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Automated pulse discrimination of two freely-swimming weakly electric fish and analysis of their electrical behavior during dominance contest

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ABSTRACT

Electric fishes modulate their electric organ discharges with a remarkable variability. Some patterns can be easily identified, such as pulse rate changes, offs and chirps, which are often associated with important behavioral contexts, including aggression, hiding and mating. However, these behaviors are only observed when at least two fish are freely interacting. Although their electrical pulses can be easily recorded by non-invasive techniques, discriminating the emitter of each pulse is challenging when physically similar fish are allowed to freely move and interact. Here we optimized a custom-made software recently designed to identify the emitter of pulses by using automated chirp detection, adaptive threshold for pulse detection and slightly changing how the recorded signals are integrated. With these optimizations, we performed a quantitative analysis of the statistical changes throughout the dominance contest with respect to Inter Pulse Intervals, Chirps and Offs dyads of freely moving *Gymnotus carapo*. In all dyads, chirps were signatures of subsequent submission, even when they occurred early in the contest. Although offs were observed in both dominant and submissive fish, they were substantially more frequent in submissive individuals, in agreement with the idea from previous studies that offs are electric cues of submission. In general, after the dominance is established the submissive fish significantly changes its average pulse rate, while the pulse rate of the dominant remained unchanged. Additionally, no chirps or offs were observed when two fish were manually kept in direct physical contact, suggesting that these electric behaviors are not automatic responses to physical contact.

1. Introduction

Social hierarchies are established and maintained by a broad range of dynamic behaviors expressed by animals during communication (Lorenz, 1981). Weakly electric fish in the genus *Gymnotus* are territorial and show marked changes in their motor and electrical behavior once a dominance hierarchy is established (Batista et al., 2012; Westby, 1975a,b). The dominant fish actively swims and explores the whole environment, while the submissive fish often remains still. Sequences of Inter Pulse Intervals (IPI) reveal highly variable patterns that are clearly distinct before and after the dominance contest (Batista et al., 2012; Westby, 1975a; Zubizarreta et al., 2015). Two critical tasks are performed by using self- and conspecific-generated electrical pulses and their feedback on fish's electrosensory system: electrolocation and electrocommunication (Black-Cleworth, 1970; Caputi et al., 2008; Castello et al., 2000; von der Emde, 2013). Detecting distortions on the stereotyped self-generated electric pulse provides information from the environment (Jun et al., 2012; Pereira and Caputi, 2010), while IPI patterns are likely used for communication among conspecifics (Forlim and Pinto, 2014).

While fish are contesting dominance, they may stop emitting pulses during variable time intervals ("offs") (Westby, 1975a,b). In some situations, instead of the stereotyped pulses, these fish can emit small electric field oscillations, known as "chirps" (Fig. 2) (Batista et al., 2012; Zubizarreta et al., 2015). Offs and chirps are often related to submission (Batista et al., 2012), physical aggression and retreat. Therefore, they might be important flags used by fish to convey submission or stress. There is strong evidence that electrocommunication has an important role in dominance definition and maintenance (McGregor and Max Westby, 1992; Westby, 1975a).

However, to assess electrocommunication, one of the main challenges is to discriminate pulses from freely interacting fish (Black-Cleworth, 1970; Letelier and Weber, 2000; McGregor and

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Max Westby, 1992). Two distinct but electrically coupled aquaria have been used to avoid this problem (Forlim and Pinto, 2014), but complex behaviors such as chirps have never been detected with such artificial setups, possibly due the lack of behavioral cues other than the electric pulses (e.g., movement and bites).

Here we report on improving a state-of-art classification technique (Matias et al., 2015), that discriminates pulses from *Gymnotus carapo* dyads, to allow automated chirp detection. We used a supervised learning algorithm, and its training required only short samples of time series with and without chirps. We applied these tools to analyze the electrical interactions during and after dominance interaction. Fish dominance roles were identified by observing behavioral cues. We analyzed data from several dyads, and discuss the changes found in the distributions of IPIs, offs and chirps, during their dominance contest. Because chirps often occur when both fish engage in close-range physical contact, we tested the hypothesis that chirps and offs could be automatically generated by the contact of electric organs when fish are touching each other. However, no chirps nor offs were observed when two fish were manually placed in physical contact, with their skins touching. This suggests that instead of related to an automatic mechanism, chirps and offs might be used to communicate important information that shapes the dominance context.

All our software is freely available (Matias and Guariento, 2016). Data from one of our dyads is also available (Guariento et al., 2016).

2. Materials and methods

2.1. Ethics statement

All experimental protocols and procedures were in accordance with the ethical principles of the Society for Neuroscience and were approved by the Committee on Ethics in Animal Experimentation of the São Carlos Institute of Physics – University of São Paulo.

2.2. Subjects and housing

Experiments were conducted on 6 healthy adult specimens of *Gymnotus carapo*, 15–25 cm long, regardless of sex. The home tanks and feeding protocol were previously described (Forlim and Pinto, 2014). All specimens were acquired from local commercial suppliers within 15 days before experiments. Four specimens were acquired on September/October 2015 and two on June 2016.

2.3. Experimental setup

The experiments with freely interacting fish were performed in a glass aquarium ($100 \times 50 \times 50$ cm) filled with tap water, and shielded by a grounded metallic mesh (Faraday cage). To induce interaction between pairs of fish, no objects were placed in the measurement aquarium, leaving no spots for hiding. The water was set at room temperature $(23 \pm 2)\,^\circ$C and the conductivity was measured before and after the experiments as $(55 \pm 5)\,\mu$S/cm. The fish were placed in this setup only during the experiments.

The Electric Organ Discharges (EODs) were measured using a three-dimensional array of 12 electrodes, each consisting of a 0.2 mm diameter stainless steel wire (Fig. 1). The electrodes were inserted through the vertices and in the mid edge of the longer sides of the measurement aquarium. Time series of 11 electrodes were differentially amplified (100 times – Texas Instruments Operational Amplifier TL07X series on inverting mode with a 10 Hz input high-pass filter) with a single common reference electrode, and digitized at 45.5 kHz by a commercial acquisition system (Digidata 1322A, Molecular Devices).

2.4. Time series from isolated fish and training protocol

To collect enough data to calculate pulse shape statistics over a wide range of positions, each fish was placed alone in the measurement aquarium to record their own EOD time series. Each fish was left freely swimming for 10 min and then induced to swim for another 10 min, by prodding the fish with a non-conductive net. These time series were then used as training examples for a protocol based on state-of-art machine learning techniques (Matias et al., 2015), as described in Section 2.7.

2.5. Dominance contest

Each pair of fish was placed simultaneously in the measurement aquarium to interact for 70 min, when a contest for dominance happened, and then, moved back to their home tanks. All the experiments were performed at night (2000–0200 h) in the dark. According to classical signatures described in the literature (Batista et al., 2012; Westby, 1975a; Zubizarreta et al., 2015), after each experiment a dominance relationship was formed. For instance, one of the fish in each dyad rapidly stop biting its conspecific and frequently swam away from their aggressive counterparts. These fish were later identified as submissive. The dominance contests were performed *before* the training protocol as defined by Section 2.4, assuring that the animals were naïve to the experimental setup.

2.6. Chirp detection

We used supervised learning to detect chirps in the time series (Fig. 2a and b). First, we built a new time series by summing the absolute values of voltage of all electrodes. Several non-overlapping sections were uniformly selected, each one 0.5 s long, and manually inspected and labeled according to two classes: (i) sections with chirp and (ii) sections without chirps. Then, N sections with chirp and $2N$ without chirp were fed as training examples for a Random Forest (Breiman, 2001) classifier. To implement this classifier as a continuous chirp detector, the training examples were segmented in sampling windows of 2000 data points (44 ms). The accuracy of the classification was measured by 5-fold cross validation (Bishop, 2006) and increased with N.

Empirically, chirps were always longer than 100 ms, i.e. 3 moving windows of 44 ms. To avoid misclassification of smaller regions, when applying the detector on the whole timeseries, chirp detection was only considered when 4 consecutive windows of 2000 data points (44 ms) were classified as containing chirps (Fig. 2c). Similarly, the end of the chirp was considered when 10 consecutive windows were classified as not containing chirps.

All chirp detection programs were written in Python and are freely available GitHub (Matias and Guariento, 2016).

2.7. Fish discrimination

Each pulse was assigned to a fish by using supervised learning, based on previous methodology (Matias et al., 2015) with minor improvements. Specifically, we applied Hilbert transform to the time series of all 11 electrodes, summed their absolute values, and identified peaks. In regions without chirps, the position of each pulse was then detected by finding the peaks of the summed signal with a threshold of 1 V. In regions containing chirp, the threshold was adaptively set as 30% of the summed signals of the maximum value in the last 300 ms. The timing of each pulse is defined when

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