

# Communication scenes of weakly electric fish recorded in natural habitats challenge sensory processing

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December 10, 2015

## Abstract

Weakly electric fish generate and sense high-frequency electric organ discharges employed in localization and communication. We used an array of submerged electrodes to record these signals and thereby automatically track multiple individual fish and their communication interactions in their natural habitat in Neotropical streams. Our long-term monitoring revealed rich social behavior and allowed to deduce the functions of specific electrocommunication signals during courtship and aggression and to quantify the statistics of natural communication scenes. Unexpectedly, in most interactions, the electroreceptor neurons will be activated only weakly, either because large distances between the fish lead to strong signal attenuation or because of frequency mismatch. This study emphasizes the importance of quantifying behavior under natural conditions in order to understand challenges faced and solved by sensory systems.

The statistics of behaviorally relevant natural stimuli provide essential context for understanding the functioning of sensory systems [1, 2]. The electric organ discharge (EOD; Fig. 1 A) generated continuously by Neotropical weakly electric fish offers a unique opportunity to monitor individual movements and communication signals (Fig. 1 B). By eavesdropping on their EOD with an electrode array (Fig. 1 C), we were able to reconstruct social interactions and evaluate the associated sensory scenes (Fig. 1 D–F, movies S9 and S10). This novel method allowed us, for the first time, to track multiple individual electric fish in their natural habitat over extended periods of time in a non-invasive way (fig. S2). Our data provide unprecedented quantitative insight into courtship and electrocommunication behavior and reveal constraints imposed on sensory processing by natural social interactions.

The gymnotiform fish *Apteronotus rostratus* generates a periodic EOD with constant and individual-specific frequency ( $EODf$ , 600–1200 Hz) that provides the basis for tracking of individual fish (movie S8). The EOD together with the fish's electrosensory system forms an active electrosense employed for localization of objects and communication [3, 4]. Brief frequency excursions called “chirps” (20–400 Hz, Fig. 1 B) function as communication signals that are emitted during courtship and aggression.

Fish were tracked during their reproductive season in a stream in the Panamanian rainforest (fig. S1). In total, we detected 54 episodes of short-distance courtship interactions between low-frequency females ( $EODf < 750$  Hz) and high-frequency males ( $EODf > 750$  Hz) occurring exclusively at night (Fig. S2). Courting was characterized by extensive production of chirps (Fig. 1 D) by both males and females—with up to 8 400 chirps per fish per night (fig. S6). Most chirps were so-called “small chirps”, characterized by short duration (<20 ms),  $EODf$  excursions of less than 150 Hz, and minimal reduction in EOD amplitude (Fig. 1 B, [5]). In courtship episodes only females emitted an additional type of chirp, the “long chirp” (Fig. 1 B), with a duration of  $162 \pm 39$  ms, a large  $EODf$  excursion of about 400 Hz ( $n = 54$ ), and a strong decrease in EOD amplitude. Per night and female we observed 9 to 45 long chirps generated every 3 to 9 minutes (1st and 3rd quartile), exclusively between 7 pm and 1 am (fig. S6). Occasionally,

courtship was interrupted by intruding males leading to aggressive interactions (Fig. 1 D–F, movies S9 and S10 show examples of both behaviors).

Courtship communication was highly structured, with female long chirps playing a central role. These long chirps were preceded by persistent chirping of the male with rates of up to 3 Hz (Fig. 2 A and fig. S4 A), exceeding maximum small chirp rates reported from laboratory observations [6, 7]. Immediately before the long chirp, the female chirp rate increased strongly from below 1 Hz to about 3 Hz within a few seconds. The male chirp rate followed rate increase of the female and the concurrent high-frequency chirping of both fish ceased after the female long chirp. Courtship episodes were characterized by close proximity of the two fish (< 30 cm, Fig. 2 B and fig. S4 B). Long chirps were consistently acknowledged by males with a doublet of small chirps emitted  $229 \pm 31$  ms after long chirp onset (Fig. 2 C). The two chirps of the doublet were separated by only  $46 \pm 6$  ms, more than seven-fold shorter than the most prevalent chirp intervals (fig. S3). Remarkably, the concurrent increase in chirp rate terminated by the female long chirp and male doublet form a specific, highly stereotyped communication motif (Fig. 2 C, audio S7).

On a sub-second timescale, male chirping was structured by the timing of female chirps (Fig. 2 D and fig. S4 C). Following a female chirp, male chirp probability first decreased to a minimum at  $84 \pm 13$  ms (t-test  $p \ll 0.001$ , effect size Cohen's  $d = 1.4 \pm 0.4$ ,  $n = 5$  pairs) and subsequently increased to a peak at  $169 \pm 9$  ms (t-test  $p \ll 0.001$ ,  $d = 1.6 \pm 0.6$ ). The precise timing of this echo response supports the functional relevance of chirping as a communication signal (see supplementary discussion). In contrast to this finding, females did not show any echo response (Fig. 2 E and fig. S4 D) — they timed their chirps independently of the males' chirps ( $d < 0.6 \pm 0.2$  within 0–250 ms). Previous lab studies reported only echo responses between males [7, 8, 9].

The stereotypical sequence of chirping and movement suggested a synchronizing function in spawning [10]. We tested this hypothesis in a breeding experiment in the laboratory [11] by continuously recording and videotaping a group of 3 males and 3 females of the closely related species *A. leptorhynchus* over more than 5 months. Scanning more than 1.3 million emitted chirps, we found 76 female long chirps embedded in communication episodes similar to those observed in *A. rostratus*. Eggs were only found after nights with long chirps (six nights). In addition, the associated video sequences triggered on female long chirps (Fig. 3, movie S11) clearly demonstrate the exclusive function of the female long chirp in synchronizing spawning. Such synchronization is critical in aquatic animals with external fertilization and is, in many species, achieved by means of visual, acoustic, and/or tactile displays, e.g. [12].

In addition to courtship chirping we observed small chirps during aggressive male-male interactions in the field (Fig. 1 E, movie S9). Occasionally, courting dyads were approached by intruding males. The resident males then usually attacked the intruder. In 5 out of 12 such situations a few small chirps indistinguishable from those produced during courtship were elicited exclusively by the retreating fish (fig. S6), suggesting an additional, possibly submissive function of small chirps in aggressive contexts [7, 13].

Our field data allow to deduce the relevant signal space for the processing of electrocommunication signals. Tuberous electroreceptors (P-units) distributed over the fish's skin encode amplitude modulations (AMs) of the fish's own EOD [14, 15, 16]. Superposition of a fish's EOD with that of a nearby fish results in a periodic amplitude modulation, a so-called beat. Both frequency and amplitude of the beat provide a crucial signal background for the neural encoding of communication signals. The beat frequency is given by the difference between the two EODf and the beat amplitude equals the EOD amplitude of the nearby fish at the position of the receiving fish (Fig. 4 A and fig. S5, [17]).

We estimated the population activity of electrosensory afferents in *A. leptorhynchus* from the standard deviation of the summed nerve activity, which is known to closely match the tuning properties of single nerve fibers [15, 16]. The P-unit population response quickly dropped

to non-detectable levels at inter-fish distances of about 50 cm (Fig. 4 B). P-unit afferents are also tuned to beat frequency and are most sensitive between 20–130 Hz (Fig. 4 E, [14, 15, 16]). All courtship chirping occurred at much higher beat frequencies (205–415 Hz, Fig. 4 G). Even though the beat amplitudes during these interactions are large due to the small distances of less than 32 cm (Fig. 4 C), such high-frequency stimuli evoke hardly discernible P-unit activity, which, however, must be the basis of the observed precisely timed chirp interactions.

In contrast, two behaviors involving intruding males occurred at much larger distances (Fig. 4 D), but within the P-units' best-frequency range (Fig. 4 H): (i) Intruding males initially often lingered at some distance from the interacting dyad (8 of 16 scenes, median duration 58.5 s; e.g., Fig. 1 D, movie S9), consistent with assessment behavior [18]. The associated distances were larger than 70 cm. (ii) The distances at which resident males started to attack intruders ranged from 20 cm to 177 cm ( $81 \pm 44$  cm,  $n = 10$ , Fig. 1 E, movie S10). At the largest observed attack distance of 177 cm the electric field strength was maximally  $0.34 \mu\text{V}/\text{cm}$ , close to minimum threshold values of about  $0.1 \mu\text{V}/\text{cm}$  measured in the laboratory [19, 20]. This demonstrates that extremely weak electrosensory stimuli close to the absolute detection threshold are successfully evaluated by the electrosensory system in natural male-male interactions. In contrast, the small number of chirps emitted during aggressive male-male interactions all occurred at short distances and within the best-frequency range, causing strongly synchronized P-unit activity [15].

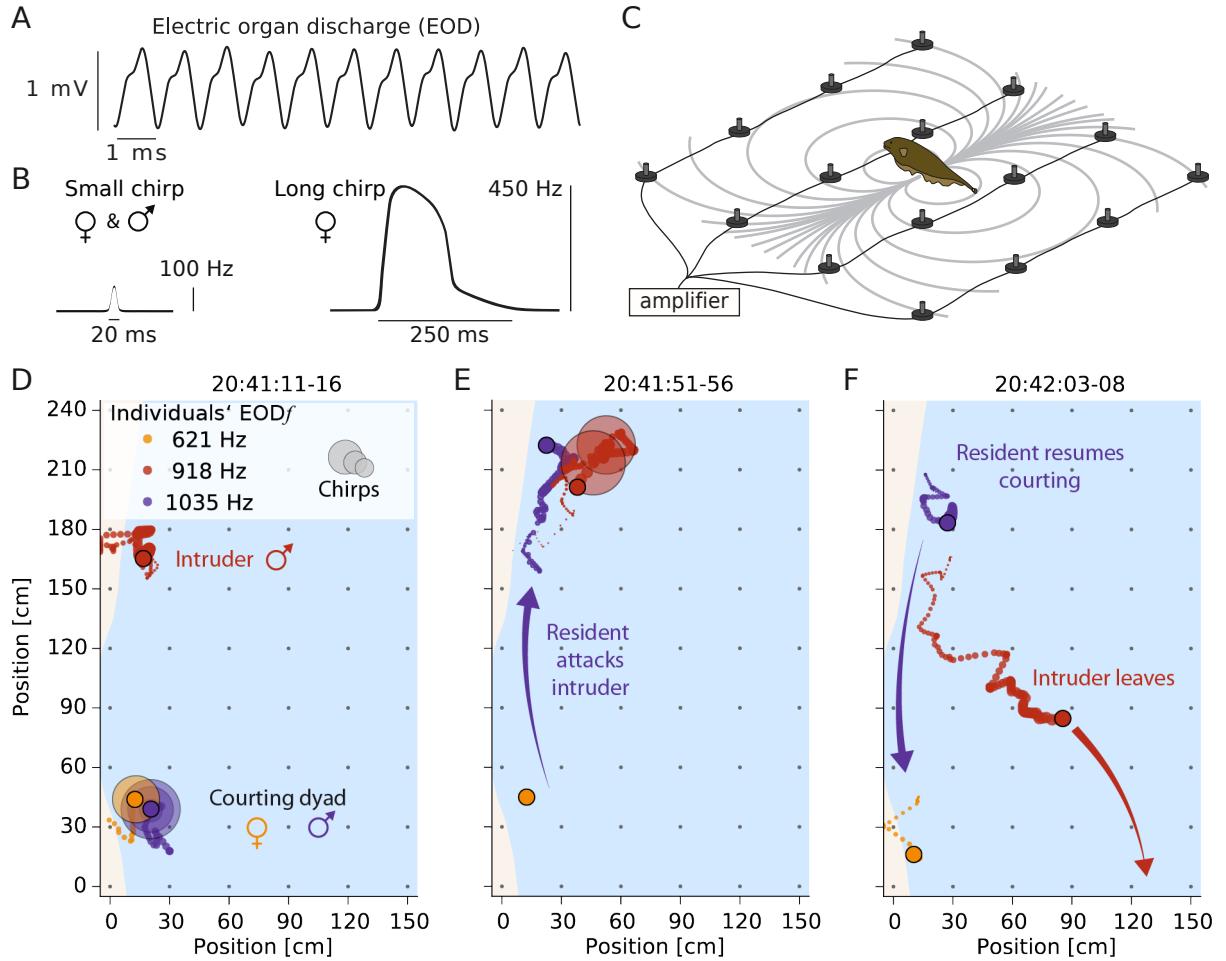
Our set of field recordings revealed unprecedented details on the nocturnal courtship and aggression behaviors of weakly electric fish freely interacting in their natural rainforest habitat. Electrocommunication signals in these contexts often barely activate electroreceptors, either because of a frequency mismatch during courtship or large interaction distances during aggressive encounters. This demonstrates the striking ability of weakly electric fish to detect, categorize, and respond to weak signals reliably within a few tens of milliseconds as indicated by echo responses (Fig. 2 D, E), chirp doublets (Fig. 2 C), and attacks (Fig. 4 D). We therefore expect neural adaptations of the electrosensory system for processing signals at the limit of its sensitivity [21, 2]. In addition, we expect the unusual frequency mismatch between courting signals and receptor tuning to be an important selective constraint on the evolution of the sexual dimorphism in EODf.

## Acknowledgments

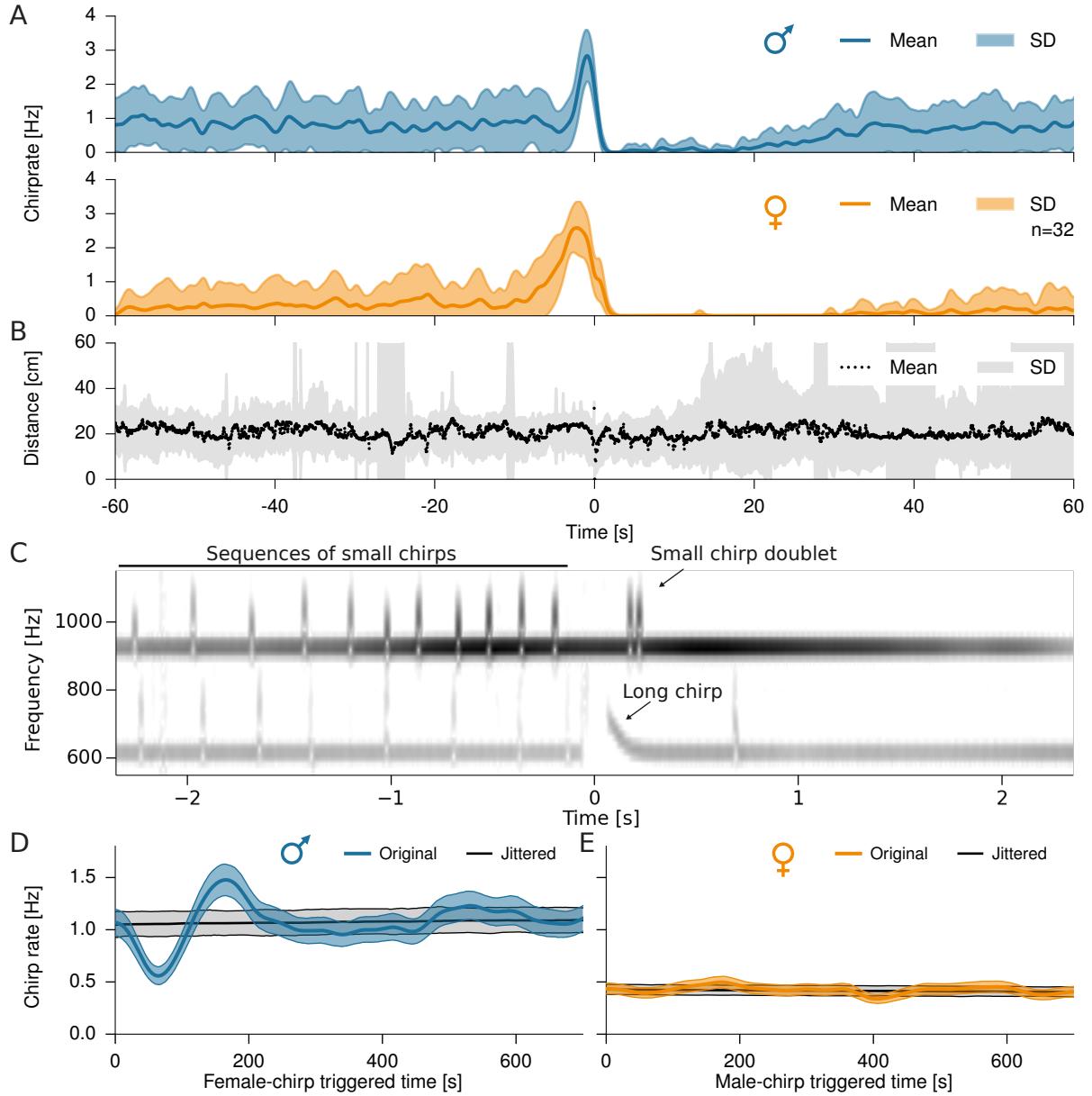
Supported by the BMBF Bernstein Award Computational Neuroscience 01GQ0802 to J.B., and a Discovery Grant from the Natural Sciences and Engineering Research Council of Canada to RK. We thank Hans Reiner Polder and Jürgen Planck from npi electronic GmbH for designing the amplifier, Sophie Picq, Diana Sharpe, Luis de León Reyna, Rigoberto González, the staff from the Smithsonian Tropical Research Institute, and the Emberá community of Peña Bijagual for their invaluable logistical support.

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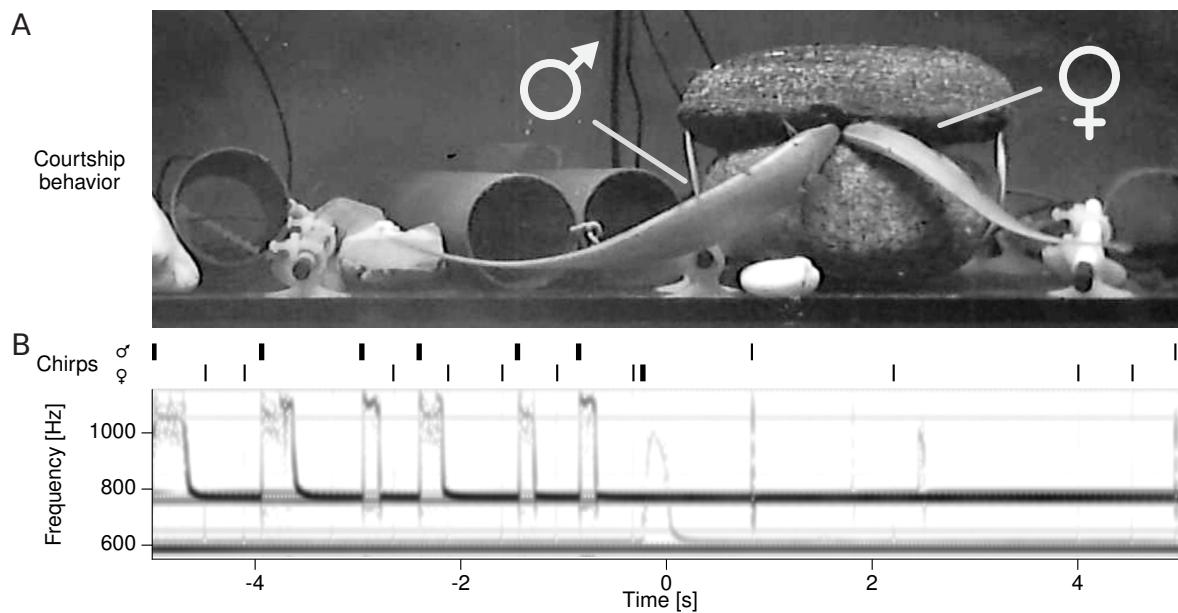
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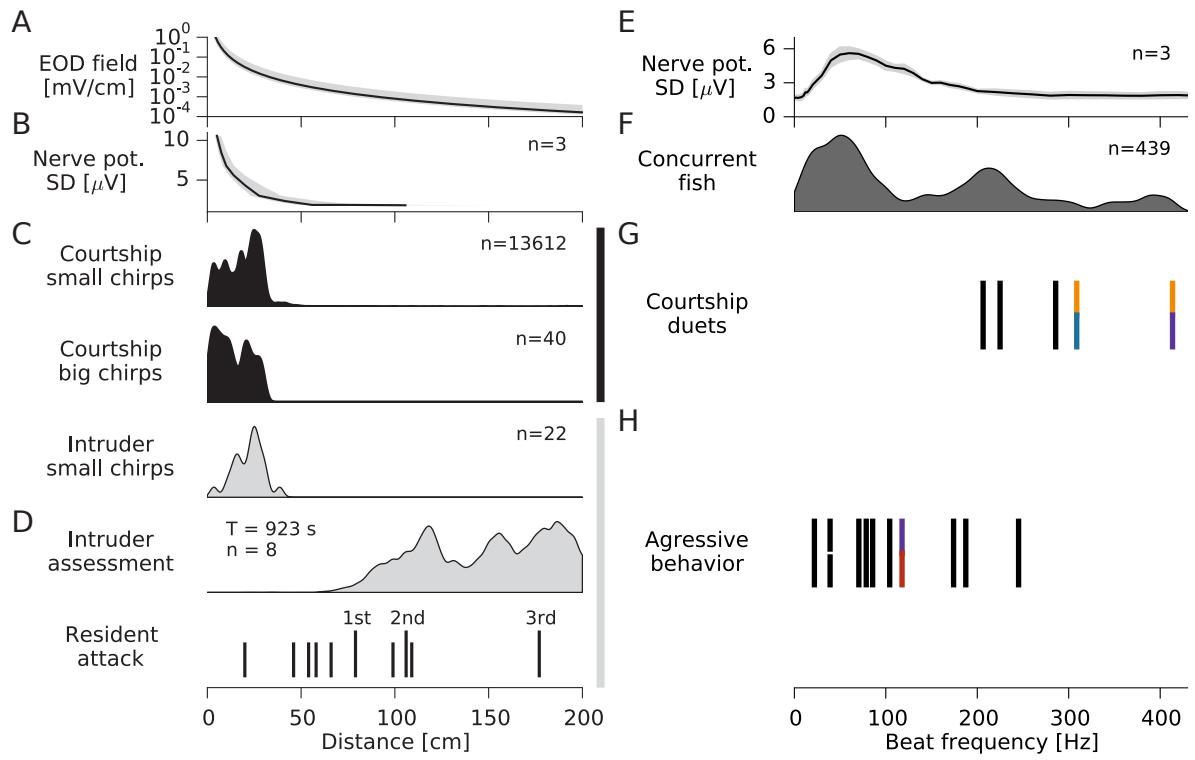
**Figure 1:** Monitoring electrocommunication behavior in the natural habitat. A) EOD waveform of *A. rostratus*. B) Transient increases of EODf, called small and long chirps, function as communication signals. C) The EOD generates a dipolar electric field (gray isopotential lines) that we recorded with an electrode array. D, E, F) Snapshots of reconstructed fish interaction (see movie S9). The current fish position is marked by filled circles. Trailing dots indicate the positions of the preceding 5 s. Colors label individual fish throughout the manuscript. Large transparent circles denote occurrence of chirps. Gray dots indicate electrode positions, and light blue illustrates the water surface. D) Courting female (orange) and male (purple) are engaged in intense chirping activity. An intruder male (red) lingers at a distance of about one meter. E) The courting male attacks the intruder (purple arrow) who emits a series of chirps and, F) leaves the recording area (red arrow).



**Figure 2:** Temporal structure of courtship chirping. A) Average rate of small chirps and B) distance between courting male and female fish before and after a female long chirp at time zero. Gray bands mark SD. C) Spectrogram (audio S7) shows EODfs of a female (620 Hz) and male (930 Hz) during courtship and their stereotyped chirping pattern: the two fish concurrently produce series of small chirps before the female generates a long chirp and the male responds with a chirp-doublet. D, E) Chirp rate of one fish relative to each chirp of the other fish (cross-correlogram, median with 95 % inter-percentile range in color). Corresponding chirp rates from randomly jittered, independent chirp times in gray. D) Male chirping is first inhibited immediately after a female chirp (at 64 ms,  $d = 1.93 \text{ SD}$ ) and then transiently increased (at 166 ms after a chirp,  $d = 1.60 \text{ SD}$ ). E) Female chirps are timed independently of male chirps (Cohen's  $d < 0.73 \text{ SD}$ ).



**Figure 3:** Synchronizing role of the female long chirp in spawning. A) Simultaneous video (snapshot of movie S 11) and B) voltage recordings (spectrogram, bottom) of *A. leptorhynchus* in the lab demonstrate the synchronizing function of the female long chirp (at time zero; lower trace with 608 Hz baseline frequency) in spawning. In contrast to *A. rostratus*, male *A. leptorhynchus* generate an additional, large chirp type before spawning (top trace with 768 Hz baseline frequency). Chirp times of the male (top) and the female (bottom) are marked by vertical bars above the spectrogram. Thick and thin lines indicate long and short duration chirps, respectively.



**Figure 4:** Inter-fish distances, beat frequencies and their encoding. A) Maximum electric field strength in dependence on distance from the emitting fish (median with total range). B) Activity of the electroreceptor population rapidly declines with distance between two fish (beat frequency 60 Hz). C) Small and long chirps in both courtship and aggression contexts are emitted consistently at distances below 32 cm. D) Intruder assessment and initiation of attacks by residents occur at much larger distances (see movie S 10). E) Tuning of electroreceptor activity to beat frequency. F) Distribution of beat frequencies of all *A. rostratus* appearing simultaneously in the electrode array ( $n = 439$  pairings, peaks at 51, 213, and 393 Hz). G) Courtship behaviors occurred at beat frequencies in the range of 205–415 Hz, far from the receptors' best frequency. Colors indicate the scenes depicted above. H) Aggressive interactions occurred at beat frequencies below 245 Hz, better matching the tuning of the electroreceptors.

## Supplementary Materials

(see extra pdf file)

**Figure S1:** Field site and position of electrode array.

**Figure S2:** Overview of weakly electric fish tracked over 25 hours.

**Figure S3:** Interchirp interval distributions.

**Figure S4:** Temporal structure of courtship chirping for a second pair of fish.

**Figure S5:** Relationship of EOD amplitude and the distance from the fish.

**Figure S6:** Ethogram

**Audio S7:** Audio trace of courtship chirping shown in Fig. 2C.

**Movie S8:** Raw data and position estimate.

**Movie S9:** Animation of the courtship and aggression behavior shown in Fig. 1D–F.

**Movie S10:** Animation of courtship behavior with multiple attempts of an intruder to approach the dyad.

**Movie S11:** Spawning in the lab.