Title: Why the brown ghost chirps at night.

Livio Oboti[1], Federico Pedraja[2], Marie Ritter[1], Marlena Lohse[1], Lennart Klette[1], Rüdiger Krahe[1]

[1]Institut für Biologie, Humboldt Universität zu Berlin, Berlin, Germany.

[2]Department of Neuroscience, Zuckerman Mind Brain Behavior Institute, Columbia University, New York, United States.

Abstract

Since the pioneering work by Moeller, Szabo and Bullock, weakly electric fish have been widely used as a model to approach the study of both spatial and social cognitive abilities in a vertebrate taxon typically less approachable than mammals or other terrestrial vertebrates. Through their electric organ, weakly electric fish generate low-intensity electric fields around their bodies with which they scan the environment and manage to orient themselves and interact with conspecifics even in complete darkness. The brown ghost knifefish is probably the most studied species due to the large repertoire of individually variable and sex-specific electric signals it produces. Their rich electric vocabulary is composed of brief frequency modulations - or *chirps* - of the oscillating dipole moment constantly emitted by their electric organ. Because chirps come in different *types*, each carrying very specific and behaviorally salient information, they can be used as references to specific internal states during recordings - of either the brain or the electric organ - or during behavioral observations. Not surprisingly, this made the fortune of this model in neuroethology during the past 7 decades. Yet, truth is, this is not necessarily all true. Although established, to date this view has been supported only by correlative evidence and, to be fair, we still do not have a convincing framework to explain why these freshwater bottom dwellers emit electric chirps. Here we provide evidence for a previously unexplored role of chirps as specialized self-directed signals used to expand conspecific electrolocation ranges during social encounters.

Introduction

Homeoactive sensing - i.e. self-directed signal production (Zweifel and Hartmann, 2020) - is often used as a strategy to enhance spatial navigation in heterogeneous and unpredictable environments. By emitting high-pitch calls (8-200 kHz; Fenton et al., 1998), bats are able to localize stationary and moving objects while flying at high speeds and in complete darkness (Jones and Holderied, 2007). Similarly, odontocete cetaceans are capable of echolocating objects by emitting very short (a few ms long) clicks covering a broad frequency spectrum (5 Hz-100 kHz). A few other mammals (shrews and tenrecs), but also bird species (oilbirds and swiftlets) and even blind humans (Griffin, 1944; Swain, 2014), are known to emit brief high-frequency echolocation calls (Konishi and Knudsen, 1979; Brinkløv et al., 2013; Gould, 1965).

In general, all biosonar systems represent a remarkable adaptation to low-light or dark environments in which other sensory modalities would offer only insufficient information required to optimize fast spatial navigation during foraging or social interactions. While call spectral features, such as amplitude and frequency range, are differently tuned to optimize the echolocation range typical of a given species, the rate at which calls are produced often depends on the proximity and the complexity of the target objects. Bats and whales, for instance, can echolocate at distances ranging between a few meters (10-20 m in bats, Fenton and Bell, 1981) to a few hundred meters (100-200 m in whales; Murchinson 1980), the range for shrews and tenrecs is much shorter (10-20 cm; Gould, 1965). In all cases, the time-course of probing signals adapts to the rate at which echoes are received (with faster echo returns implying closer distance to the target; Schnitzler et al. 2003). As a direct

consequence, in complex environments call frequency increases while call duration is strongly reduced (Schnitzler et al., 2003; Thomas and Jalili, 2004; Siemers et al., 2009).
But navigating in the dark is not only about detecting sounds. Electrosensory systems offer analogous advantages, yet at the same time present fundamental differences. The combined capability of detecting and producing bioelectric fields allows weakly electric fish to passively detect bioelectric sources but also to actively sense interferences of their own field with surrounding objects (Lissmann 1958, Lissmann and Machin 1958, Bullock and Chichibu 1965). This detection can be considered almost instantaneous, if compared to the echo detection of acoustic calls, and is possible only within relatively short ranges. Brown ghost knifefish are one of the most studied weakly electric fish. They belong to the gymnotiform group (as the electric eel) and occupy benthic habitats in the Amazon river basin (Crampton, 2019). Brown ghosts generate quasi-sinusoidal electric fields at relatively high frequencies (600-900 Hz) by means of modified motor projection neurons forming a so-called electric organ, located in the tail. For this reason, the electric fields generated by these species are also referred to as electric organ discharges (EODs).
Together with a limited number of other taxa (Rose, 2004), gymnotiforms possess the unique capability to localize bioelectric sources (such as small prey or other conspecifics) using only electrosensory information (i.e. passive electrolocation; Rose, 2004). Compared to echolocation, passive electrolocation is possible only at shorter ranges (0-5 cm; Lissmann and Machin, 1958; Von der Emde, 1999; Knudsen, 1974) and it is more sensitive to signal degradation (Benda, 2020; Nelson and MacIver, 2006).
Conversely, active electrolocation (i.e. the assessment of the location of an object based on the detection of its interference with actively generated electric fields) allows to extend the *active space* of electrolocation (Hopkins and Westby, 1986; Brenowitz, 1982) slightly beyond this range: since EODs spread roughly equally in all directions, all affected surrounding objects (preys, substrate, vegetation) are simultaneously detected as they cast their electric image on the fish skin (up to 10-12 cm away from the skin; von der Emde, 1999; Snyder et al., 2007).
But the real advantage of active electrolocation can be appreciated in the context of social interaction: when two fish approach each other, the alternating constructive and destructive interaction of their EODs results in an amplitude- and phase-modulated signal (also referred to as *beat*) detectable by each of them within a 30-40 cm radius (see appendix 1 *Detecting beats at a distance*; Knudsen, 1975; Fotowat et al., 2013; Henninger et al., 2018; Benda, 2020). The frequency of such beat modulations is equal to the frequency difference (DF) of the two sinewave signals. Because each brown ghost has a different EOD frequency (at 25°C female range 500-750 Hz, male range 750-950 Hz, Bastian et al., 2001) the beat frequency can be used not only to sense an approaching conspecific but also to determine its identity and sex (low DFs = same sex, high DFs = opposite sex).
In summary, weakly electric fish are capable of passively detecting preys and bioelectric sources in close proximity to their body (0-5 cm), but also inanimate objects, by actively sensing the instantaneous interferences they cause to their EODs (6-12 cm; Chen et al., 2005; Snyder et al., 2007; Fotowat et al., 2013). In addition, by detecting amplitude modulations of their EOD induced by other EOD emitting sources (i.e. the beat), they can instantaneously detect the presence of other conspecifics at distances of up to 30-40 cm (Fotowat et al., 2013; Henninger et al., 2018; Silva A., personal communication). Although the possibility of detecting conspecifics beyond beat-ranges has been reported for up to 150-200 cm (Henninger et al., 2018; Fortune et al., 2020), it is not clear whether this effect is necessarily due to high electroreceptor sensitivity to bioelectric signals (Knudsen, 1974; Kawasaki, 1997) or to input from other sensory modalities (i.e. chemical, auditory, visual).
Importantly, since the strength of electric fields decays with the squared distance (see appendix 1), even at short range EODs are more prone to anisotropic degradation due to the presence of noise and interfering underwater materials. Such problems may arise even during fast and near range interactions, when the complex reciprocal interference generated by different EOD sources gets distorted, weakened and potentially disrupted. This may occur due to the presence of objects, heterogeneous materials or simply by fish motion, all altering

the electric field geometry in unpredictable ways (Gómez-Sena et al., 2014; Yu et al., 2019). Moreover, since the detection of other EOD-emitting fish occurs at a sampling rate equal to the beat frequency, closely related frequencies (i.e. low DFs) will be perceived with a low temporal resolution (Assad et al., 1997; Kelly et al., 2008; Babineau et al., 2006).

Thus, although detecting conspecifics by detecting the beat extends the brown ghost electrolocation range to distances a few times its body length, interacting EODs cause rather diffuse and heterogeneous interferences (Kelly et al., 2008; Fotowat et al., 2013) which may lose directional information already at 20 cm (i.e. loss of head-tail asymmetry and approximation to dipole fields; Knudsen, 1975), thus making the processing of this information imprecise and prone to errors (Pedraja et al., 2016; Milam et al., 2019; Yu et al., 2019).

The picture is further complicated by the presence of another critical source of beat interference: communication signals. Brown ghosts produce brief EOD frequency modulations - also called *chirps* - which can be broadly classified in at least 4 different types, based on their duration and the amplitude of the chirp frequency modulation (Engler et al., 2000; Zupanc et al., 2006). Different types of chirps are thought to carry different semantic content based on their occurrence during either affiliative or agonistic encounters (Larimer and MacDonald 1968; Bullock 1969; Hopkins 1974; Hagedorn and Heiligenberg 1985; Zupanc and Maler 1993; Engler et al. 2000; Engler and Zupanc 2001; Bastian et al., 2001).

Yet, since chirps can only be detected as beat perturbations (Petzold et al., 2016), this signaling is likely to interfere with electrolocation and with any other information detected through the beat (i.e. the location of other EOD emitting conspecifics) for the following reasons: first, chirps do interfere with the beat and, in fact, this interference has been proposed to be decoded into behaviorally meaningful inputs already by peripheral electrosensory processing (Benda et al., 2005, 2006); second, chirp-triggered beat interference is probably occurring most of the time as the majority of chirps seem to be produced when fish are in close proximity with each other, within beat detection ranges (< 50 cm; Zupanc et al., 2006; Hupé and Lewis 2008; Henninger et al., 2018; see appendix 1); third, this interference is exponentially amplified the closer fish get to each other, implying its effects are most dramatic when communication and electrolocation are most likely to be temporally overlapping (Yu et al., 2012; Fotowat et al., 2013). How brown ghosts succeed in the complicated task of assessing conspecific location through blurred electric beat interferences, at the same time communicating through chirping signals highly distorting them, is not known.

In this study, we propose a solution to this problem by providing evidence for a previously unexplored function of chirping in weakly electric fish. Since we began this study with the aim of conducting a thorough validation of the behavioral correlates of chirping, we first show that the choice of different chirp types does not significantly correlate with any particular behavioral or social context. It is instead correlated to the biophysical properties of underwater electric fields and to the spatial arrangement of interacting EODs. We gathered a large dataset of more than 54.000 chirps, obtained from staged social pairings, playback experiments and behavioral assessments of fish locomotory activity. By using high-speed infrared video recordings synchronized with multi-channel voltage data acquisition, we analyzed chirps considering both their effects on beat processing and their spatial attributes, i.e. the spatial location of the interacting fish. Analysis of cross-correlation of chirp time-series, chirp type transition sequences and of behavioral responses to playback chirps were used to assess the presence of meaningful correspondences. Finally, by recording fish in different environmental settings characterized by different levels of electrical “visibility”, we provide validating evidence in support of a new functional hypothesis to explain why brown ghosts chirp at night: homeoactive sensing.

Results

Experience is the main factor affecting chirp variability

During social interactions, communication signals are often related to immediate aspects of behavioral physiology. According to this, correlations between signals and behavioral contexts are often indicative of signal meaning (Flack and De Waal, 2007). We predicted that, if behavioral meaning can be attributed to different types of chirps, as posed by the prevailing view (e.g., Hagedorn and Heiligenberg, 1985; Larimer and MacDonald, 1968; Rose, 2004), one should be able to identify clear correlations between behavioral contexts and chirp types. To this aim, we recorded fish pairs in different contexts broadly divided in three groups based on the experience with the recording environment (*tank experience*): *residents* (experienced), *intruders* (naive) and *equal* (pairs in which both fish were naïve). Additional sub-groups were identified based on other factors underlying a pair social relationship (*context*): social experience, simulated breeding conditions, dominance status and physical accessibility (N = 130 fish pairs, see methods for details).

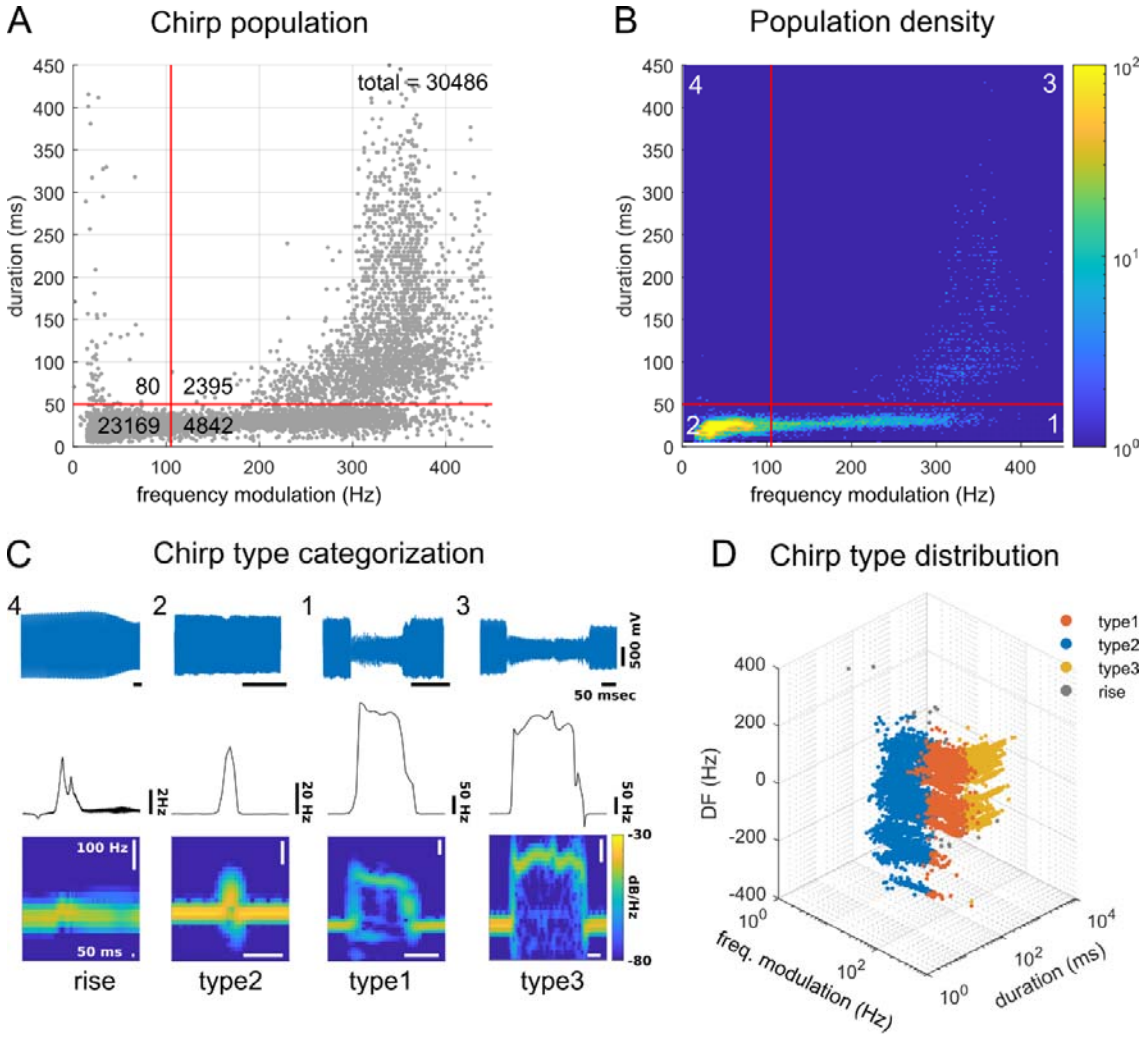

Figure 1. Chirp type categorization based on FM parameters*. A: Chirp distribution by frequency modulation (Hz) and duration (ms). The cut-off values between different chirp*

types are shown in red: duration 50 ms and modulation 105 Hz. These are based on the distribution density (B) of the whole population of chirps (rounded median duration = 50 ms), rounded 2nd quantile for frequency modulation = 105 h; N chirps = 30486; N chirping fish = 130, N fish pairs = 78). C: Representative examples of the 4 different chirp categories (voltage data on the first row, instant frequency on the second and spectrogram on the third). D: Scatter plot showing the distribution of different chirp types by DF (frequency difference between sender and receiver fish): note the gradual change in chirp type composition (color coded) at different DF values. Due to the sex difference in the brown ghost EOD frequency, negative DF values correspond mainly to females, positive values to males.

Chirps have been previously categorized in different types based on the extent and the duration of their frequency modulation (in Hz and ms, respectively; Engler et al., 2000). Since only broad ranges have been defined for each type of chirp (Engler et al., 2000; Zupanc et al., 2006), with sensible variations in different recordings (Zakon et al., 2002; Bastian et al., 2001), in this study we decided to categorize chirps by individuating rough cut-off values based on the distribution density of a large population of chirps. These were obtained from all recorded fish pairs in these first experiments (N = 30486 chirps; rounded value for duration = 50 ms, corresponding to the 50% quantile, rounded value for frequency modulation = 105 Hz, corresponding to the 75% quantile). Based on these cut-off values, chirps could be categorized in short (with small - type 2 - or large - type 1 - frequency modulation) and long (with small - rises - or large - type 3 - frequency modulation; **Figure 1**). Slower events, such as gradual frequency changes (or gradual frequency rises; Engler et al., 2000), were more sporadic and were not considered in this analysis.
Overall, the majority of chirps were produced by male subjects, in comparable amounts regardless of environmental experience (resident, intruder or equal; **Figure S1A**, **C)**, social status (dominant vs subordinate; **Figure S1B**) or social experience (novel vs experienced; **Figure S1D**). Interestingly, this sex difference was reversed when fish were allowed to interact freely, as opposed to fish whose interaction was limited due to the presence of a plastic mesh divider (divided vs free; **Figure S1D, E**). Sex dimorphisms were found also in the types of chirp produced, mainly in the case of male-to-female exchanges (MF; **Figure S2C, D**), confirming previous accounts of marked sex dimorphisms in both the number and the type of chirps produced by brown ghosts (Dye, 1987; Zupanc and Maler,1993; Dulka and Maler, 1994).
To better define the main factors underlying chirp variability, we used a 3D factor analysis for mixed categorical and numerical data (FAMD) to evaluate for each recorded chirp several parameters relative to the interacting fish (both *sender* and *receiver*) and their respective EODs (see methods for details; **Figure 2A**). The model obtained was further adjusted in order to include the most significant variables (those explaining above 20% of the total variance). The least number of variables required to explain the highest percentage of variance (N = 3: *tank experience*, *DF* and *context*) was obtained by further simplifying the model through subsequent iterations (percent of variance explained 52.314%). Overall, this analysis indicated that environmental and social experience, together with beat frequency (DF) are the most important factors explaining chirp variability (**Figure 2B**). Analysis of the distribution of individual chirps revealed some cluster segregations in correspondence of resident-intruder or novel-experienced fish pairs, while less evident clustering can be seen for other variables (e.g., social status). Notably, no obvious cluster segregation was found between sender and receiver fish of either sex, as cluster centroids overlap in both cases. Type 2 chirps (see marginal chirp type distributions in **Figure 2C**) are mainly localized around resident fish and novel encounters (**Figure 2C**); type 1 and type 3 chirps seem to be equally distributed between naive and experienced pairs (see kernel distribution plots in **Figure 2C**); rises seem to be clustered more around female subjects, regardless of status and experience (**Figure 2C**). Of particular interest is the segregation of chirps produced by fish pairs subject to water changes to simulate the onset of the breeding

season, with (breeding-naive/experienced-divided) or without tank dividers (breeding-experienced-free). The proximity of the latter to chirps produced by naïve and freely swimming mixed-sex pairs (no courtship-naïve-free) suggests that either locomotion and/or social contact might explain this segregation much better than breeding status. Collectively, these results confirm the strong effect of EOD frequency difference (DF) on chirp type distribution (**Figure 1**; Bastian et al., 2001; Petzold et al., 2016) and reveal that experience is a significant factor determining chirp heterogeneity. In addition, they suggest chirp production might be affected by locomotory activity and behavioral variables linked to direct close-range social interaction.

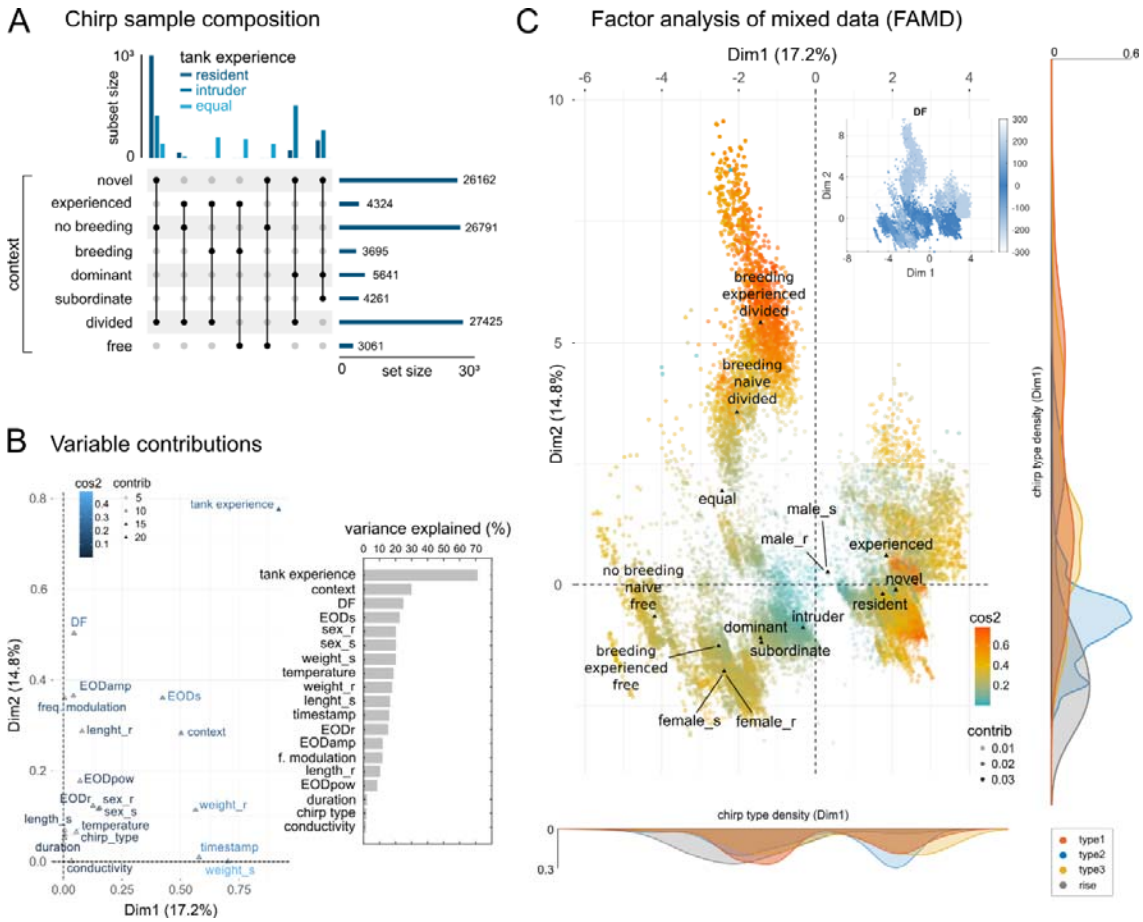

Figure 2. Factor analysis of mixed data (FAMD) – social contexts and tank experience. *A: Diagram showing the total number of chirps produced in different types of social interaction. These are color coded based on "tank experience" (upper bar plot: resident = 1-week tank experience, intruder = new to the tank, equal = both new) and sorted in different "context" categories (labeled on the left side). These categories refer to the reciprocal experience of each fish pair (novel or experienced), their hierarchical status (dominant or subordinate), the type of interaction (divided = behind a plastic mesh barrier, free = freely swimming) and the simulated breeding season (based on water conductivity levels: high conductivity = ca. 400 µS, no breeding; low conductivity = ca. 100 µS, breeding) at which the interaction takes place (see methods for details). B: Scatter plot showing the contribution of all variables considered: triangles indicate the coordinates of the variable centroids, their contribution ("contrib") is coded by color intensity, whereas the quality of their representation on the transformed coordinates is coded by color hue ("cos2"). Note how "tank experience",*

“context” and “DF” stand out of the variable group. Estimates of the total variance explained indicate that tank experience, together with DF and context, are the most important factors explaining chirp variance. C: Representation of chirps in the transformed coordinates. The clustering is based on qualitative coordinates (tank experience, context and chirp type). Cluster distance represents the correlation among variables. The marginal plots show the kernel distribution of the chirp population by chirp type (legend on the bottom right). Labeling chirps by DF shows how chirps can be meaningfully clustered based on this parameter (inset top right).

Chirping is mainly a self-referenced behavior

A crucial and highly debated aspect of communication signals is the presence of conserved motifs that can serve as unequivocal signifiers for the specific context in which they are produced (Berwick et al., 2011; Nieder and Mooney, 2020). Specificity could reside in the order of these motifs - or syllables - or in whole "phrases" having their own peculiar sequence of composing elements, as suggested by studies on bats (Bohn et al., 2008) cetaceans (Dunlop, 2017) and primates (Slocombe and Zuberbüler, 2005a,b; Notman and Rendall, 2005). In weakly electric fish, specific chirp sequences have been proposed to be salient to specific behavioral contexts (Henninger et al., 2018; Dye, 1987), and even emotional states (Triefenbach and Zakon, 2008; Smith et al., 2013; Silva et al., 2013). To validate this idea and assess whether specific chirp transitions could be considered signatures for different types of behavioral interactions, we counted all possible chirp transitions in the chirp time-series recorded. This was done first independently (each subject considered separately; **Figure S2**) and then interactively (considering the sender-receiver transitions; **FIgure 3**).

Considering all recordings independently, the most common transition observed is “type 2”-”type 2”, except for the case in which males are chirping to females (MF; **Figure S2B**), probably a direct consequence of the wider variety of chirp types used by males during this type of interaction (**Figure S3**). Nonetheless, neither chirp transitions nor chirp type ratios were differently distributed in any other group (**Figure S2**). Rather, the proportion of different chirp types and the transition frequency from one type to the others were remarkably similar, regardless of the context and the sex of the interacting fish. At an individual level, the most common chirp sequence was a repeat of type 2 chirps in both males and females, with type composition being only slightly more heterogeneous for male senders (**Figure 3A**, **B**). Chirp-type patterning at the individual level is therefore highly conservative and not connected to any of the behavioral contexts considered.

Nonetheless, it is possible that meaningful chirp transitions (i.e. correlated to a specific behavioral context) are to be identified only when considering time series interactively (Chow et al., 2015; Fröhlich, 2017). For instance, recordings from brown ghosts, both in the field and in captivity, suggested the presence of correlations between male small chirps (type 2) and female larger chirps (type 3; Henninger et al., 2018). Meaningful correlations have been also suggested to consist more broadly in temporal correlations between time-series, with chirps produced with a preferred latency by two interacting subjects. Yet, different authors reported different latency values (Zupanc et al., 2006, latency 500-1000ms; Hupé and Lewis 2008, latency 200-600 ms; Henninger et al., 2018, latency 165 ms). These discrepancies - either due to population differences or differences in the recording environments - certainly do not suggest the presence of stereotyped temporal patterning.

Using our data, a first assessment of temporal chirp correlation was made by calculating cross-correlation indices for all chirp time-series produced during differently matched sex pairs (**Figure 3C**).

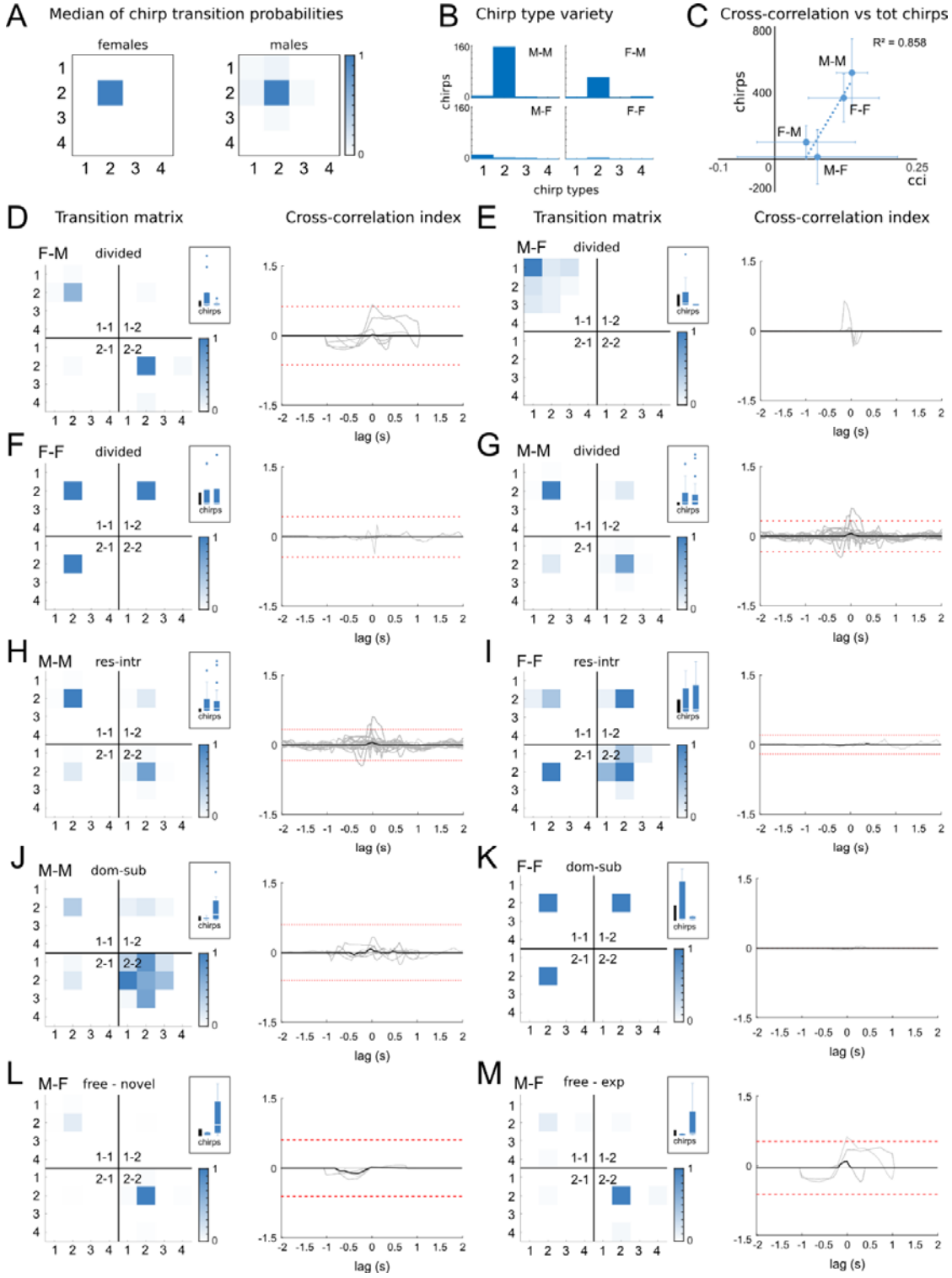

Figure 3. Chirp time-series correlations during social interactions*. A: Chirp transition maps representing the normalized median transition probability of all possible chirp type pairs calculated for female and male fish, considered individually. B: Number of chirps per type produced by fish pairs according to different sex pairings. C: Plot showing the linear correlation between cross-correlation indices (cci) of chirp time-series and the total amount of chirps produced during differently matched sex pairs (coefficient of determination*

Pearson's R^2 = 0.858). D-M: Transition matrices showing the normalized median of the transition frequency of all possible chirp-type transitions, produced by females (N = 38) and males (N = 92). For each given type of social pair, the identity of each fish is indicated by different ID numbers in the different map quadrants: 1-1 = fish1 to fish1, 1-2 = fish1 to fish 2, 2-1 = fish2 to fish1, 2-2 = fish2 to fish2. When ambiguous, the sex of the fish follows the same order of the fish ID. The presence of higher chirp-transition frequencies in the 2nd and 4th quadrants of the matrices (labeled with 1-1 and 2-2) indicates a substantial independence between chirp time-series (i.e. lack of temporal correlation). On the right side of each matrix, a cross-correlogram shows the result of a cross-correlation analysis of chirp sequences recorded in all the pairs of the group (50ms binning). No strong evidence of periodicity was found in any of the groups considered (except for a few cases in the MM group). Only pairs in which both fish were chirping were used. The red dotted lines in the cross-correlograms indicate a 3 standard deviation boundary used threshold for statistical significance. The negative values of the cross-correlation indices indicate lags at which sequences are less similar to each other. The boxplot insets display the total number of chirps produced by each type of fish pair (boxplot scale bar = 100).

Although highly variable among fish pairs, the temporal correlation of chirp series seems to depend quite strongly on the number of chirps produced (**Figure 3C;** Zupanc et al., 2006, max estimated chirp rate 0.4/sec; Hupé and Lewis 2008, max estimated chirp rate 0.53/sec; Henninger et al., 2018, max estimated chirp rate 3/sec) raising the question of whether the reported instances of higher temporal correlations could be just a direct consequence of higher chirp rates. Nonetheless, chirp transitions were further assessed considering both senders and receivers interactively for different sex pairings (M-M, M-F, F-M, F-F). Transition frequencies were mapped onto four quadrants defined by fish identity (1 or 2) and temporal order: 1-1, 1-2, 2-1 and 2-2.

What stood out most consistently from this comparison was the much lower (or sometimes completely absent) extent of sender-receiver chirp transitions (**Figure 3D-G**), as shown by the higher transition probabilities along the 1-1 and 2-2 quadrants of the transition probability maps. According to this, cross-correlation indexes are very low or near zero levels in all cases (**Figure 3D-G**). Although slightly different interaction rates were observed in different contexts (**Figure 3H-K**), in all cases the most frequent chirp transitions were self-referenced (1-1 or 2-2). Although within FF pairs 2-1 and 1-2 interactions seem to be more frequent, this is simply due to the much lower number of chirps produced by this sex (see boxplot insets for comparison). Chirp time-series cross-correlation indices were extremely low in all cases, confirming a significant self-reference of chirping in our recorded series.

Eventually, the absence of conserved temporal correlations could also depend on the type of behavioral interactions and the proximity at which they take place (Dunlap, 2002). Social distance in particular could explain the high chirp-rates previously observed during courtship (Hagedorn and Heiligenberg 1985; Henninger et al., 2018) and short-range agonistic interactions between fish confined in a relatively small aquarium (Hupé and Lewis 2008) as opposed to the more sporadic chirping observed during encounters over wider spatial ranges (Henninger et al., 2018). Alternatively, the higher chirp temporal cross-correlation could be due to neuro-endocrine effects of courtship or other mating-related factors.

To test whether cross-correlation would be significantly affected during courtship, we simulated the onset of the reproductive season by gradually lowering water conductivity over the course of a 40 day period (from 400 mS to 100 mS, Kirschbaum, 1979). As a result of this treatment at least 75% of the females used in this experiment (N = 8) showed visible signs of egg maturation. Mixed-sex pairs (N = 8 pairs) held in such conditions were then allowed to interact freely, in absence of any tank divider. As a control group we paired males and females in absence of any water conductivity change ("novel" vs "experienced", **Figure 3L-M**). Although female fish produced a surprisingly higher number of chirps in these conditions (**Figure S1F**), only minimal signs of cross-correlation could be detected, without

reaching on average the threshold for statistical significance (**Figure 3M**). Collectively, these results indicate the use of a very limited variety of chirp type transitions in different behavioral contexts and the lack of any correspondence between contexts and specific chirp type repertoires. More importantly, these results show that - at least in the behavioral conditions analyzed here - chirp time series produced by different fish during paired interactions are consistently independent of each other.

Invariant chirp responses to chirping playback EOD mimics

Communication signals often acquire their meaning due to pre-existing associations between sensory cues and behavioral states (Owren et al., 2010). These correspondences can be often uncovered through playback experiments as they are a more direct means to assess signal value or meaning, compared to simpler correlative observations.
For this purpose, we adopted a variation of the classic "chirp chamber" approach (e.g., Dye, 1987; Bastian, 2001; Dunlap and Larkins-Ford 2003) and assessed fish responses to chirping signals in freely moving conditions. This allows to evaluate fish decisions to approach or avoid EOD sources as a possible measure of signal value (Fugere and Krahe, 2010). Fish were allowed to swim in a 30 x 80 cm aquarium at whose opposite ends two 13 x 30 cm playback compartments were created using plastic mesh barriers. Playback electrodes were positioned parallel to the longest tank axis and ca 10 cm apart. Electric stimuli were EOD mimics at different frequency steps (DFs) of both positive and negative sign, relative to the fish's own EOD frequency (±240Hz, ±160Hz, ±80Hz, ±40Hz, ±20Hz, ±10Hz, ±5Hz, 0Hz). The EOD playback intensity was set at 1mV/cm at ca. 5 cm distance (which is in the range of natural field strengths of this species). Playback sessions were organized in 4 different *modes*, based on the pattern of frequency modulations contained (mode 0 = no chirps, mode 1 = type 1 chirps, mode 2 = type 2 chirps, mode 3 = rises). Each playback trial lasted ca. 60 sec with 180-sec inter-trial intervals (see methods for details).
Fish responses to plain sinewave EODs confirmed previously reported findings: fish respond with large chirps (type 1 and type 3) to high DFs and small chirps (type 2) to low DF signals (**Figure S4)**, with males producing more chirps than females (**Figure S5**; Bastian et al., 2001; Engler and Zupanc, 2001). Notably, a transient decrease in chirping was observed in the immediate temporal surround of playback chirps (modes 1-3, **Figure 4, S6**). This effect was similar for all chirp types and seemed to be limited to the short time around chirp occurrence. Accordingly, a more pronounced decrease in chirping was observed during 3 Hz trains of type 2 chirps, possibly due to the higher frequency at which these events were repeated (**Figure 4C**). Apart from this, fish responses to chirping EODs were hardly distinguishable from non-chirping EOD mimics in terms of number of chirps produced (**Figure S5**).
Analysis of chirp time course further revealed two interesting details: first, while type 1 and type 3 chirps seem to be randomly scattered throughout the whole trial (60 s), the number of type 2 chirps seems to increase over time. This trend was independent of the type of playback and is therefore intrinsically related to the use of this particular type of chirp. All chirp types are subject to a brief decrease only in concurrence with 2 Hz trains of type 2-chirp playback (**Figure S6**). Second, the vast majority of rises was produced right at the end of the playback signal, regardless of whether or not playback chirps were occurring (**Figure S6**). Overall, these results agree with previous reports showing an increase of type 2 chirps over time during paired interactions (Hupé and Lewis 2008) and the presence of a short delay between consecutive chirps produced by different subjects (Hupé and Lewis 2008; Henninger et al., 2018). The consistent occurrence of rises at the end of the playback signal has also been reported previously (Kolodziejski et al., 2007). Nonetheless, these findings clearly suggest a disjoint of chirping from social valence or specific behavioral meaning, either in terms of number of chirps produced or relative to the types of chirps produced. The strong symmetrical dependency of chirp production on positive and negative beat frequencies (DFs; **Figure S4**; Bastian et al., 2001; Engler and Zupanc, 2001; Triefenbach and Zakon, 2007; Kolodziejski et al., 2007) and the lack of specificity in the response of

brown ghosts to playback chirps suggests that, in this species, chirping is primarily conditioned by beat parameters.

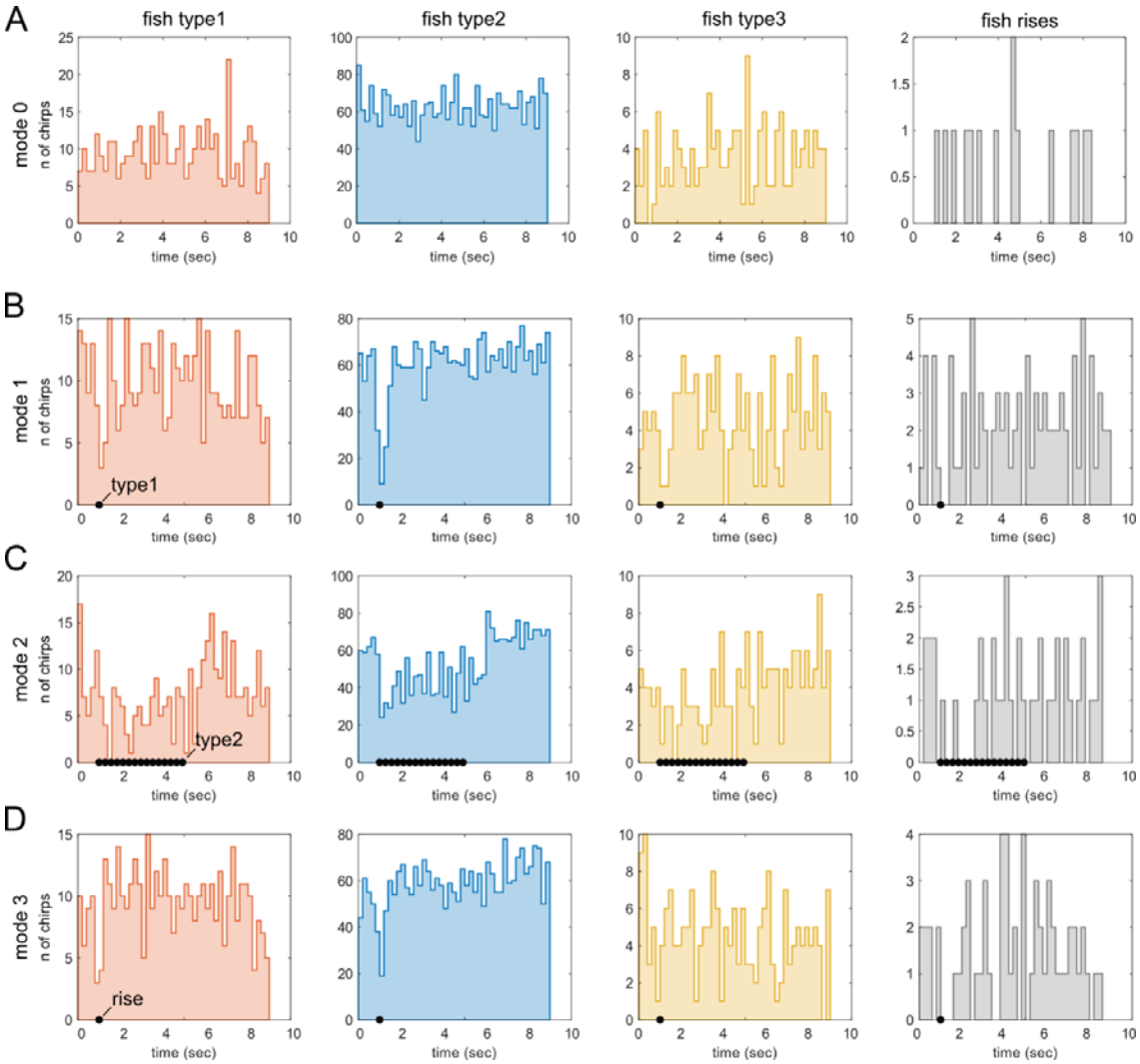

Figure 4. Playback chirps temporarily suppress chirping in freely swimming fish. *A-D: Peristimulus time-histograms (PSTH) of chirps produced by 16 fish (8 females and 8 males) during playback experiments. Results for different types of chirps are displayed in different columns while each row is related to chirps produced in response to a given playback mode (mode 0 = plain sinewave, mode 1 = type 1 chirps at 0.2 Hz, mode 2 = type 2 chirps at 3 Hz, mode 3 = rises at 0.2 Hz; see methods for details). The PSTHs show that the main effect of playback chirps on the chirps produced by the test subjects is a brief temporal suppression (accentuated when chirps are repeated in trains, C) but no sign of any significant temporal correlation, except for a transient suppression.*

Chirp type choice can be predicted by chirp interference with beat periodicity

Since brown ghost electroreceptors are highly tuned to the fish's own EOD (Scheich and Bullock, 1974; Zakon, 1986), they are directly affected by the EOD amplitude modulation

and their firing rates are entrained by the beat periodicity (Benda et al., 2005). It follows that any frequency modulation will result in alterations of the periodic electroreceptor firing and therefore will be detected as an abrupt change in electroreceptor activity (Benda et al., 2006). This perturbation of the beat is scaled by mainly 3 parameters: the extent of the frequency modulation, its duration and the underlying DF (or beat frequency) at which such modulation is produced (Benda, 2006). It follows that chirps of different types will have different effects on electroreceptor firing based on the frequency of the interacting EODs (i.e. the beat frequency; Walz et al., 2013).

Although the DF-dependency of chirp-induced beat perturbations has been proposed to be at the basis of a differential behavioral value of chirps at different beat frequencies (Benda et al., 2006; Walz et al., 2013), given that chirp type heterogeneity correlated more significantly with beat frequency than with any other variable linked to behavior, we posit that chirp function is likely to be related mainly to the physiological aspects of beat processing by the chirping individual. This hypothesis is further supported by the correlation existing between interacting EOD frequencies (or DF) and chirp variance (Figure 2B, Figure S3).

To further validate this idea, we have computed an estimate of the interference caused by a chirp on the beat periodicity (*beat interference*). This was done by calculating the ratio between the duration of the beat cycles affected by the chirp (beat cycle outliers) and the total duration of the beat cycles detected within a fixed time window (700 ms). Such an estimate of beat interference was made for a wide range of Gaussian chirps (duration 10-400 ms, modulation range 0-400 Hz) on a wide range of beat frequencies (±300 Hz; **Figure 5A**). For each chirp, the interference values obtained for 4 different phases (90° steps) were averaged. As a result, we obtained a three-dimensional space on which recorded chirps (N = 30486 chirps) could be localized and matched to their putative interference.

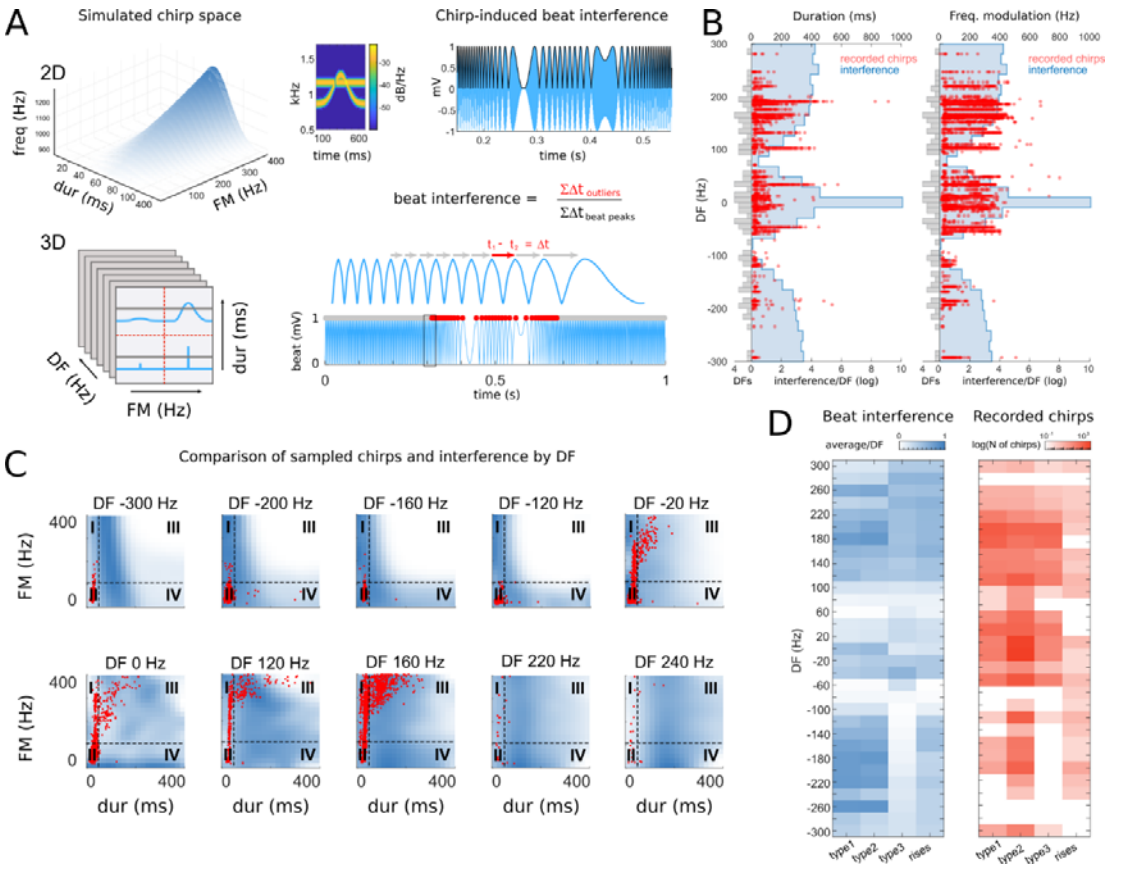

Figure 5. Chirp interference with the beat regularity. *A: Artificial chirps were generated on an 862 Hz EOD baseline covering a 0-400 ms duration range and a 0-400 Hz peak*

amplitude. An additional EOD signal was added to the chirping signal (-300-300 Hz DF range) to simulate a wide range of beats. The sign of the DF is referred to the baseline reference EOD. The beat interference induced by chirps was calculated as the ratio of beat peak durations affected by a chirp on the total duration of the beat peaks within a 700 ms time window (see methods for details). B: Duration and frequency modulation histograms or recorded chirps (N = 30486) sorted by DF and matched to their estimated beat interference. The gray histograms on the ordinate axis represent the beat frequencies sampled. C: The heatmaps show examples of chirp-induced beat interferences (color coded in blue) calculated at different DF values. Real chirps produced at the same DF are overlaid in red. D: Comparison of average chirp-induced beat interferences sorted by type (left map, blue) and the actual observed chirps (log N, right map in red).

What we found is that chirps were indeed often localized in the areas of maximum interference, at different beat frequencies: at low DFs chirps were localized mainly in the type 2 range, while at positive DFs the areas corresponding to larger chirps were more frequently populated by recorded chirps (see scatter plots on the side in **Figure 5B**). Notably, by calculating the normalized cumulative interference for each chirp type, a high degree of matching was found between the DF values at which the interference is higher and those at which chirps were actually recorded (**Figure 5C**).
In general, chirp interference seems variable across different DF values, suggesting that chirps are probably chosen based on beat parameters in order to exert a tangible effect on the electrosensory system (**Figure 5D**). A comparison of chirp interference across different chirp types (**Figure 5D**) further revealed that type 2 chirps at low or even negative DFs may induce similar interference levels as larger chirps at positive beat frequencies, which could perhaps explain the lower occurrence of larger chirps in low frequency fish (being more often in negative DF encounters). In addition, areas of lower interference seem to be symmetrically localized around 60 Hz, the frequency level to which electroreceptors are most sensitive (Bastian, 1981) and at which chirp occurrence was lower (**Figure 5B-D**). Overall, these results corroborate the idea that chirp production may be constrained mainly by the effects chirps have on the beat.

Chirping is significantly affected by locomotion and social distance

We sought to gather more evidence in support of this notion by looking for associations between chirping and locomotion during the exploration of a beat source. This is in fact a context in which beats exert highly variable effects on the electrosensory system due to fish locomotory activity (Fotowat et al., 2013). We thus set this experiment in an enriched environment to evaluate other aspects of locomotor and social behavior which have been neglected in our previous experiments and which could possibly explain the high interindividual chirping variability (e.g. thigmotaxis, novelty- or shelter-seeking, sociability).
Fish were allowed to swim freely (for 30 min) in a 160 x 80 cm arena containing shelters (plastic tubes and plants), the social stimulus - and also the “beat source” - consisted of a conspecific caged in a mesh tube located on one side of the arena. A “novel object” (a 1 x 5 cm rod of graphite) was hidden behind a barrier and used to test novelty seeking. Shelters consisted of transparent PVC tubes and plastic plants. Different regions of interest (ROIs) were used to score the occurrence of fish swimming behavior around the shelter area, the tank walls (within 5 cm distance), the novel object and the conspecific (**Figure 6A**). The time spent swimming in the arena’s open space (5 cm away from the walls) was also evaluated. Behavioral observations were conducted taking into account the subject’s sex, as it is the behavioral variable more directly correlated to DF and beat frequency.

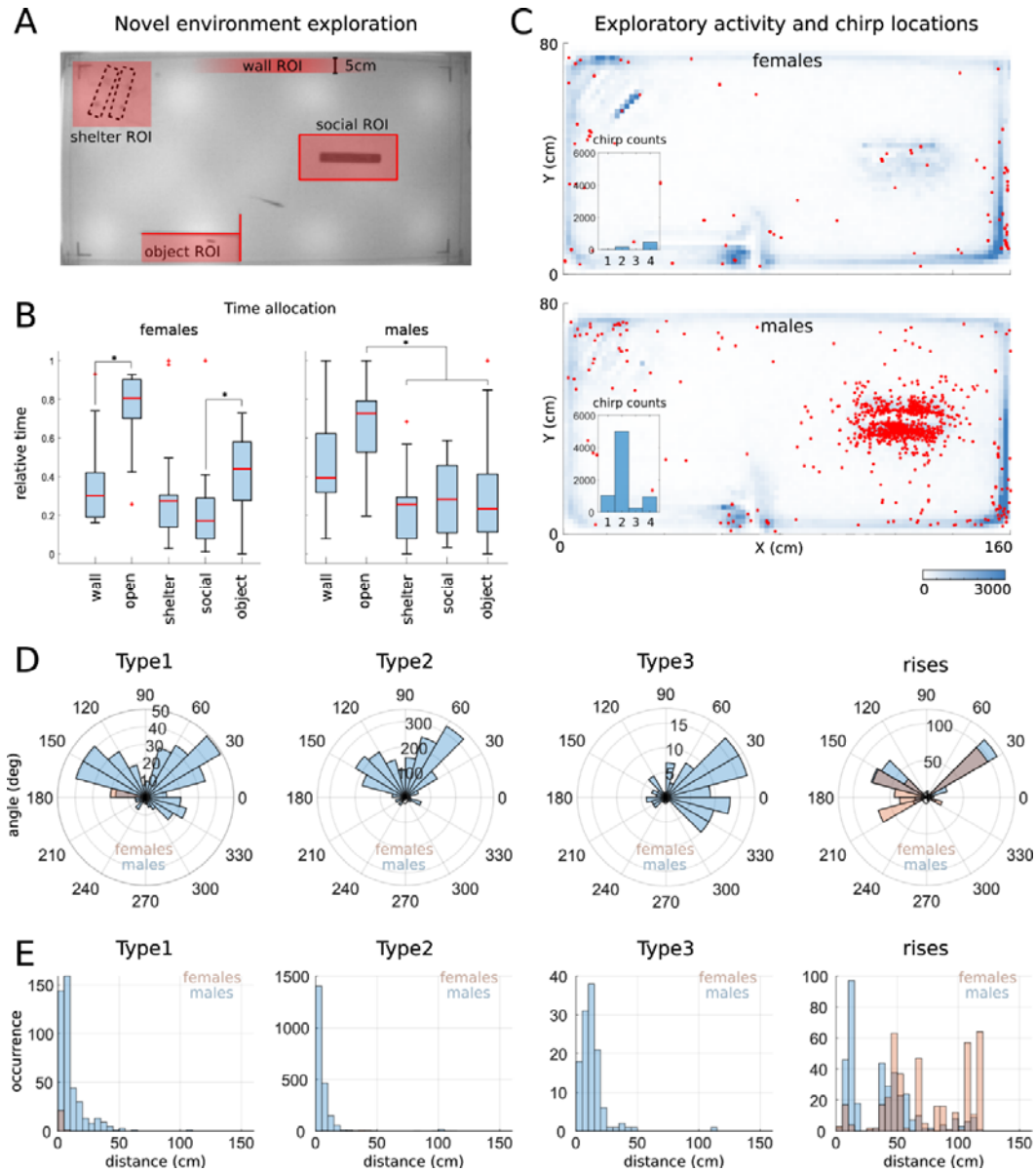

Figure 6. Chirping during novel environment exploration*. A: Diagram of the recording arena showing the definition of different regions of interest (ROIs) identified by 1) the presence of shelters (PVC tubes), 2) the proximity to the tank walls (distance < 5 cm), 3) the presence of a fish (caged in a mesh tube), 4) the presence of an "unknown/novel" conductive object (a 3 cm piece of graphite). B: Proportion of time spent in the different ROIs (N = 14 females, Friedman X^2 22,9 $p < 0.001$ wall vs open $p < 0.001$, social vs object $p = 0.02$, open vs shelter/social/object < 0.01; N = 15 males, Friedman X^2 19,8 $p < 0.001$ wall vs open $p = 0.049$, wall vs shelter/object $p < 0.05$, open vs shelter/social/object $p < 0.001$). C: Chirp locations (red) overlaid to the heatmaps showing the average swimming activity of females and males. D: Polar histograms showing the angles between the two fish during chirping. Angles are referred to the X-axis and are sorted based on chirp type and sex (male = blue, female = red). E: Histograms of the chirping distances in males (blue) and females (red) relative to different types of chirps.*

We found that female brown ghosts spent more time swimming in the open space areas of the arena, as opposed to swimming closer to the tank walls (thigmotaxis; **Figure 6B**). Female subjects also spent more time investigating the novel object than the conspecific (**Figure 6B**). Male behavior showed a different trend as males spent comparatively more time swimming close to the tank walls and more time in contact with conspecifics (**Figure 6B**). Possibly as a direct consequence of this, they also produced a higher number of chirps, compared to their female counterparts. Notably, most of the chirps were produced around the caged conspecific (**Figure 6C**). Analysis of the angles and distances of fish during chirps revealed that first, chirps were mostly produced when fish were located at 30°, 150° or 330° angles relative to the axis defined by the mesh tube (**Figure 6D**), and second, most chirps were produced at a distance of less than 25-50 cm (**Figure 6E**). While the angle bias could be possibly due to the circular loops described by fish swimming around the conspecific, the rather limited range at which chirps are produced could be explained by the range of the EOD field (beat amplitude reaches 1% at around 30 cm; Fotowat et al., 2013; Henninger et al. 2018; Benda, 2020; see box *Detecting beats at a distance*) and confirms previous observations (Hupe and Lewis, 2008; Zupanc et al., 2006; Henninger et al., 2018).

Interestingly, chirps of different types were not all produced at similar angles: while type 1 and type 2 chirps seem to be more often produced at 30-150 angles, type 3 chirps - produced in this experiment only by males - seem to be used in the ±30° range (i.e. on the right side of both the arena and the plane representing the angles). Rises - in both males and females - seemed more often produced at multiples of 30° located on the left side of the cartesian plane (30°, 150° and 210°). All chirp types, except for rises, were produced within a 50 cm radius from the conspecific fish (**Figure 6E**). Rises were produced almost ubiquitously, by both sexes, and most of the time at locations far away from the caged fish (**Figure 6C**, **Figure 6E**).

Factor analysis (FAMD) confirmed the already known sex-dimorphisms in chirp distribution (males chirp more than females and more often towards other males, **Figure S7A**). Collectively, caged fish produced more chirps than freely swimming subjects. In addition, variables known to be related to beat frequency (such as DF, EOD frequency, sex, chirp type) explain a good percentage of chirp variance (73,7% of variance explained as opposed to the 55% explained including all other variables 55%; see also **Figure 2B**). A relatively high loading on principal components was also found for variables associated with locomotion, such as distance and time spent exploring different tank areas (**Figure S7B**). Accordingly, chirp clustering is affected by the EOD frequency difference of the emitting fish (DF) and by distance (**Figure S7C**, insets).

Modelling of field current lines and iso-potential lines of dipole models of our fish allows to visualize more intuitively how both chirp angles and distances can be explained by the electric field geometry (**Figure 7A**). Most chirps (90%) in fact are produced within distances corresponding to the 1% sinewave field intensity (roughly 30 cm; **Figure 7B**) and are positioned in correspondence to the areas of the highest density of current field lines (compare **Figure 6D** with **7A**). The correlation between beat range and chirping is even more evident when chirp distances are compared to the values of sinewave intensity measured at different distances (**Figure 7C**, **D**). To assess whether, within the same range of distances, chirps have a detectable effect on randomly generated beats, we have estimated the interference caused by different chirp types on representative sinewave pairs using the reference values obtained from our test recordings (**Figure 7D**). Not surprisingly, we found a linear and direct correlation of chirp-induced beat interference with beat decay over a distance which simply indicates that chirps affect the beat within beat-detection ranges (**Figure 7E-H**). These results are nonetheless useful to confirm that our interference estimates are meaningfully related to beat detection.

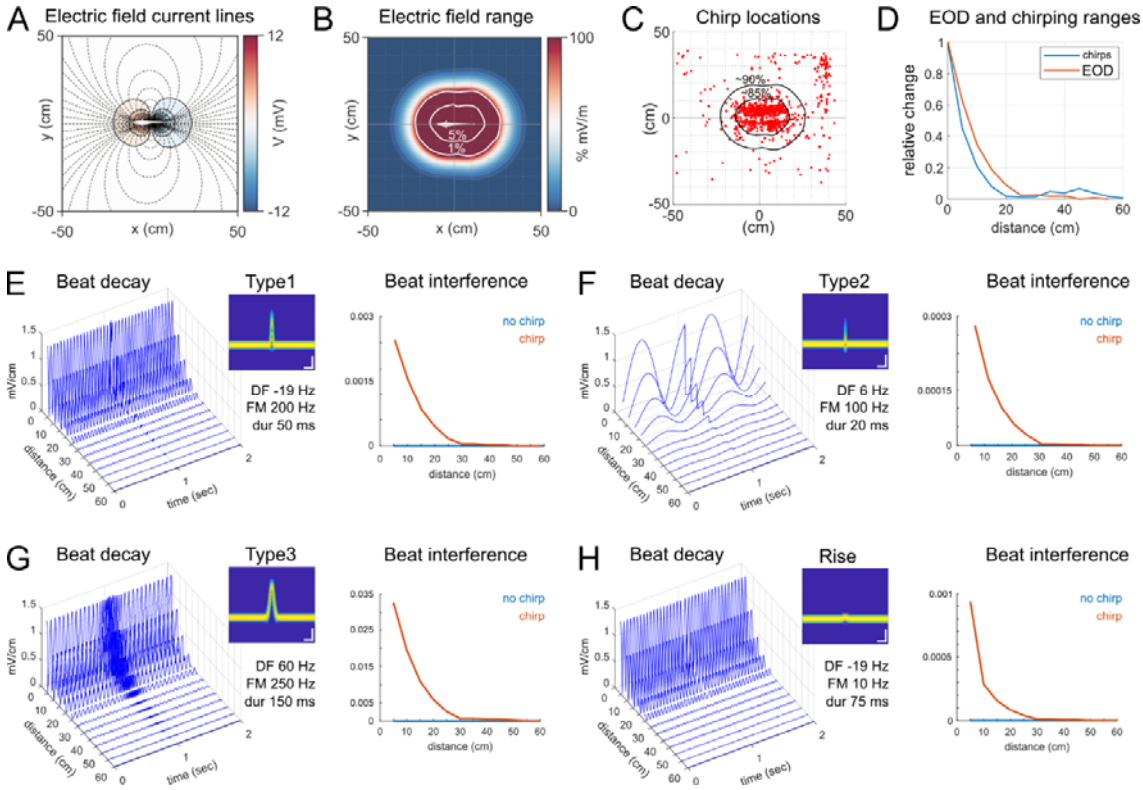

Figure 7. Chirps interfere with the beat within the electric field range. *A: Electric field generated by a 16 cm dipole modeling an electric fish of the same size. Iso-potential lines are shown for the near-field range in different colors, based on field polarity. Current is represented by the dashed lines, perpendicular to them. B: Electric field intensity mapped around the same modeled fish. The level lines represent the 1% and 5% intensity of the electric field generated by the ideal fish. C: Scatter plot of chirp locations. The overlay is centered at the origin and corresponds to 90% and 85% of all chirps produced, respectively. D: Plot showing the intensity range of an EOD mimic calculated at 22-25 °C and 200 µS (red) and the distribution range of chirps emitted by real fish (blue), for comparison. E-H: 3D plots showing the ideal beats calculated for different sinewave pairs during different chirp types and plotted over distance. The 2D plots on the side represent the beat interference (calculated using a threshold of 1% of max beat amplitude) caused by each chirp type over distance. Scale bars in the spectrograms are 100 ms and 100 Hz.*

Differences in the beat interference elicited by chirps of different types were found: the interference induced by type 1 chirps is almost one order of magnitude higher than the estimates of the same parameter for type 2 chirps but one order of magnitude lower than the beat interference induced by type 3 chirps. Rises induce interferences in the range of type 1 chirps. Interestingly, these estimates are somehow inversely correlated with the relative abundance of the 4 chirp types in these recordings (**Figure S8**) suggesting that those being used more often are also those less effective in interfering with a beat.

Overall these results indicate that during social encounters, all types of chirps, but not rises, are produced within a short distance. This possibly may explain why female subjects, apparently less motivated in exploring social stimuli, also produce considerably fewer chirps. More broadly, these findings indicate that chirping, besides being strongly correlated to the frequency of the beat generated by the interacting fish, is also significantly affected by locomotion and by spatial relationships between subjects within beat detection ranges. This further corroborates the hypothesized role of chirps in beat processing.

Effects of environmental complexity on chirping

Based on these results we hypothesized chirps could be used as probes to fine-tune the detection of EOD sources (i.e. conspecifics) during social interactions. According to this hypothesis, one would expect that increasing levels of environmental complexity will result in higher chirp rates. To test this idea, we used both playback experiments and recordings of interacting fish pairs. We aimed at assessing the responses of brown ghosts to EODs in presence of environmental clutter which would distort electric fields in a tangible but unpredictable way (von der Emde, 2006).
We first recorded social interactions using the same divided tank configuration as in our previous recordings. Fish pairs (N = 6) were recorded in 3 different conditions: 1) with lights turned ON and no object in the tank, except for the mesh divider placed in between the 2 fish (lights ON - clear), 2) with lights switched OFF (lights OFF - clear) and 3) with lights OFF and the addition of clutter consisting of shelter PVC tubes and plastic plants filling almost entirely the 2 tank compartments (lights OFF – cluttered; **Figure 8A**).

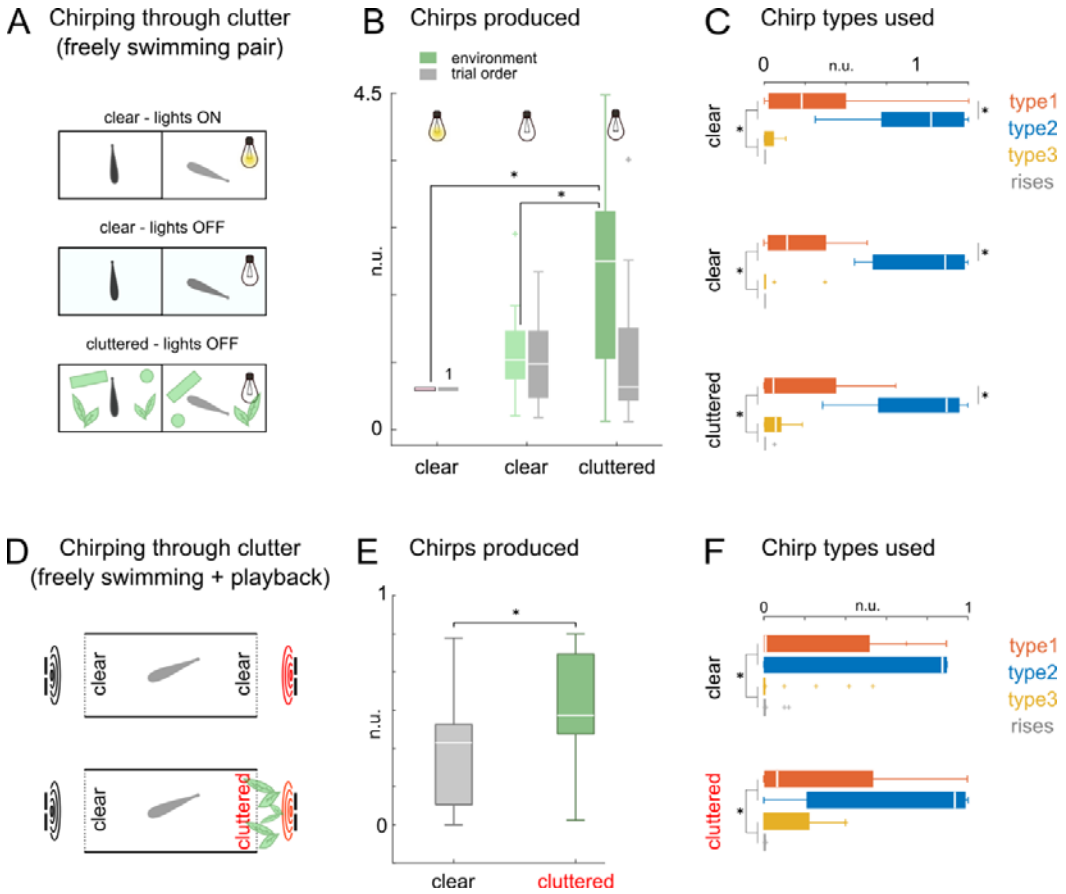

Figure 8. Effect of environmental clutter on interacting fish pairs. *A: Recording of fish pairs in environments of different sensory complexity: lights ON = clear tank environment and direct illumination, lights OFF = clear tank environment, no illumination, lights OFF + clutter = no illumination and cluttered environment. B: Total amount of chirps produced in each condition and normalized on the lights ON session (brown plots; Friedman's p = 0.053; lights ON vs lights OFF + clutter = 0.024, lights OFF vs lights OFF + clutter = 0.051). Chirp counts relative to trials sorted in chronological order are shown in gray. C: The box plots*

*show the normalized chirp counts relative to each session (lights ON, Friedman's X^2 = 21.9 p < 0.001, pairwise comparisons 1*2 p = 0.006, 1 vs 3, 1 vs 4, 2 vs 3, 2 vs 4 p < 0.001; lights OFF Friedman's X^2 = 21.8 p < 0.001 pairwise comparisons 1 vs 2, 1 vs 3, 1 vs 4, 2 vs 3, 2 vs 4 p < 0.001; clutter Friedman's X^2 = 19.5 p < 0.001 pairwise comparisons 1 vs 2, 1 vs 4, 2 vs 3, 2 vs 4 p < 0.001). D: Results of playback experiments in which EOD mimics were either directly detectable through a fine mesh barrier (clear) or more indirectly due to a barrier of plastic plants interposed between the mesh and the EOD source (cluttered). Clear and cluttered trials were presented in random succession (N fish = 6, 10 trials each, 60 sec ITI). E: Total chirp counts in the 2 conditions are normalized on the total amount of chirps produced by each subject (Wilcoxon, p = 0.025). F: Boxplots showing the chirp type composition of each condition (clear Friedman's X^2 = 17.4 p < 0.001 pairwise comparisons 1 vs 3 p = 0.048, 1 vs 4, 2 vs 3, 2 vs 4 p < 0.001; cluttered Friedman's X^2 = 29.9 p < 0.001 pairwise comparisons 1 vs 3, 1 vs 4, 2 vs 3, 2 vs 4 p < 0.001; Wilcoxon type 1 clear*type 1 clutter p = 0.034).*

Fish pairs were recorded for 15 minutes with no previous knowledge of either the environment or their social partner. Trials were shuffled in a randomized order and were spaced by 5-minute inter-trial intervals. Chirp counts were evaluated at the end of the trials. As expected, the total chirp rate increased in absence of light and even further when clutter was present in the environment (**Figure 8B**). In all cases, type 2 chirps were the most often produced type (**Figure 8C**). A slight (non-significant) increase in type 2 and type 3 chirps was observed under cluttered conditions. These results clearly indicate that chirping is affected by environmental complexity, since in these recordings aggression- or courtship-related behaviors were either absent or randomly sorted in all three conditions and both fish had equal environmental and social experience.
Nonetheless, even in conditions of low "electrical detectability", fish can sense each other using visual cues, olfactory and mechanical cues induced by water movements during swimming. Therefore, chirp production in these experiments could also be affected by a range of sensory inputs co-occurring in an unnaturally confined space and for the whole duration of the recordings (15 min).
To rule out these possibilities, we conducted playback experiments in a larger enclosure (160 x 50 cm), allowing fish to detect playback EODs from a wider range of distances. Playback electrodes were not directly accessible but placed behind a mesh barrier located at either side of the enclosure (**Figure 8D**). Environmental clutter (plastic plants) was interposed between the electrodes and the mesh divider at a location randomized for each trial. We found that chirp rates increased in response to playback signals specifically when they were partially concealed by clutter **Figure 8E**). Notably, this effect seems to be more evident for larger chirps (type 1 and type 3; **Figure 8F**) although combined effects of chirp types and clutter have not been found. Overall, these results indicate that chirping is significantly affected by the presence of environmental clutter partially disrupting - or simply obstructing - beat detection during locomotion. They further confirm electric signals alone are sufficient to trigger the chirping increase in cluttered conditions. Although all chirp types seem to be affected, type 2 chirps are the most common chirp type produced also in cluttered conditions. The enhanced use of chirping during locomotion around partially concealed EOD sources fully supports the proposed idea of chirps as self-referenced probing signals to optimize the electrolocation of conspecifics.

Discussion

In this study, we have analyzed the factors implied in the production of EOD frequency modulations (chirps) by brown ghosts (a South American species of wave-type weakly electric fish). We have collected a large chirp dataset by recording EODs in several types of social interactions to assess the effect of territory ownership, social hierarchy, sex,

reproductive state and social experience. We have used this data to identify the most significant factors explaining chirping and found that by far the strongest predictors are DF (beat frequency) and experience (environmental and social). We found that chirp patterns are ubiquitously constituted by repeats of chirps of the same type, depending on the beat frequency (DF). Fish responses to playback of different types of chirps are indistinguishable both in terms of electric signaling as well as in terms of exploratory behavior (approach/avoidance). Behavioral analysis of freely swimming fish indicated that chirping occurs always at relatively short-ranges and most of the time in association with an active social approach. Based on these results, we propose that chirps are used as self-referenced signals (sensory probes) used to enhance beat-detection and thus the processing of beat-related spatial information.

Existing functional hypotheses

Since the earliest reports (Larimer and MacDonald, 1968), chirps have been assigned behavioral meaning as communication signals based on the following observations: gymnotiform fish chirp mainly during social encounters; males of one of the gymnotiform species with the highest chirping rates, the brown-ghost knifefish, chirp considerably more than females (Dye 1987, Bastian et al. 2001, Tallarovic and Zakon 2002, Dunlap and Larkins-Ford 2003, Kolodziejski et al. 2007) and are also considerably more aggressive; chirps produced during male-male encounters (often agonistic) in the same species are smaller (i.e. shorter duration and lower frequency excursion; type 2) than chirps produced during female-male encounters (courtship-related); high chirp rates are observed in mating pairs too, with a relatively higher production of large chirps (i.e. type 1 and type 3; Hagedorn and Heiligenberg, 1985; Henninger et al., 2018). This led to the hypothesis of small chirps being implied in agonistic interactions while larger chirps were considered pertaining to different aspects of courtship and mating.

This idea raises the following concerns: first, social encounters do not only involve communication but also higher levels of locomotion and reciprocal exploration. Second, the multifactorial effects of beat frequency cannot be easily separated in this species: beat frequency affects both the production of different chirp types and correlates with sex in brown ghosts which makes it difficult to separate the biophysical properties of EOD detection from other neuroendocrine or behavioral factors. Third, observations in a few species are generalized to all other gymnotiformes without testing for species adaptations or differences (if chirps contain some sort of semantic code, this is likely to be shared - at least partially - by related species; Turner et al., 2007; Smith et al., 2013; Petzold et al., 2016). Last but most importantly, despite the hypothesized role of small chirps in modulating aggressive encounters or larger chirps being part of a courtship repertoire, no causal evidence in support of these claims has ever been provided.

Yet, the idea of chirps being used as *probes* is not entirely new: due to the tight correlation between chirp features and beat frequency (mainly chirp frequency modulation), Benda and coauthors previously hypothesized a role of chirps in determining the sign of the beat frequency (Walz et al., 2014). This indeed represents an ambiguous parameter as two opposite frequency differences result in the same beat frequency (e.g., in presence of 3 individual EODs, EOD1 = 750 Hz, EOD2 = 800 Hz and EOD3 = 700 Hz, for the fish producing EOD1 the other two may be indistinguishable). However, since any frequency modulation of EOD1 will have different effects on the beat frequency relative to EOD2 and EOD3, chirps could indeed solve the problem of disambiguating the sign of DF (which in brown ghosts could mean no less than discriminating between male and female conspecifics). However, it is also possible to obtain beat frequency information more reliably with a more gradual and slow change in the fish's own EOD frequency (which knifefish are capable of producing, see for instance Rose and Canfield, 1991 for more details). More importantly, the frequency at which chirps are produced does not seem to realistically match

such an aim (at low-beat frequencies, it is not rare for a fish to chirp more than 100 times per minute).

More recent studies have further suggested that patterns of specific chirp types could be a salient signal used for communication purposes (Henninger et al., 2018). However, such behavioral observations were limited to small groups of individuals and - as above mentioned - no causal relationship between chirps and behavior was provided. Results obtained from our recordings do not support this possibility, as fish pairs acclimated to breeding conditions used very similar and non-patterned chirp repertoires (similar also to fish pairs recorded in non-breeding conditions). One exception to the monotony of the chirping repertoires observed in this study is given by male-female pairs: in this case, type 1 and type 3 chirps are used more often, while the relative amount of type 2 chirps is lower (**Figure 3, Figure S1C,D**). However, this effect is likely to be a result of the combined effect of the pronounced dependency of chirp type on DF together with the higher chirp rates observed in males (i.e. if chirp variety increases with DFs and males chirp more, chirp type variety will be higher when males interact with high beat frequencies). Since our playback experiments showed no difference in the response of both sexes to EOD mimics containing type 1 chirps (**Figure S4**) it is likely that chirp type choice is dictated by self-referenced sensory requirements and not by different semantic contents. This idea is further corroborated by the known symmetric distribution of chirp types within the ±400 Hz DF range (Bastian et al., 2001; Engler and Zupanc, 2001; Triefenbach and Zakon, 2008; Kolodziejski et al., 2007). Based on our beat interference estimates, we proposed that the occurrence of the different types of chirps at more positive DFs (such as in male-to-female chirping) may be explained by their different effect on the beat (**Figure 5D**), an idea which seems congruent with published electrophysiological studies (Benda et al., 2006; Walz et al., 2013).

Inconsistencies between behavior and hypothesized signal meaning

In our playback experiments we observed no differences in the responses of fish of both sexes to playback stimuli, regardless of whether they contained a particular chirp type or not: test subjects chirped at comparable rates in response to all types of EOD mimics (containing type 1 chirps, type 2 chirps or rises), confirming the main factor determining type choice is DF. Notably, rises reliably occurred at the end of the playback stimulations (as also reported by Kolodijesky et al., 2007), which could be explained by a role in probing signal presence in case of abrupt interruptions or weakening (either caused by objects occluding temporarily the EOD field - clutter - or by playback interruption, as in the case of rises).

Arguably, fish could display invariant responses to EOD mimics simply because these stimuli are only close - yet nonrealistic - copies of more natural stimuli. However, EOD waveform and harmonic components – which make natural EODs different from playback mimics - do not seem to play a role in EOD discrimination as EOD frequency alone does (Dunlap and Larkins-Ford, 2003; Fugere and Krahe, 2010). Moreover, artificial EODs are widely used in electrophysiological studies as they elicit comparable responses to natural stimuli in the electrosensory system (e.g., Benda et al., 2006, Marsat et al., 2009). In line with our behavioral data, electrophysiological recordings conducted at all main nodes of the electrosensory pathway did not show consistent chirp-type specific responses in either peripheral or central brain areas (Metzen and Chacron, 2017; Allen and Marsat, 2018; Metzen et al., 2020). This confirms at a physiological level that brain responses to different chirp types do not show any evidence of response specificity. Instead, the temporal correlation between chirps and other events (locomotion-related) seem to be more salient factors, as recently proposed for midbrain circuits whose phasic responses to moving objects are used to timestamp their occurrence during spatial navigation (Wallach et al., 2018). Recordings from interacting fish pairs confirmed the absence of any significant correlation between chirp type choice and behavioral context (**Figure S2**) although chirp variance is significantly affected by this factor (**Figure 2**). This may suggest that the effect of context is mainly detectable on the number of chirps produced (**Figure S1**), rather than the type. Previous experiments reported the use of rises (larger events compared to those we

have labeled as such) during agonistic interactions (Hupé & Lewis, 2008; Raab et al., 2021). However, it is not clear whether these are aggressive signals (as proposed by Hupé and Lewis, 2008) or de-escalating submissive signals (as proposed by Raab et al., 2021). In our recordings rises are produced more or less with equal probability by female and male subjects (42.5% in males, 57.5 in females) and yet, compared to other chirp types, their relative probability is higher in females (1.2% in females vs 0.12% in males), although female brown ghosts are less frequently engaged in aggressive interactions. Their consistent occurrence at the end of playback stimuli rather suggested a probing function, in response to a sudden removal of the EOD mimic. In addition, although type 2 chirps are considered to be significantly correlated with aggression levels (Zupanc, 2002; Hupé and Lewis, 2008; Triefenbach and Zakon, 2008), our results do not support their role as aggressive signals: type 2 chirps are produced at higher rates at low beat frequencies (a feat of any encounter in which fish with similar EOD frequency approach each other and which has nothing to do with their intentions), they are more often produced by newly introduced fish (which are not necessarily more aggressive but rather more explorative; **FigureS1A**, **D**), less in experienced fish (which also move less; **Figure S1A**, **D**), more by males when separated from females (males are more motivated to interact socially; **Figure 6**) and more in females when freely swimming (females are more motivated to escape when exposed to males; **Figure 6**, personal observation relative to the breeding experiment). Overall, suggesting the main factors determining their number are the activity levels and the need to localize the other fish. Although it is true that type 2 chirps are mostly produced by males (99.16%), which tend to be also more aggressive than females, when compared to other chirp types, the sex difference is reversed: most chirps produced by females (95%) are type 2 chirps, whereas in males type 2 production reaches only 73%. Eventually, chirp type choice seemed to be largely unbiased by the behavioral context in which chirps were recorded. The main factor seemingly explaining its variance is rather beat frequency (low DF values are associated with larger numbers of chirps of this type), as can be clearly seen by plotting all chirps according to modulation range, duration and beat frequency (DF; **Figure 1D** or **Figure 5E**).

Conclusions

Although our results do not completely rule out a role of chirps in electrocommunication (probing cues could be used for instance as proximity signals to advertise a subject's presence, deter further approaches or coordinate synergistic behaviors as during spawning, Henninger et al., 2018), the high self-correlation of chirp time-series and the lack of any meaningful behavioral correlate of chirping, seems to suggest that this is an unlikely scenario for the majority of chirping observed. Moreover, the rate of chirp production (extremely variable) and the sparseness of the production of chirp types other than type 2 chirps, would make such a communication channel very unreliable and semantically too limited to be compatible with the functions so far attributed to chirps. Our behavioral experiments strongly suggest a role of chirps in homeoactive sensing. In echolocating species, active sensing has been shown to clearly depend on environmental complexity and spatial features (Fenton and Bell, 1981; Siemers et al., 2009; Fouda et al., 2018). As probing cues, chirps of different types could be used by knifefish adaptively, based on the features of the foreign EOD source (frequency, position, location, orientation, distance, etc.). Here, we emphasize the role of chirps as homeoactive signals - as opposed to alloactive - to underline the fact that chirps represent active modulations of signal frequency possibly aimed at optimizing the otherwise passive beat detection occurring constantly and unintentionally (Zweifel and Hartmann, 2020). To our knowledge, this would be a unique biological example of how underwater object localization can be enhanced instantaneously by sinewave FM probes without the assessment of echo-delays (a similar strategy is currently used in continuous-wave frequency-modulated - CWFM - radars to maximize signal-to-noise ratio and improve object distance estimates; Gurgel and Schlick, 2007, Sandström and Akeab, 2016). By providing a novel and more parsimonious explanation to explain chirping behavior, this

study revives the debate on the reasons why these nocturnal bony fish evolved this signaling system. Ideally, returning to such an old question in the light of the most recent advancements will reignite the discussion and perhaps inspire a reconsideration of the evolutionary forces that brought about the electrosensory system (electrolocation vs communication). It would emerge yet another fascinating facet of how spatial orientation can be efficiently achieved in the absence of visual, acoustic or olfactory information.

Materials and Methods

Animals

A total of 130+30+16+20 *Apteronotus leptorhynchus* of both sexes - age ranging between 2-3 years - were obtained from tropical fish importers and housed in individual 80x35x40 cm aquariums under a 12/12 light cycle. Fish were allocated to the different experiments as follows: 130 for the assessment of context-dependent effects on chirping, 16 fish were used for the playback experiments, 30 fish were used in the novel environment exploration assay, 12 fish were used to assess the environment effects on chirping and 8 to assess the impact of clutter on the fish responses to playback EODs. In all groups both sexes were equally represented. Throughout the experiments, the water was continuously filtered, water conductivity was maintained between 150-300 µS cm^{-1}, temperature between 23-26 °C and pH between 6-8. The hometank environment consisted of PVC shelter tubes and plastic plants. The fish were fed 3 times a week with red mosquito larvae. Prior to each experiment, the fish's EOD frequency (EODf), body length and weight were measured. To identify their sex, the EODf was normalized to a water temperature of 25.0 °C using a Q_{10} of 2 (Dunlap and Ragazzi, 2015; Dunlap et al., 2000). The limitation of this approach is that females cannot be distinguished from immature males with absolute certainty, since no post-mortem gonadal inspection was carried out. Nevertheless, fish with a normalized EODf higher than 750 Hz are considered males and those with lower frequencies are considered females (Dunlap et al., 2000).

Recordings of fish pairs and playback experiments

Equipment

All technical equipment used in the playback experiments is listed in the table below.

Device	Model	Manufacturer
Amplifier	DPA-2FSL and DPA-2FS	npi electronic GmbH
Attenuator	839 attenuator	KAY elemetrics Corp.
Computer	OptiPlex 7050	Dell Technologies Inc.
Data acquisition system	NI 6211 USB	National instruments
Infrared illuminators	CM-IRP6-940 nm	CMVision
Stimulus isolator	ISO-STIM 01M	npi electronic GmbH
Infrared video camera	GS3-U3-41C6NIR-C	FLIR

Experimental setup and groups

Fish pairs were recorded in 2 adjacent chambers of tripartite 80x35x40 cm aquariums. Tank compartments were separated by a plastic mesh barrier to allow electrical but no physical interaction. EOD recordings were conducted using 2 graphite electrodes placed on opposite sides of the tank (1 pair per compartment). EOD recordings were assigned to the following categories based on the type of experience fish had with either the test aquarium or the paired subjects: *resident*, *intruder*, *dominant*, *subordinate*, *novel*, *experienced*, *courtship*, *no courtship*, divided and *free*. *Resident* fish were housed for 1 week alone in the same setup, before being paired with *intruder* fish. Dominance was assessed by means of shelter-competition tests prior to EOD recordings. These tests consisted in 30 min long trials in which fish pairs were allowed to freely interact and compete for the occupancy of a plastic shelter tube positioned in one of the 3 compartments of a tripartite aquarium (1 pair per aquarium). The fish spending more time in the shelter tube was considered to be the *dominant* one, the other the *subordinate*. Fish were selected such that one fish was always 3-5 grams heavier than the other, to ensure the predictable outcome of the competition and to limit aggressive displays to a minimum (Jennions and Backwell 1996; Umbers et al. 2012). In these and other recordings, fish were considered "*experienced*" after at least 1 week of pairing, as opposed to "*novel*" (just paired). This means that residents, intruders, dominant and subordinate fish were all assigned to the category "novel", whereas the category "experienced" included only resident and intruder fish, due to their longer pairing period (1 week). In a separate set of recordings, male and female pairs were subject to water conductivity changes to simulate the onset of the breeding season: during the course of a 4 weeks period water conductivity was lowered from 400 to 100 µS cm^{-1} (novel fish in high conductivity water = *no breeding*, experienced fish in low conductivity water = *breeding season*). Although, the term "breeding" here refers to the context and not to the actual behavioral repertoire displayed by the fish, this treatment resulted in 6/8 female fish to show evident signs of developed eggs (no post-mortem exam was used to confirm egg presence in the other 2 fish). At the end of this period, fish were allowed to swim freely and interact with no mesh barriers (experienced fish in low conductivity water = breeding + free). Recordings from such pairs were compared with naïve male-female pairs freely swimming in high conductivity (400 µS cm^{-1}) waters (naïve fish in high conductivity water = no breeding + free). Although even in this case fish were paired to their tank partners for longer times, due to the different treatment they have been subject to, they have not been included in the "experienced" category.

Amplifier and recording settings

EOD recordings (3 channels) were amplified through DPA-2FSL and DPA-2FS amplifiers (*npi electronics*) with a gain of 200, low pass filtered at 10 kHz, high-pass filtered at 100 Hz. The acquisition sampling rate was kept at 20 kHz in all recordings.

Playback experiments

All playback experiments were conducted in 80x35x40 cm glass aquariums in a dark room. Water conductivity and temperature were similar to the fish housing conditions and ranged between 200-300 µS cm^{-1} and 23-26 °C, respectively. Water was pre-heated using commercially available heaters (EHEIM 3612 heating rod 50W). At one end of the tank, a mesh barrier separated the playback electrode from the fish. Electrodes were placed 10 cm apart and 1 cm away from the barrier. This layout ensured that playback stimuli consisted in

more naturalistic electric fields (Kelly et al., 2008). Electrodes were randomly placed at either side of the aquarium, in different experiments, to avoid playback location biases.

Three pairs of recording electrodes were placed in the fish compartment in accordance with an “IX” layout: 1 pair was parallel to the short tank side (“I”) the other 2 pairs were oriented oblique and perpendicularly (“X”). This layout ensured that the fish EOD was always detectable, regardless of fish movement and body orientation. Prior to commencing the study, the stimulus amplitude was calibrated to have a field intensity of approximately 1 mV/cm (amp value in MATLAB = 0.1, attenuation = 45 dB).

Playback sinewave stimuli were designed in MATLAB and their frequency based on the measured fish EOD frequency prior to trial onset. Stimuli were then delivered via a 6211-USB DAQ (National Instruments) to the aquarium through a stimulus isolator (ISO-STIM 01M, npi electronics). The stimuli played back were modified sinewaves mimicking conspecific EODs which frequencies were calculated as differences (DF) from the fish own EOD frequency: -240, -160, -80, -40, -20, -10, -5, 0, 5, 10, 20, 40, 80, 160, 240 (Hz). Each of these playback stimuli was delivered in 4 different modalities distinguished by their chirp content (modes, see Table1 below): mode0 contained no chirps, mode1 contained type 1 chirps, mode2 with type 2 chirps and mode3 contained small rises (abrupt frequency rises). Each playback trial (50 s with a 5 s time of fade-in and fade-out) had a 180 s inter-trial interval. Each playback session consisted in a randomized sequence of 15 x 4 playback trials (1 min + 3 min each) which lasted for 4 h.

Table1. Playback chirp parameters.

mode	duration [ms]	freq. mod. [Hz]	Chirps
0	-	-	no chirps
1	approx. 120	500	0.1 Hz for 50 s (1 chirp every 10 s = 5/trial)
2	approx. 20	120	2 Hz for 5 s (n = 10), every 10 s (n=10x5)
3	approx. 60	130	0.1 Hz for 50 s (1 chirp every 10 s = 5/trial)

Video tracking

During playback experiments but also novel environmental exploration assays, freely swimming fish were recorded at 40 FPS using an infrared USB camera (Grasshopper3, FLIR, model: GS3-U3-41C6NIR-C). The camera was set to acquire a frame every 500 EOD samples (1 sec = 20000 samples) through a TTL channel of the digital acquisition device (NI USB 6211 National Instruments). Swimming trajectories were extracted from the videos using the software BioTracker after converting the files from H264 to AVI (https://www.igb-berlin.de/en/biotracker). Playback experiments began around 1 p.m., after the onset of the animals lights OFF phase (12 AM lights ON, 12 PM lights OFF). Test subjects were acclimated for 30 min to the test aquarium, before trial onset.

Beat decay estimates

The beat dissipation range in our experimental conditions was estimated by recording the interaction of two EOD mimics (a static reference electrode pair and a moving source) at increasing distances and in the same water conditions as in our recordings (conductivity 200 µS and temperature 25°C). Both the reference and the moving EOD mimic were scaled to natural fish signals (1mV/cm). Recordings were made moving the electrode pair delivering the moving EOD source within a 0-60 cm distance range, with 5 cm steps and parallel to the reference electrode. The EOD field intensity generated by both reference and moving electrodes was recorded at the extremities of the reference pair.

Environmental manipulations

Fish pairs were exposed to changing environmental conditions in the experiments illustrated in Figure 8. Recordings in lights ON, OFF and cluttered conditions were carried out in 80x35x40 cm aquariums divided in 2 compartments by a plastic mesh divider. During *lights-ON*, even illumination of both tank compartments was provided via a Leica LED illuminator located on a shelf above the aquarium (Leica CLS 150 XE Microscope Cold Light Source, 150W). During *lights-OFF* and *cluttered* conditions, a drape obtained from a blackout curtain was used to fully cover the tank (room lights were switched OFF and daylight lowered by curtains). Cluttered conditions were exactly the same as *lights-OFF* with the addition of floating and submerged plastic shelter tubes and plants heterogeneously arranged in order to fill up each compartment.

Data analysis

Chirp detection

Chirps were detected in a two-step process consisting in a first automatic detection followed by a manual validation. The first step implied a measurement of the power density in the FFT of the recorded signal (FFT parameters: window size = 2^12; overlap = 90%) within the range set by the fundamental frequency and the first harmonic component (EODf-5 and 2xEOD-100 Hz). Chirps were detected as peaks in the power density. The manual validation consisted into a survey of the detected chirps to eliminate false positives. False negatives were estimated at a rate lower than 10%. Although the two electrode pairs used in each aquarium would detect EODs from both fish, chirps were properly assigned to the sender based on signal intensity (EODs generated by a fish will have higher intensity if recorded by electrodes placed in the same compartment).

Chirp categorization

	Frequency excursion < 105 Hz	Frequency excursion > 105 Hz
Duration > 50 msec	AFR	Type 3 chirp
Duration < 50 msec	Type 2 chirp	Type 1 chirp

Factor analysis mixed data - I

Factor analysis was conducted using the R package FactoMiner ("Analyse factorielle de donnees mixtes." Revue Statistique Appliquee. LII (4). pp. 93-111. Josse and Husson, 2008). A total of 30486 sampled chirps were used. For each chirp, the following qualitative and quantitative variables were considered: freq_modulation (peak of EOD instant frequency during chirp), chirp duration (instant frequency peak duration), EOD frequency of the sender (EODs), EOD frequency of the receiver (EODr), EOD_amplitude (amplitude of the EOD sinewave during chirp), power (chirp triggered EOD intensity), water temperature, water conductivity, chirp timestamp, weight and length of the sender fish, weight and length of the receiver fish, sender and receiver sex, status of the sender and receiver fish (experience with the recording aquarium), fish ID (to account for interindividual variability), chirp type (categorized as above), state (based on the experience with the other fish of the pair).
A first model (3 dimensions, model#1, dim1 17.46%, dim2 15.76%, dim3 10.67%) was made considering all variables. Subsequent models were calculated progressively reducing the

number of variables (down to 3, model#11, dim1 23.17%, dim2 20.4%, dim3 14.35%) by eliminating at each iteration those with eigenvalues lower than the mean average contribution. Another separate model was calculating excluding the variable categorical variable chirp type as it was one of the least explaining the total variance (model#6, dim1 31.83%, dim2 29.16%, dim3 13.16%).

Factor analysis mixed data - II

To assess the correlations among swimming related variables and chirps, a total of 7893 chirps were used. For each chirp, the following qualitative and quantitative variables were considered: wall (i.e. percentage of time spent near the tank wall), open (i.e. percentage of time spent in the tank open space), shelter (i.e. percentage of time spent in the shelter area), social (i.e. percentage of time spent within a rectangular area 10 cm around the conspecific location), object (i.e. percentage of time spent investigating the novel object), timestamp (the time at which chirps were produced), chirp type, fish ID, EOD frequency of the freely swimming fish, EOD frequency of the caged fish, sex of the chirp sender, sex of the chirp receiver, DF (i.e. EOD frequency difference between sender and receiver), distance (i.e. distance between sender and receiver), average speed of the sender, angle between sender and receiver (referred to the a horizontal axis delineated by the tube), actual interference (interference estimate weighed on the distance), interference (max beat interference possible by a given chirp).

Chirp transitions and time-series cross-correlation

The number of chirp transitions present in each recording was measured by searching in a string array containing the 4 chirp types, all their possible pairwise permutations. The number of each chirp-type transition was then used to create a 4x4 frequency matrix. Chirp transition diagrams were obtained by calculating the median of such frequency matrices calculated for different fish pairs. The resulting matrices were used as input arguments for a discrete-time, finite-state, time-homogeneous Markov chain modelling using MATLAB. Chirp time series were extracted from each recording and binned (50 ms) to evaluate the cross-correlation index within a +-2 s lag window.

Chirp interference

Chirp interference was calculated as the total duration of outlier beat peaks after calculating the beat envelope for each chirp/DF combination. Gaussian chirps were generated on top of a constant frequency carrier of 862 Hz (arbitrarily chosen) and the beat resulting from a paired sinewave with a ±300 Hz frequency range. The frequency modulation and duration of gaussian chirps were determined by setting the sigma and the amplitude of the peak and by measuring the actual peak size of the signal instant frequency (“medfreq” MATLAB function). This was obtained from the FFT analysis of the chirping signal (window size 2^12, 90% overlap, sampling frequency 20kHz). For each chirp, beat peaks were detected searching for local maxima in the signal first order envelope. Outliers in the beat peak durations were subsequently detected using a MATLAB built-in function (“isoutlier”) with the “quartile” settings to include peak durations on both tails of the peak distribution. The interference value for each chirp was obtained dividing the cumulative outlier peak duration by the overall duration of the beat peaks. The duration of each beat peak corresponded to the difference in time between consecutive peaks. In this way, the effect of a chirp could be weighted on

the number of peaks (i.e. the beat frequency). The MATLAB code used to generate these chirps can be found online (ref).

Statistics on chirp counts

The number of chirps produced during the experiments assessing the effect of environmental factors on chirp rate production were compared using the Friedman's test (non-parametric ANOVA for repeated measures) as data were not following a gaussian distribution.

Acknowledgements

We would like to thank Len Maler, Jan Benda and Jan Grewe for the insightful discussions during the course of this study. We are grateful to Len Maler and Ben Arthur for their constructive comments to the first manuscript draft. Research funding was provided by the Cluster of Excellence NeuroCure and by Humboldt-Universität zu Berlin to R.K..

Author contributions

L.O. designed the experiments, collected and analyzed the data and wrote the first draft of the manuscript. M.R, performed part of the resident-intruder experiments. M.L. recorded fish in simulated breeding conditions. L.K.performed the playback experiments. F.P. provided the electric field models and prepared the relative figure panels. R.K. contributed with discussions throughout the course of the study and provided feedback on the manuscript drafts.

Conflict of interest statement

The authors declare no conflict of interest.

References

Allen KM, Marsat G (2018) **Task-Specific Sensory Coding Strategies Are Matched to Detection and Discrimination Performance**. *J Exp Biol* **221**:jeb170563. doi: 10.1242/jeb.170563. https://doi.org/10.1242/jeb.170563.

Assad C (1997) **Electric Field Maps and Boundary Element Simulations of Electrolocation in Weakly Electric Fish** https://doi.org/10.7907/2XH4-JV86.

Babineau D, Longtin A, Lewis JE (2006) **Modeling the Electric Field of Weakly Electric Fish** *Journal of Experimental Biology* **209**:3636–51. https://doi.org/10.1242/JEB.02403.

Bastian J (1981) **Electrolocation. II. The Effects of Moving Objects and Other Electrical Stimuli on the Activities of Two Categories of Posterior Lateral Line Lobe Cells in Apteronotus Albifrons** *Journal of Comparative Physiology* https://doi.org/10.3/JQUERY-UI.JS.

Bastian J, Schniederjan S, Nguyenkim J (2001) **Arginine Vasotocin Modulates a Sexually Dimorphic Communication Behavior in the Weakly Electric Fish Apteronotus Leptorhynchus** *The Journal of Experimental Biology* **204**:1909–23. https://doi.org/10.1242/JEB.204.11.1909.

Benda J, Longtin A, Maler L (2005) **Spike-frequency adaptation separates transient communication signals from background oscillations** *J Neurosci* **25**:2312-21. doi: 10.1523/JNEUROSCI.4795-04.2005.

Benda J, Longtin A, Maler L (2006) **A Synchronization-Desynchronization Code for Natural Communication Signals** *Neuron* **2**: 347–58. https://doi.org/10.1016/j.neuron.2006.08.008.

Benda J (2020) **The Physics of Electrosensory Worlds** In: Fritzsch, B. (Ed.) and Bleckmann, H. (Volume Editor), The Senses: A Comprehensive Reference, vol. 7. Elsevier, Academic Press, pp. 228--254

Berwick RC, Okanoya K, Beckers GJL, Bolhuis JJ (2011) **Songs to Syntax: The Linguistics of Birdsong** *Trends in Cognitive Sciences* **3**:113–21. https://doi.org/10.1016/J.TICS.2011.01.002.

Bohn KM, Schmidt-French B, Ma ST, Pollak GD (2008) **Syllable Acoustics, Temporal Patterns, and Call Composition Vary with Behavioral Context in Mexican Free-Tailed Bats** *The Journal of the Acoustical Society of America* **3**:1838–48. https://doi.org/10.1121/1.2953314.

Brenowitz EA (1982) **The Active Space of Red-Winged Blackbird Song** *Journal of Comparative Physiology* **47**:511–22.

Brinkløv SM, Fenton B, Ratcliffe JM (2013) **Echolocation in Oilbirds and Swiftlets** *Frontiers in Physiology* **4**: 123. https://doi.org/10.3389/fphys.2013.00123

Bullock TH, Chichibu S (1965) **Further Analysis of Sensory Coding in Electroreceptors of Electric Fish** *Science* **148**:664-5. https://doi.org/10.1126/science.148.3670.664-c.

Bullock TH (1969) **Species Differences in Effect of Electroreceptor Input on Electric Organ Pacemakers and Other Aspects of Behavior in Electric Fish** *Brain, Behavior and*

Evolution **2**:102–18. https://doi.org/10.1159/000125816.

Chen L, House JL, Krahe R, Nelson ME (2005) **Modeling signal and background components of electrosensory scenes** *J Comp Physiol* **191**:331-45. https://doi.org/10.1007/s00359-004-0587-3.

Chow CP, Mitchell JF, Miller CT (2015) **Vocal Turn-Taking in a Non-Human Primate Is Learned during Ontogeny** *Proceedings of the Royal Society* **282**:20150069. https://doi.org/10.1098/RSPB.2015.0069.

Crampton WGR (2019) **Electroreception, Electrogenesis and Electric Signal Evolution** *Journal of Fish Biology* **95**: 92–134. https://doi.org/10.1111/JFB.13922.

Dulka JG, Maler L (1994) **Testosterone Modulates Female Chirping Behavior in the Weakly Electric Fish, Apteronotus Leptorhynchus** *Journal of Comparative Physiology* **174**:331–43. https://doi.org/10.1007/BF00240215.

Dunlap KD, Smith GT, Yekta A (2000) **Temperature Dependence of Electrocommunication Signals and Their Underlying Neural Rhythms in the Weakly Electric Fish, Apteronotus Leptorhynchus** *Brain, Behavior and Evolution* **55**:152–62. https://doi.org/10.1159/000006649.

Dunlap K (2002) **Hormonal and Body Size Correlates of Electrocommunication Behavior during Dyadic Interactions in a Weakly Electric Fish, Apteronotus Leptorhynchus** *Hormones and Behavior* **41**:187–94. https://doi.org/10.1006/HBEH.2001.1744.

Dunlap KD, Larkins-Ford J (2003) **Diversity in the Structure of Electrocommunication Signals within a Genus of Electric Fish, Apteronotus** *Journal of Comparative Physiology* **189**:153–61. https://doi.org/10.1007/s00359-003-0393-3.

Dunlap KD, Ragazzi MA (2015) **Thermal Acclimation and Thyroxine Treatment Modify the Electric Organ Discharge Frequency in an Electric Fish, Apteronotus Leptorhynchus** *Physiology & Behavior* **151**:64–71. https://doi.org/10.1016/J.PHYSBEH.2015.06.036.

Dunlop RA (2017) **Potential Motivational Information Encoded within Humpback Whale Non-Song Vocal Sounds** *The Journal of the Acoustical Society of America* 141:2204–13. https://doi.org/10.1121/1.4978615.

Engler G, Fogarty CM, Banks JR, Zupanc GKH (2000) **Spontaneous Modulations of the Electric Organ Discharge in the Weakly Electric Fish, Apteronotus Leptorhynchus: A Biophysical and Behavioral Analysis** *Journal of Comparative Physiology* **186**:645–60. https://doi.org/10.1007/S003590000118.

Engler G, Zupanc G (2001) **Differential Production of Chirping Behavior Evoked by Electrical Stimulation of the Weakly Electric Fish, Apteronotus Leptorhynchus** *Journal of Comparative Physiology* **187**:747–56. https://doi.org/10.1007/S00359-001-0248-8.

Dye J (1987) **Dynamics and Stimulus-Dependence of Pacemaker Control during Behavioral Modulations in the Weakly Electric Fish, Apteronotus** *Journal of Comparative Physiology* **161**:175–85. https://doi.org/10.1007/BF00615239.

Fenton MB, Bell GP (1981) **Recognition of Species of Insectivorous Bats by Their Echolocation Calls** *Journal of Mammalogy* **62**:233–43. https://doi.org/10.2307/1380701.

Fenton MB, Portfors CV, Rautenbach IL, Waterman JM (1998) **Compromises: Sound Frequencies Used in Echolocation by Aerial-Feeding Bats** *Canadian Journal of Zoology* **76**:1174–82. https://doi.org/10.1139/CJZ-76-6-1174.

Flack JC, De Waal F (2007) **Context Modulates Signal Meaning in Primate Communication** *Proceedings of the National Academy of Sciences of the United States of America* **104**:1581–86. https://doi.org/10.1073/pnas.0603565104

Fortune ES, Andanar N, Madhav M, Jayakumar RP, Cowan NJ, Bichuette ME, Soares D (2020) **Spooky Interaction at a Distance in Cave and Surface Dwelling Electric Fishes** *Frontiers in Integrative Neuroscience* **14**:51. https://doi.org/10.3389/fnint.2020.561524

Fotowat H, Harrison RR, Krahe R (2013) **Statistics of the Electrosensory Input in the Freely Swimming Weakly Electric Fish Apteronotus Leptorhynchus** *Journal of Neuroscience* **33**:13758–72. https://doi.org/10.1523/JNEUROSCI.0998-13.2013.

Fouda L, Wingfield JE, Fandel AD, Garrod A, Hodge KB, Rice AN, Bailey H (2018) **Dolphins Simplify Their Vocal Calls in Response to Increased Ambient Noise** *Biology Letters* **14**: 20180484. https://doi.org/10.1098/RSBL.2018.0484.

Fröhlich M, Müller G, Zeiträg C, Wittig RM, Pika S (2017) **Gestural Development of Chimpanzees in the Wild: The Impact of Interactional Experience** *Animal Behaviour* **134**:271–82. https://doi.org/10.1016/J.ANBEHAV.2016.12.018.

Fugère V, Krahe R (2010) **Electric Signals and Species Recognition in the Wave-Type Gymnotiform Fish Apteronotus Leptorhynchus** *Journal of Experimental Biology* **213**:225–36. https://doi.org/10.1242/JEB.034751.

Gómez-Sena L, Pedraja F, Sanguinetti-Scheck JI, Budelli R (2014) **Computational Modeling of Electric Imaging in Weakly Electric Fish: Insights for Physiology, Behavior and Evolution** *Journal of Physiology-Paris* **108**: 112–28. https://doi.org/10.1016/J.JPHYSPARIS.2014.08.009.

Gould E (1965) **Evidence for Echolocation in the Tenrecidae of Madagascar** *Proceedings American Phil Society* **109**:352–60. https://doi.org/10.2307/986137.

Griffin DR (1944) **Echolocation by Blind Men, Bats and Radar** *Science* **2609**: 589–90. https://doi.org/10.1126/SCIENCE.100.2609.589

Gurgel KW, Schlick T (2007) **Compatibility of FMCW modulated HF surface wave radars with radio services**, *International Radar Symposium* IRS 2007, pp. 255–258, Cologne, Germany,

Hagedorn M, Heiligenberg W (1985) **Court and Spark: Electric Signals in the Courtship and Mating of Gymnotoid Fish** *Animal Behaviour* **33**:254–65. https://doi.org/10.1016/S0003-3472(85)80139-1.

Henninger J, Krahe R, Kirschbaum F, Grewe J, Benda J (2018) **Statistics of Natural Communication Signals Observed in the Wild Identify Important Yet Neglected Stimulus Regimes in Weakly Electric Fish** *J Neurosci.* **38**:5456-5465. https://doi.org/10.1523/JNEUROSCI.0350-18.2018

Hopkins CD (1974) **Electric Communication: Functions in the Social Behavior of Eigenmannia Virescens** *Behaviour* **50**:270–305. http://www.jstor.org/stable/4533613.

Hopkins CD, Westby GWM (1986) **Time Domain Processing of Electric Organ Discharge**

Waveforms by Pulse-Type Electric Fish *Brain, Behavior and Evolution* **29**:77–104. https://doi.org/10.1159/000118673.

Hupé GJ, Lewis JE, Benda J (2008) **The Effect of Difference Frequency on Electrocommunication: Chirp Production and Encoding in a Species of Weakly Electric Fish, Apteronotus Leptorhynchus.** *Journal of Physiology-Paris* **102**:164–72. https://doi.org/10.1016/J.JPHYSPARIS.2008.10.013.

Jennions M (1996) **Residency and Size Affect Fight Duration and Outcome in the Fiddler Crab *Uca Annulipes*** *Biological Journal of the Linnean Society* **57**:293–306. https://doi.org/10.1006/BIJL.1996.0017.

Jones G, Holderied MW (2007) **Bat Echolocation Calls: Adaptation and Convergent Evolution** *Proceedings of the Royal Society* **274**:905–12. https://doi.org/10.1098/RSPB.2006.0200.

Kawasaki M (1997) **Sensory Hyperacuity in the Jamming Avoidance Response of Weakly Electric Fish** *Current Opinion in Neurobiology* **7**:473–79. https://doi.org/10.1016/S0959-4388(97)80025-6.

Kelly M, Babineau D, Longtin A, Lewis JE, Kelly (2008) **Electric Field Interactions in Pairs of Electric Fish: Modeling and Mimicking Naturalistic Inputs** *Biological Cybernetics* **98**:479–90. https://doi.org/10.1007/S00422-008-0218-0.

Kirschbaum F (1979) **Reproduction of the Weakly Electric Fish Eigenmannia Virescens (Rhamphichtyidae, Teleostei) in Captivity** *Behavioral Ecology and Sociobiology* **4**:331–55. https://doi.org/10.1007/BF00303241.

Knudsen EI (1974) **Behavioral Thresholds to Electric Signals in High Frequency Electric Fish** *Journal of Comparative Physiology* **91**:333–53. https://doi.org/10.1007/BF00694465.

Knudsen EI (1975) **Spatial Aspects of the Electric Fields Generated by Weakly Electric Fish** *Journal of Comparative Physiology* **99**:103–18. https://doi.org/10.1007/BF00618178.

Kolodziejski JA, Sanford SE, Smith GT (2007) **Stimulus Frequency Differentially Affects Chirping in Two Species of Weakly Electric Fish: Implications for the Evolution of Signal Structure and Function** *Journal of Experimental Biology* **210**:2501–9. https://doi.org/10.1242/jeb.005272.

Konishi M, Knudsen EI (1979) **The Oilbird: Hearing and Echolocation** *Science* **204**:425–27. https://doi.org/10.1126/SCIENCE.441731.

Larimer JL, MacDonald JA (1968) **Sensory Feedback from Electroreceptors to Electromotor Pacemaker Centers in Gymnotids** *Journal of Physiology* **214**:1253–61. https://doi.org/10.1152/AJPLEGACY.1968.214.6.1253.

Josse J, Husson F (2008) **FactoMineR: An R Package for Multivariate Analysis** *Journal of Statistical Software* **25**:1-18. https://doi.org/10.18637/jss.v025.i01.

Lissmann HW (1958) **On the function and evolution of electric organs in fish** *Journal of Experimental Biology* **35**:156-191. https://doi.org/10.1242/jeb.35.1.156

Lissmann HW, Machin KE (1958) **The Mechanism of Object Location in Gymnarchus Niloticus and Similar Fish** *Journal of Experimental Biology* **35**:451–86.

https://doi.org/10.1242/JEB.35.2.451.

Marsat G, Proville RD, Maler L (2009) **Transient Signals Trigger Synchronous Bursts in an Identified Population of Neurons** *Journal of Neurophysiology* **102**:714–23. https://doi.org/10.1152/jn.91366.2008

Metzen MG, Chacron MJ (2017) **Stimulus Background Influences Phase Invariant Coding by Correlated Neural Activity** *ELife* 6:e24482 https://doi.org/10.7554/ELIFE.24482.

Metzen M, Hofmann V, Chacron MJ (2020) **Neural Synchrony Gives Rise to Amplitude- and Duration-Invariant Encoding Consistent With Perception of Natural Communication Stimuli** *Frontiers in Neuroscience* **14** https://doi.org/10.3389/FNINS.2020.00079.

Milam OE, Ramachandra KL, Marsat G (2019) **Behavioral and Neural Aspects of the Spatial Processing of Conspecific Signals in the Electrosensory System** *Behav Neurosci* **133**:282-296. https://doi.org/10.1037/bne0000320.

Murchison AE (1980) **Detection Range and Range Resolution of Echolocating Bottlenose Porpoise (Tursiops Truncatus)** In *Animal Sonar Systems* Eds. Busnel RG, Fish JF, Springer, Boston, MA, pp 43–70. https://doi.org/10.1007/978-1-4684-7254-7_3.

Nelson ME, MacIver MA (2006) **Sensory Acquisition in Active Sensing Systems** *Journal of Comparative Physiology* **192**:573–86. https://doi.org/10.1007/S00359-006-0099-4.

Nieder A, Mooney R (2020) **The Neurobiology of Innate, Volitional and Learned Vocalizations in Mammals and Birds** *Philosophical Transactions of the Royal Society of London* **375**:20190054. https://doi.org/10.1098/RSTB.2019.0054.

Notman H, Rendall D (2005) **Contextual Variation in Chimpanzee Pant Hoots and Its Implications for Referential Communication** *Animal Behaviour* **70**:177–90. https://doi.org/10.1016/J.ANBEHAV.2004.08.024.

Owren MJ, Amoss RT, Rendall D (2011) **Two Organizing Principles of Vocal Production: Implications for Nonhuman and Human Primates** *American Journal of Primatology* **73**:530–44. https://doi.org/10.1002/AJP.20913.

Pedraja F, Perrone R, Silva A, Budelli R (2016) **Passive and active electroreception during agonistic encounters in the weakly electric fish Gymnotus omarorum**. *Bioinspir Biomim.* **11**:065002. doi: 10.1088/1748-3190/11/6/065002. PMID: 27767014.

Petzold JM, Marsat G, Smith GT (2016) **Co-Adaptation of Electric Organ Discharges and Chirps in South American Ghost Knifefishes (Apteronotidae)** *Journal of Physiology* **110**: 200–215. https://doi.org/10.1016/J.JPHYSPARIS.2016.10.005.

Raab T, Bayezit S, Erdle S, Benda J (2021) **Electrocommunication Signals Indicate Motivation to Compete during Dyadic Interactions of an Electric Fish** *The Journal of Experimental Biology* **224**:jeb242905. https://doi.org/10.1242/JEB.242905.

Rose GJ, Canfield JG (1991) **Discrimination of the Sign of Frequency Differences by Sternopygus, an Electric Fish without a Jamming Avoidance Response** *J Comp Physiol A* **168**:461–67.

Rose GJ (2004) **Insights into Neural Mechanisms and Evolution of Behaviour from**

Electric Fish." *Nature Reviews Neuroscience* **5**:943–51. https://doi.org/10.1038/nrn1558.

Sandström SE, Akeab IK (2016) **A study of some FMCW radar algorithms for target location at low frequencies** *Radio Science* **51**:1676–1685, doi:10.1002/2016RS005974

Scheich H, Bullock TH (1974) **The Detection of Electric Fields from Electric Organs** In *Electroreceptors and Other Specialized Receptors in Lower Vertebrates*, Eds. Fessard A. Heidelberg: Springer, pp 201–56

Schnitzler HU, Moss CF, Denzinger A (2003) **From Spatial Orientation to Food Acquisition in Echolocating Bats** *Trends in Ecology & Evolution* **18**: 386–94. https://doi.org/10.1016/S0169-5347(03)00185-X.

Siemers BM, Schauermann G, Turni H, von Merten S (2009) **Why Do Shrews Twitter? Communication or Simple Echo-Based Orientation** *Biol Letters* **5**:593–96. https://doi.org/10.1098/RSBL.2009.0378.

Silva AC, Perrone R, Zubizarreta L, Batista G, Stoddard PK (2013) **Neuromodulation of the Agonistic Behavior in Two Species of Weakly Electric Fish That Display Different Types of Aggression** *Journal of Experimental Biology* **216**:2412–20. https://doi.org/10.1242/JEB.082180.

Slocombe KE, Zuberbühler K (2005) **Agonistic Screams in Wild Chimpanzees (Pan Troglodytes Schweinfurthii) Vary as a Function of Social Role** *Journal of Comparative Psychology* **119**:67–77. https://doi.org/10.1037/0735-7036.119.1.67.

Slocombe KE, Zuberbühler K (2005) **Functionally Referential Communication in a Chimpanzee** *Current Biology* **15**:1779–84. https://doi.org/10.1016/J.CUB.2005.08.068.

Smith GT, Krahe R, Fortune E (2013) **Evolution and Hormonal Regulation of Sex Differences in the Electrocommunication Behavior of Ghost Knifefishes (Apteronotidae)** *Journal of Experimental Biology* **216**:2421–33. https://doi.org/10.1242/JEB.082933.

Snyder JB, Nelson ME, Burdick JW, MacIver MA (2007) **Omnidirectional sensory and motor volumes in electric fish** *PLoS Biology* **192**:2671-2683.

Tallarovic SK, Zakon HH (2002) **Electrocommunication Signals in Female Brown Ghost Electric Knifefish, Apteronotus Leptorhynchus** *Journal of Comparative Physiology* **188**:649–57. https://doi.org/10.1007/S00359-002-0344-4.

Thomas JA, Jalili M (2004) **Review of echolocation in insectivores and rodents.** In *Echolocation in Bats and Dolphins*. Eds. Thomas JA, Moss C, Vater V, The University of Chicago Press, pp. 547–564.

Turner CR, Derylo M, De David Santana C, Alves-Gomes JA, Smith GT (2007) **Phylogenetic Comparative Analysis of Electric Communication Signals in Ghost Knifefishes (Gymnotiformes: Apteronotidae)** *Journal of Experimental Biology* **210**:4104–22. https://doi.org/10.1242/JEB.007930.

Triefenbach FA, Zakon HH (2008) **Changes in Signalling during Agonistic Interactions between Male Weakly Electric Knifefish, Apteronotus Leptorhynchus** *Animal Behaviour* **75**:1263–72. https://doi.org/10.1016/J.ANBEHAV.2007.09.027.

Umbers KDL, Tatarnic NJ, Holwell GI, Herberstein ME (2013) **Bright Turquoise as an Intraspecific Signal in the Chameleon Grasshopper (Kosciuscola Tristis)** *Behavioral*

Ecology and Sociobiology **67**:439–47. https://doi.org/10.1007/S00265-012-1464-7/FIGURES/2.

von der Emde G (1999) **Orientation in the dark: brain circuits involved in the perception of electric signals in mormyrid electric fish** *Eur J Morphol.* 37:200-5. doi: 10.1076/ejom.37.2.200.4747.

von der Emde G (2006) **Non-Visual Environmental Imaging and Object Detection through Active Electrolocation in Weakly Electric Fish** *Journal of Comparative Physiology* **192**:601–12. https://doi.org/10.1007/s00359-006-0096-7.

Wallach A, Harvey-Girard E, Jun JJ, Longtin A, Maler L (2018) **A Time-Stamp Mechanism May Provide Temporal Information Necessary for Egocentric to Allocentric Spatial Transformations** *ELife* 7. https://doi.org/10.7554/ELIFE.36769.

Walz H, Grewe J, Benda J (2014) **Static Frequency Tuning Accounts for Changes in Neural Synchrony Evoked by Transient Communication Signals** *Journal of Neurophysiology* **112**:752–65. https://doi.org/10.1152/jn.00576.2013

Walz H, Hupé GJ, Benda J, Lewis JE (2013) **The Neuroethology of Electrocommunication: How Signal Background Influences Sensory Encoding and Behaviour in Apteronotus Leptorhynchus** *Journal of Physiology-Paris* **107**:13–25. https://doi.org/10.1016/J.JPHYSPARIS.2012.07.001.

Yu N, Hupé G, Garfinkle C, Lewis JE, Longtin A (2012) **Coding Conspecific Identity and Motion in the Electric Sense** *PLOS Computational Biology* 8:e1002564. https://doi.org/10.1371/JOURNAL.PCBI.1002564.

Yu N, Hupe G, Longtin A, Lewis JE (2019) **Electrosensory Contrast Signals for Interacting Weakly Electric Fish** *Frontiers in Integrative Neuroscience* **13**: 36. https://doi.org/10.3389/FNINT.2019.00036/BIBTEX.

Zakon HH (1986) **The Electroreceptive Periphery** In *Electroreception*, edited by W. Bullock, Theodore H., Heiligenberg, 103–56. Wiley Online Library.

Zakon H, Oestreich J, Tallarovic S, Triefenbach F (2002) **EOD Modulations of Brown Ghost Electric Fish: JARs, Chirps, Rises, and Dips** *Journal of Physiology, Paris* **96**:451–58. https://doi.org/10.1016/S0928-4257(03)00012-3.

Zupanc G, Maler L (1993) **Evoked Chirping in the Weakly Electric Fish Apteronotus Leptorhynchus: A Quantitative Biophysical Analysis** *Canadian Journal of Zoology* **71**: 2301–10. https://doi.org/10.1139/Z93-323.

Zupanc GKH, Sîrbulescu RF, Nichols A, Ilies I (2006) **Electric Interactions through Chirping Behavior in the Weakly Electric Fish, Apteronotus Leptorhynchus** *Journal of Comparative Physiology* **192**:159–73. https://doi.org/10.1007/S00359-005-0058-5/FIGURES/6.

Zupanc G (2002) **From oscillators to modulators: behavioral and neural control of modulations of the electric organ discharge in the gymnotiform fish, Apteronotus leptorhynchus** *Journal of Physiology, Paris* **96**:459-472.

Zweifel NO, Hartmann MJZ (2020) **Defining 'Active Sensing' through an Analysis of Sensing Energetics: Homeoactive and Alloactive Sensing** *Journal of Neurophysiology* **124**: 40–48. https://doi.org/10.1152/jn.00608.2019