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

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Abstract

Pulse-type weakly electric fish present a rich repertoire of spatio-temporal electrical patterns used in electrolocation and electrocommunication. Common characteristic patterns, such as pulse rate changes, offs and chirps, 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 describe the statistical changes of some communication patterns during a dominance contest of freely moving Gymnotus carapo dyads. Quantitative analysis was possible by using home-made software for automated pulse discrimination and chirp detection. In all freely interacting dyads chirps were signatures of subsequent submission, even when they occurred early in the contest. However, offs were not exclusive of the submissive fish, but more frequent and longer on those. Both results are in agreement to previously reported manual analysis, validating our automated analysis. We show that the submissive fish slows down its average pulse rate while the dominant keeps it almost unchanged during and after the dominance is established, in all experiments performed. Additionally, we analyzed if the direct interference of electric organs could cause offs and chirps. But none were found by simply forcibly keeping fish touching each other, regardless of their relative position or interaction time.

Keywords: Neuroethology, Electrocommunication, Inter Pulse Interval, Pulse Discrimination, Chirp Detection, Off, Random Forest, Dominance Contest, Submissive, Gymnotus, Pulse Type Electric fish

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1. Introduction

Social hierarchies are established and maintained by a broad range of dynamic behavioral traits expressed by animals during their communication (King, 1973; Lorenz, 1981). *Gymnotus* sp. are territorial weakly electric fishes and gender does not influence the outcome in their contest for dominance (Batista et al., 2012). Their motor and electrical behavior change dramatically once a hierarchy of dominance is established (Batista et al., 2012; Westby, 1975a,b). Dominant fish tend to actively swim around, exploring the environment, while the submissive frequently remains still. A self-generated electrical pulse is a stereotyped discrete event and its shape can be used as a signature to identify each fish (Matias et al., 2015; McGregor and Westby, 1992). However, 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 electoreceptors network: 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 brings surroundings information (Jun et al., 2012; Pereira and Caputi, 2010), while exchanging IPI patterns are likely used for communication among conspecifics (Forlim and Pinto, 2014).

While fish are disputing the dominance, they may stop emitting pulses during variable time intervals (‘offs’) (Westby, 1975a,b). In opposition to the pulse-type common behavior they may also show complex transient oscillations of the generated electric field (‘chirps’) (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 inform submission or stress. These are strong evidences that electrocommunication has an important role in dominance (McGregor and Westby, 1992; Westby, 1975a).

However, to address electrocommunication, one of the main challenges is to discriminate pulses from freely interacting fish (Black-Cleworth, 1970; Letelier and Weber, 2000; McGregor and Westby, 1992). Nevertheless, even in physically separated fish, it is possible to induce some interaction by electronically coupling the aquariums (Forlim and Pinto, 2014), and observe some offs, but no chirp.

Here we report on improving a state-of-art classification technique (Matias et al., 2015), that discriminates pulses from *Gymnotus carapo* dyads, to include automated chirp detection. It was implemented by using a supervised learning algorithm, and its training required only short samples of time series with and without chirp. We applied these tools to analyze the electrical interactions during and after their conflict for dominance. Fish roles were identified *a posteriori* 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. We also report on experiments where fish were kept still to verify if chirps and/or offs could be produced by the interference of their
electric organs in direct contact.

All the discrimination and chirp detection programs were written in C++ and Python and are freely downloadable at GitHub [Matías and Guariento 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 carried on 8 healthy adult specimens of Gymnotus carapo, 15–25 cm long, regardless of sex. The housing and feeding are the same described in [Forlim and Pinto 2014]. All specimens were acquired from local commerce less than 15 days from experiments.

2.3. Experimental setup

The freely interacting experiments were performed in a glass aquarium (100×50×50 cm) filled with tap water and insulated in a metallic mesh grounded Faraday cage. The water was at room temperature (23 ± 2)°C and the conductivity was measured before and after the experiments as (55 ± 5)µS/cm. Fish were placed in this setup only during the experiments, and no hiding places were present.

Spatio-temporal electric organ discharge (EOD) were measured using a three-dimensional array of 12 electrodes, each consisting in a 0.2 mm diameter stainless steel wire inserted through the aquarium silicone glue. The electrodes were placed on the vertices and on the mid edge of the longer side of the aquarium (Fig. 1). Time series of 11 electrodes were differentially amplified (100 times) with a single common reference electrode, digitized at 45.5 kHz by a commercial acquisition system (Digidata 1322A, Molecular Devices), and stored in computer disk for future analysis.

2.4. Dominance contest

All the experiments took place at night (from 8 PM to 2 AM), in the dark, and both fish were simultaneously placed into the measurement aquarium. The specimens were left to interact for 1h10’ and then moved back to the housing.

Dominance contests happened before the training protocol (See section 2.5), so the animals were naïve to the experimental setup. Dominance was determined a posteriori by observation of the behavioral movement cues after the experiment and by the number of offs on the IPI time series of each fish.
2.5. Training protocol

After their dominance contest, each fish was placed alone on the aquarium. Such lonely fish acquired data were used to train the machine learning protocol, so it could identify their particular pulse signatures, allowing to associate pulse and emitter on the dominance contest data (Matias et al., 2015).

Each fish were left freely swimming for 10 minutes and then, with the help of a non-conductive fish net, induced to swim for another 10 minutes, to ensure enough pulse shape statistics over a wider range of positions.

2.6. Chirp detection

We have used supervised learning to detect when chirps happened in the time series (Figure 2). First, to evidence chirps, we built a new time series by summing the absolute values of voltage in all electrodes. Several non-overlapping sections were uniformly selected, each one 0.5 s long, and manually inspected and labeled according to two classes: as presenting or not-presenting chirps. Then we select N of the presenting chirp sections, and 2N of the non-chirp ones. These labeled sections were used to train a Random Forest (Breiman, 2001) classifier (composed of 200 decision trees), after segmented in smaller 2000 (44 ms) data samples windows. The accuracy of the classification was obtained by 5-fold cross validation (Bishop, 2006) and increased with N. As an example, for a given time series, for $N \sim 30$ all chirps ($\sim 300$) were correctly detected.

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. Mainly, we now apply Hilbert transform to the time series of all 11 electrodes, sum...
Figure 2: Example of a chirp - a single electrode is shown. **Unshaded:** Both fish are firing. **Shaded:** Detected as chirp: only one of the fish is firing and the other one emitted a chirp, which can be seen as noisy-like oscillations of the signal baseline.

their absolute values, and look for peaks. Position of each pulse is defined at the instant of maximum at each peak found. This way, pulse detection is independent on the fish position in the aquarium [Jun et al., 2012]. We also implemented a Graphical User Interface (GUI) to verify and manually correct the few pulses wrongly classified.

2.8. **Bound interaction of electric organs**

Members of a dyad from the dominance contest experiments were manually held and rubbed against each other, with their bodies oriented in parallel, anti-parallel and orthogonal. Each relative position was maintained by 2–5 minutes. A pair of dipole electrodes were placed near to each fish to record and save its electric activity for posterior analysis, using commercial apparatus (A-M Systems 1700 differential AC amplifier, Digidata 1322A, Molecular Devices).

3. **Results**

3.1. **Impact of chirp detection on fish discrimination**

Automatic chirp detection trained for a single dyad proved to be effective to generalize detection to all other dyads, with high accuracy.

Having chirps detected, fish pulse discrimination could be also improved by changing the threshold for pulse detection (Figure 3). Without chirp detection, a few misdetected pulses could produce a sequence of spurious detections until both fish have their pulse properly classified. The current accuracy of the whole protocol is higher than 97%, which allows quick manual corrections through the GUI.
Figure 3: Fish discrimination was improved by changing the threshold for spike detection on chirp regions. **Bottom left:** Time series of inter pulse intervals before the chirp detection. **Bottom right:** Time series of inter pulse intervals of the same region after the chirp detection. **Top:** Corresponding time series acquired in one of the electrodes represented on figure 1 and pulse discrimination before (below) and after (above) chirp detection and threshold adaptation.
Figure 4: IPI time series with of two fish freely swimming in the same aquarium. **Left/Red:** Dominant **Right/Blue:** Submissive. Submissive IPI dynamics change around 400 s (7 min), and become stable again after 1000 s (16 min). Pulses were discriminated using the methodology described in section 2.2.

Table 1: Average and standard deviation of IPIs (in milliseconds) for all fish dyads. Results from three separate conditions are shown: each fish by itself (column Training), during the first 400 s of interaction (column First 400 s), and after 1000 s (column After 1000 s). For each condition, we show the the average IPI and its standard deviation.

| Dyad # | Status   | Training (ms) | First 430 s (ms) | After 1000 s (ms) |
|--------|----------|---------------|------------------|-------------------|
| 1      | Dominant | 15.8 ± 1.3    | 14.0 ± 4.1       | 13.8 ± 4.0        |
| 1      | Submissive | 15.1 ± 0.8  | 16.8 ± 8.3       | 38.5 ± 578.0      |
| 2      | Dominant | 15.1 ± 0.8    | 14.54 ± 6.9      | 14.9 ± 14.6       |
| 2      | Submissive | 15.8 ± 1.3  | 17.7 ± 58.6      | 16.9 ± 43.0       |
| 3      | Dominant | 15.3 ± 1.1    | 19.1 ± 1.9       | 18.5 ± 4.2        |
| 3      | Submissive | 19.8 ± 1.5  | 16.8 ± 116.9     | 19.7 ± 246.8      |
| 4      | Dominant | 25.3 ± 5.3    | 20.8 ± 1.0       | 20.0 ± 1.4        |
| 4      | Submissive | 20.7 ± 1.6  | 22.9 ± 1.2       | 23.7 ± 10.2       |
| 5      | Dominant | 16.8 ± 1.2    | 16.5 ± 38.0      | 16.5 ± 20.0       |
| 5      | Submissive | 17.3 ± 0.9  | 163.0 ± 370.5    | 98.8 ± 2508.0     |
| 6      | Dominant | 27.6 ± 1.3    | 22.6 ± 27.6      | 26.5 ± 6.1        |
| 6      | Submissive | 24.1 ± 2.3  | 28.3 ± 25.7      | 35.1 ± 268.3      |

3.2. Dynamics of electrical behavior during dominance contest

Dominant and submissive fish change their electrical behavior differently over the course of the dominance dispute (Figure 4). We have discriminated 6 fish dyads during the first hour from their first encounter on the experimental aquarium (Figure 4, for instance). The dominant fish almost always remains with IPIs patterns mostly unchanged, while the submissive becomes slower and only stabilizes after several minutes (around 16min in Figure 4).

Discarding chirps and offs, during the first hundreds of seconds there is little difference in the histograms of IPIs of both fish (Figure 5a,b). After a transient phase, the submissive fish increases its IPIs, while the dominant remains mostly unchanged (Figure 5c,d). After both IPI time series stabilizes, submissive has a significantly higher average (Mann-Whitney U, p < 2%, Figure 5c,d). Although the changing times of the IPI histograms can slightly vary from one dyad to another, their qualitative dynamics is preserved.

Chirps and offs heavily skew the distribution of IPIs of the submissive fish. Their dynamics is depicted in Figure 6: the submissive fish starts to present
more and more chirps and offs, which are represented as higher values of IPIs in the histogram. There were extreme cases where the submissive fish remained silent for almost a minute.

There were no apparent correlations in the IPI average and variance when comparing two fish alone and after the dominance is established (Table 1). This shows that the dynamic changes observed in figures 5 and 6 are the result of the dispute for dominance, and not due to intrinsic characteristics of the fish. In many cases, intermediary average IPI of the dominant became larger, but the final average always remained below that of the submissive fish.

3.3. Chirps are not produced by approximation and interference of the electric organs

Since chirps occur when the fish are closely interacting while disputing for dominance, one hypothesis of their emergence could be that physical contact elicited interference between electric organs. In the experiments with bound
interaction, the animals increased their firing rate, which is expected, but presented no off nor chirp, indicating that these submissive cues are conspecific dependent and related to spontaneous communication, and do not happen due to electrical interferences, regardless the proximity and the time of their interaction.

4. Discussion

Electrocommunication in pulse-type electric fish (especially in many fish of the genus Gymnotus - Crampton et al. [2013]) is scaffolded by a very rich repertoire of electrical behaviors, most of them, only present when fish are freely interacting. However, detecting and discriminating electrical activity of interacting similar fish is a challenging task if their movements are not severely restricted.

We have developed a system to analyze pulse trains of two electric fish freely swimming and interacting in the same aquarium. Our system is capable of

Figure 6: Inter Pulse Intervals (IPI) on a coarser time scale. **Left:** Dominant fish almost never shows chirps or moments of silence. **Right:** Distribution of IPIs of the submissive fish becomes skewed as chirps and moments of silence appear. (a) and (b): Histograms for the first 430 seconds. (c) and (d): Histograms for time between 430 and 1000 seconds. (e) and (f): Histograms for times greater than 1000 seconds.
automatically: (i) detect moments of chirp, and (ii) discriminate which fish emitted each pulse.

This technique allowed us to observe how the IPI distributions of both fish changed over time in a fine-grained time scale. Comparing the contest with after the dominance, these statistics for dominant fish consistently remained almost unchanged (with very similar average and variance), while the statistics of the submissive fish changed to larger average IPI (see Table 1). Particularly, the submissive fish from dyads 4, 5 and 6 in table Table 1 showed great number of chirps and offs, evidenced by their higher standard deviation.

We found that submissive fish always changed their electrical behavior to higher average IPIs, in order to avoid using the same average of the dominant, as suggested by previous studies (Batista et al., 2012; Westby, 1975a; Zubizarreta et al., 2015). One hypothesis for this fact is that the submissive fish is likely accommodating its electric sensing to the presence of a dominant, because their pulse detection pathway have cells with long refractory period of about 10ms (Nogueira and Caputi, 2011).

Automatic chirp detection demonstrated being extremely helpful in long time series, considerably decreasing manual tagging efforts. It also improved pulse detection and accuracy, avoiding propagation of detection errors. Due to the generality of the chirp electric signature, it was possible to implement an efficient detector based on few manually classified samples. Such methodology allows to analyze very long IPI sequences from interacting fish, which is of fundamental importance for investigating electrocommunication and complex hierarchical behaviors (Mosqueiro et al., 2016).

Although the whole process seem computationally expensive, we have optimized our algorithms in order to allow them to run in a single off-the-shelf personal computer: chirp detection and pulse discrimination of an one hour contest experiment takes a little more than one day, including manual corrections and inspection. Our codes have a friendly GUI and are freely available on GitHub (Matias and Guariento, 2016).

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