm}^{-1}$ water to a depth of 5 cm. This procedure isolated the electric waveform itself, ruling out the effects of visual, chemical or other behavioral signals that may also contribute to the decision-making process.

We found that all fish followed the field lines. Considering previous reports of electrotaxis (Scudamore and McGregor 1993, Curtis and Stoddard, 2003), we first tested whether there is a preferred approximation path. We assigned each individual one of four possible scores resulting from subtracting tail choices from head choices and compared the sign of the score distribution against a random null hypothesis (Fig. 2; $P=2^{-10}$, $N=10$ in each case). These results confirm that the field lines of the signals serve as a tracking guide to orient the fish to approach from behind and arrive abreast a conspecific. As the score distributions were almost identical, we infer that there is not a sex-related effect size (Fig. 2).

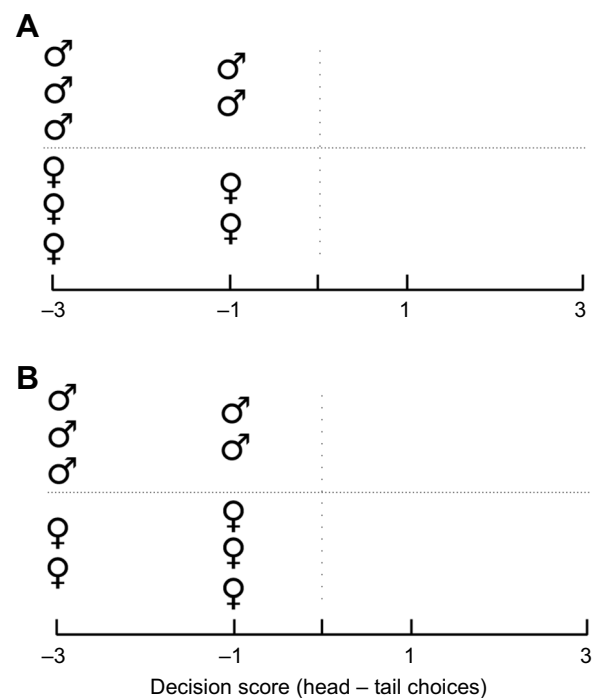


Fig. 2. Field line tracking. Fish of both sexes were exposed to electric fields characteristic of (A) female and (B) male fish. Decision scores were calculated as head choices minus tail choices. Each individual is represented by a symbol corresponding to their sex.

Male and female fish prefer the tail stimulus of the opposite sex

In the second and third group of experiments, we specifically explored the preference of individuals for the time course of the signals of the same or the opposite sex when the stimulus field was uniform. The decision-making experiment consisted of two series of forced-choice trials tested in a Y-maze with a common stem and branches of constant shape and area.

Prior to forced-choice experiments, we evaluated the electric images caused by sex-specific stimuli along the rectilinear branches and at the decision point. We recorded the maximum adjacent electric field at 16 different equally separated points along the body of a 15 cm fish aligned with the Y-maze stem axis (Fig. 2A, left). The head–body gradient of the rms value of the stimulus was not significantly different for the male and female signals at any recording point (Fig. 3B). In both cases, the largest amplitude was found at the snout and the reversal point was at about one-third of the fish length, at a position 5 cm posterior to the snout. It should be noted that when inverting the stimulus polarity, neither the longitudinal profile of local rms signals nor the power spectral density of the signals changed, but the polarity of the time courses was inverted. Therefore, the time course of the stimulus signal, considered as a whole, is the only feature that depends on the fish's approach direction relative to the stimulation dipole.

The time course of received sexual signals was also evaluated in more detail by constructing their phase portraits (i.e. field derivative versus electric field). While we found clear sexual differences, the portraits for each of the sexual stimuli were similar all along the fish body when signals were normalized by local amplitude. Fig. 3C,D compares portraits at the snout and a position 4 cm caudal to the snout. Both peaks of the local stimulation field (Fig. 3A, left) and the second and third peaks of the derivative were larger in female signals when stimulus signals were normalized such that

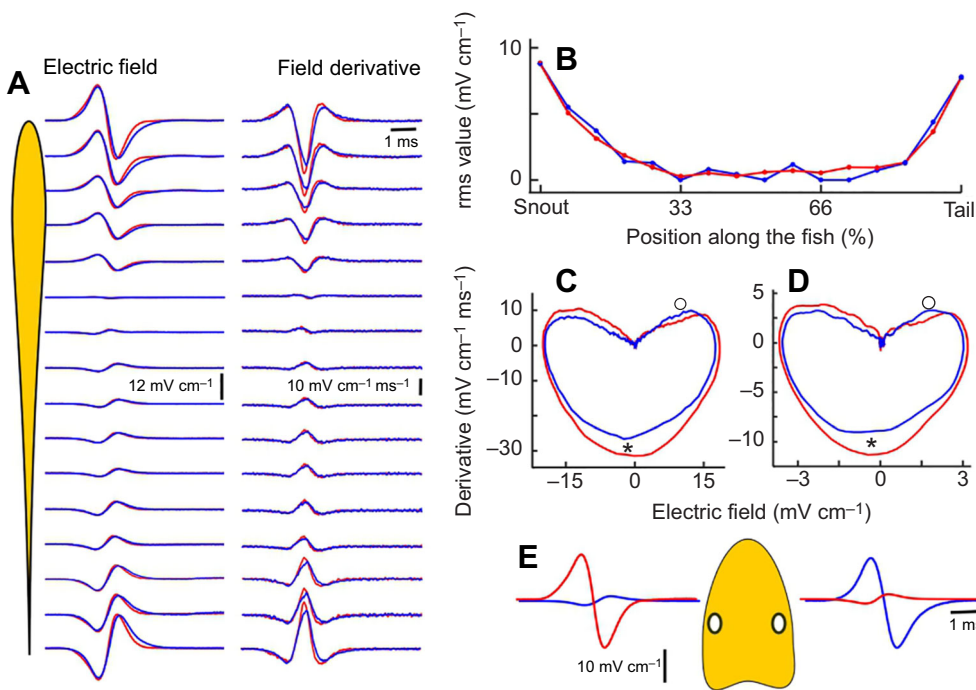


Fig. 3. Evaluation of the relevant parameter of the stimulus image used by the fish for decision making.

Female signal, red; male signal, blue. (A) Left: juxta-cutaneous local electric field. Right: time derivative of the juxta-cutaneous field. (B) Stimulus strength measured as the root mean square (rms) value, as calculated over a 10 ms period. (C,D) Phase portraits showing the similarity of the waveforms at the snout (C) and 4 cm posterior to the snout (D). Symbols indicate the difference between male (blue) and female (red) in the second (asterisks) and third (circles) peaks of the time derivative of the stimuli. (E) Stimulation at both sides of the head, with fish facing the decision point such that the male signal comes from the right and the female signal comes from the left maze branches.

they contained the same energy (Fig. 3A, right). The largest sexual difference was observed at the second peak of the stimulus derivative, being 20% greater in females than in males. At the decision point, we also found a very large side-to-side difference in the time course at the head region, with the largest juxta-cutaneous field on the stimulus side and an inversion of the polarity on the contralateral side (Fig. 3E).

Decision-making experiments consisted of 11 trials for tail-heading stimuli and one for head-heading stimuli carried out for each of the 10 fish. In the set of trials made with tail-heading stimuli, all 10 fish swam towards the branch point and made a decision to swim along the length of one of the branches in an interval shorter than 15 min after stimulus onset. None of the fish swam towards the branching point after 1 h of a head-heading stimulus playback, at which point trials were ended. This indicates a significantly larger effectiveness of stimulus polarity, consistent with the findings in the open field and previous studies (Waddell and Caputi, 2020b). As the strength of head-heading and tail-heading stimulus signals was the same for both polarities, and the field was uniform, this result indicates that the tracking cue is the surface gradient, over the skin, of some parameter of the transcutaneous stimulus time course. Further, in tail-heading stimuli conditions, we found a significant difference between male and female decision scores, calculated as female signal choices minus male signal choices. All females had a negative score, indicating a preference for male signals, and all

males had a positive score, indicating a preference for female signals (Fig. 4). While the probability of preferring either sex under a null random hypothesis is 0.5, the unanimous preference for the opposite sex in the 10 studied fish yielded a probability of 0.5^{10} under the null hypothesis. This low probability, along with the clear-cut separation of the signs of score choices between sexes, clearly indicates sex-specific EOD recognition (Fig. 4).

DISCUSSION

Brachyhypopomus gauderio live in sympatry with other electric fish of the same genus (*Brachyhypopomus bombilla*, *Brachyhypopomus draco*) and from other genera (*Gymnotus*, *Eigenmannia*, *Rhamphichthys*; J.C.W., unpublished data), and so to ensure reproductive success, they must be able to find a mate in the muddy waters they inhabit. In a previous report, we showed that they are able to distinguish between electric fields of conspecifics and allospecifics (Waddell and Caputi, 2020b). Here, we show that these fish are able to evaluate the local field of every single EOD in order to find a path that allows them to locate a conspecific of the opposite sex. Our results are compatible with the hypothesis that during social interaction, sexually dimorphic *B. gauderio* individuals are able to identify a sex-specific waveform parameter of the received transcutaneous current and have a preferential attraction to the opposite sex.

What are the relevant signals for electrotaxis and sexual discrimination?

Biophysical experiments show that in a uniform field the fish's body conductance causes a steep head-body gradient of signal emitted by a conspecific, and that under these conditions the fish is able to swim in a given direction, depending on the stimulus waveform. This means that the fish is able to evaluate the field direction by measuring the head-body gradient of a conspecific waveform parameter. The relevant image parameter evaluated by the fish in order to follow the field lines must significantly vary with field inversion as, in concordance with a brief previous report (Curtis and Stoddard, 2003), we found a tail-preferent approach and that the



Fig. 4. Sexual signal choice. Across all trials, all males have a net preference for female signals, and all females have a net preference for male signals. Each individual is represented by a symbol corresponding to their sex. For statistical purposes decision scores were calculated as the difference between same-sex choice and opposite-sex choice ($P=0.0009765$ under the null hypothesis of no preference).

stimulus in the decision maze only triggers electro taxis when it is applied in the tail-heading direction. It cannot be either the signal amplitude or the power spectral density, as these are equivalent for head-heading and tail-heading stimuli. Instead, polarity sensitivity parameters of the stimulus such as the main peak of the derivative (i.e. the peak-to-peak transition slope), positive-negative order of the peaks at the skin, and relative peak size (both associated with the sign of the maximum derivative) may contribute to waveform discrimination. In addition, as the second and third peaks of the derivative (asterisk and circle in Fig. 3C,D) significantly differ in male and female signals. While males prefer signals with steeper slopes, females prefer more gradual ones. This mechanism requires receptor tuning to the stereotyped pulsatile carrier characteristic of the species, with enhanced sensitivity for the maximum negative derivative. This receptor preference for skin-inward transition currents has been described by early encoding studies (Bennett, 1971) and is in agreement with heuristic modeling of electroreceptors (Cilleruelo and Caputi, 2012). Although receptor encoding has not been studied in relation to sexual differentiation in this species, in the absence of contrary evidence, derivative peaks should be considered the most likely candidates for encoding sex.

Our data also suggest that the fish evaluates the rostro-caudal and side-to-side difference in the stimulus time course. While the rostro-caudal difference may provoke a forward movement, the side-to-side difference may provoke a reactive turn, coinciding with the side-to-side head movements observed while the fish tracks the signal (Movie 1). In summary, a simple rule expressed as the conspecific signal triggers a negative feedback movement tending to reduce side-to-side and rostro-caudal stimulus differences in intensity may account for the tracking behavior. A similar mechanism was suggested for sex and position identification in mormyrids (Hopkins, 1986) and has been tested in artificial electrosensitive agents (Boyer et al., 2012). The geometry of the maze at the decision point places the peak of the sex-specific competing images on each fish side. The sex-related responsiveness of electroreceptors leads to a preferential turn to the side of the most effective stimulus. This side-to-side assessment of stimulus direction may be analogous to the way in which humans orient their head toward the interlocutor during verbal communication.

A single EOD may carry multiple messages

More generally, our finding implies that embedding both species and sex information in the signal carrier is a good communication strategy as it liberates carrier modulation in other dimensions to be used for independent messages. Sexual cues have been found embedded in the EODs of other electric fish, including both pulse Gymnotiformes (Gavassa et al., 2013; Hopkins et al., 1990; Shumway and Zelik, 1988) and pulse Mormyridae (Crawford, 1992; Hopkins and Bass, 1981; Kirschbaum and Schugardt, 2002; Kramer, 1997). This is also the case in Gymnotiformes that emit a continuous sine wave-like electric signal, in which frequency encodes the sexual differentiation condition of the individual (Dunlap et al., 1998; Hopkins, 1974; Mills et al., 1992; Zakon and Smith, 2009).

This may be an economical use of the stereotyped waveform that allows for other aspects of the electric field to be modified to provide additional information. For example, receptor tuning to a specific feature of a conspecific carrier (in this case the waveform shape) may allow the animal to encode additional messages in the modulation of the EOD's time series. Another known sexual code is the modulation of the EOD amplitude (Gavassa et al., 2013), shape (J.C.W., unpublished) and timing (Silva, 2002), as males increase

EOD amplitude during the night and display chirps, whereas females respond with long pauses. In addition, the way in which courtship involves near-field discharges may add new information to both fish (A. C. Silva and A.A.C., unpublished).

Here, we clearly demonstrate that electric signals alone are sufficient for sexually dimorphic electro taxis. The encoding of multiple messages within the signal communication carrier (Johnstone, 1996; Johnstone and Grafen, 1992; Kokko, 1997) merits further exploration in Gymnotiformes, because in addition to the advantages of the electric carrier (e.g. relative ease of recording, quantifying and artificially generating the EOD), they are also a diverse taxa described by robust phylogenetic analyses (Tagliacollo et al., 2016; Crampton, 2019).

We should note that we cannot rule out the use of other signaling modalities as a way to provide redundancy, to generally enhance communication efficacy via 'backup signals' (Johnstone, 1996); for example, visual differences (such as sexual dimorphism of the caudal filament) (Giora and Malabarba, 2009) or other non-electric displays, such as pheromones, in recognition and sexual selection.

The embedding of species, sex and position in the same communication message is certainly not exclusive to electric fish or the electric sense. Information regarding sex is embedded in other signaling modalities, and in a diverse array of taxa that includes sound-emitting fish (Kasumyan, 2008), frogs (Gerhardt and Huber, 2002), moths (Cardone and Fullard, 1988), bats (Grilliot et al., 2009), birds (Cooney et al., 2019; Price, 2015), and the human voice, which ranges from bass to soprano. Sex recognition is often embedded in permanent characteristics of a self-generated carrier, for example, female primates typically generate vocalizations with a higher pitch than those of males (Puts et al., 2016). In this manner, individuals can be recognized by conspecifics and simultaneously provide information relevant to sexual selection (including, but not limited to, reproductive status and/or an individual's quality) and also emit and receive messages encoded in carrier modulation. This 'multiple messages' approach (Johnstone, 1996; Zambre and Thaker, 2017) of embedding both species and sexual identity in the communication carrier is a mechanism that allows species to express complex information in the same signal. The electrosensory system in particular is intriguing because in addition to multiple messages, it separates messages via two distinct approaches: with electroreceptor tuning identifying sex and species information, and the prereceptor mechanism, caused by the effect that the conductive body shape has on the field, indicating signaler-receiver orientation.

Conclusions

Here, we provide evidence that sex and orientation are encoded in each EOD image. Both sexes swim towards a conspecific in a manner that optimizes the orientation of the head-body gradient towards the tail-converging field lines and minimizes the side-to-side gradient. As image processing is sufficient for determining field direction, 'field hunting', if it occurs, may be complementary. When two sexually dimorphic signals compete, fish preferentially orient toward the opposite sex. The encoding of additional messages within the EOD signal, such as short-range evaluation of the near electrosensory field (A. C. Silva and A.A.C., unpublished), rapid or slow waveform shape change (e.g. chirps or circadian rhythms) (Silva et al., 2013; Gavassa et al., 2013; J.C.W., unpublished), or timing modulation of the EOD (Silva et al., 2013), merits further exploration.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: J.C.W., A.A.C.; Methodology: J.C.W., A.A.C.; Software: J.C.W., A.A.C.; Validation: J.C.W., A.A.C.; Formal analysis: J.C.W., A.A.C.; Investigation: J.C.W., A.A.C.; Resources: A.A.C.; Data curation: J.C.W.; Writing - original draft: J.C.W., A.A.C.; Writing - review & editing: J.C.W., A.A.C.; Visualization: J.C.W., A.A.C.; Supervision: A.A.C.; Project administration: A.A.C.; Funding acquisition: A.A.C.

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