The Journal of Experimental Biology 213, 225-236
Published by The Company of Biologists 2010
doi:10.1242/jeb.034751

Electric signals and species recognition in the wave-type gymnotiform fish *Apteronotus leptorhynchus*

V. Fugère and R. Krahe
Department of Biology, McGill University, Montreal, QC, Canada, H3A 1B1
Author for correspondence (vincent.fugere@mail.mcgill.ca)

Accepted 23 September 2009

SUMMARY

Gymnotiformes are South American weakly electric fish that produce weak electric organ discharges (EOD) for orientation, foraging and communication purposes. It has been shown that EOD properties vary widely across species and could thus be used as species recognition signals. We measured and quantified the electric signals of various species using a landmark-based approach. Using discriminant function analysis to verify whether these signals are species specific based on different signal parameters, we found that the EOD waveform is a more specific cue than EOD frequency, which shows large overlap across species. Using *Apteronotus leptorhynchus* as a focal species, we then performed a series of playback experiments using stimuli of different species (varying in frequency, waveform, or both). In an experiment with restrained fish, we found, in contrast to what we predicted, that the choice of stimulus waveform did not affect the production of communication signals. In an experiment with free-swimming fish, the animals spent more time near the playback electrodes and produced more communication signals when the stimuli were within their conspecific frequency range. Waveform again had no measurable effect. The production of communication signals correlated with the frequency difference between the stimulus and the fish's own EOD, but approach behavior did not.

Key words: species recognition, communication, weakly electric fish, electric signals, *Apteronotus*.

INTRODUCTION

Species recognition mechanisms can contribute to speciation processes and maintain reproductive isolation in assemblages of sympatric species (Ryan and Rand, 1993). When hybridization is selected against, species-specific communication signals can evolve and become incorporated into mating rituals so as to maintain pre-zygotic isolation and prevent maladaptive matings. Mate finding *via* species-specific signals has been demonstrated in a great variety of animal taxa and sensory modalities, including the electrosensory system of weakly electric fish (e.g. Amorim et al., 2008; Feulner et al., 2009; Macedonia and Stamps, 1994).

Gymnotiform fish from South America and mormyriform fish from Africa possess an eletrogenerative organ in the caudal part of their body which, when discharged, produces a weak electric field in the surrounding water (Bennett, 1971). Using an array of electroreceptors on their skin, these fish can sense perturbations of their self-generated electric fields caused by objects in their environment (for reviews see Zupanc and Bullock, 2005; Moller, 1995). This electrosense is used for navigation and foraging purposes (electrolocation) as well as for intraspecific and interspecific communication (electrocommunication). Weakly electric fish offer an excellent model to explore species recognition because electrocommunication signals are easily quantified and compared across individuals, populations and species (e.g. Turner et al., 2007; Crampton et al., 2008). Also, most species of electric fish are nocturnal and inhabit turbid water habitats in which they appear to rely largely on the electrosensory system (Moller, 1995; Hagedorn, 1986); this almost complete reliance on a single sensory modality greatly reduces the dimensionality of the species recognition problem. In addition, a wealth of neurobiological information is available on electrosensation in weakly electric fish (for reviews, see Bell and Maler, 2005; Hopkins, 1988; Kawasaki, 2005) so that physiological investigations of species recognition can be added to evolutionary and behavioral studies in order to gain a more thorough understanding of the problem at both the ultimate and proximate levels.

Electric signals vary greatly across gymnotiforms (for a review, see Crampton and Albert, 2006). Gymnotiform species can be divided in two major groups based on an especially salient difference in the electric field they produce. 'Pulse-type' fish generate electrical pulses separated by relatively long, often irregular silent pauses, whereas 'wave-type' fish produce continuous, quasi-sinusoidal signals (reviewed in Zupanc and Bullock, 2005). Among wave-type fish, electric organ discharges (EODs) vary in both frequency and waveform (Crampton and Albert, 2006). EOD frequency (EODf), the fundamental frequency of the discharge, is the number of EOD cycles per second (measured in Hz). EOD waveform is the shape of the discharge when viewed on an oscilloscope or computer screen and is determined by the harmonic content of the discharge and the phase relationships between these spectral components. In addition, wave-type EODs can be modulated in communication contexts *via* an increase or decrease in frequency and/or amplitude, which led to the description of many communication signals known as 'chirps', 'rises' and 'interruptions' (for a review, see Zakon et al., 2002). Chirps, for example, are brief increases in discharge frequency produced by some species of wave-type fish for intraspecific communication in aggressive and courtship contexts (Bastian et al., 2001; Hagedorn and Heilgenberg, 1985; Hupé and Lewis, 2008). EODf, EOD waveform and EOD modulations often vary systematically across individuals, sex, reproductive states and social position, and the respective role of these EOD parameters in intraspecific communication has been studied by many authors (for

reviews, see Moller, 1995; Stoddard et al., 2006). For example, if a male *Apteronotus leptorhynchus* is presented with an electrical stimulus 5 Hz below its own EODf, it will produce 'small chirps' (small, transient increases in EODf), whereas a stimulus of 200 Hz below the male EODf will lead to the production of 'big chirps' (large, transient increases in EODf); because male and female *A. leptorhynchus* discharge at different frequencies, with males on average 200 Hz above females, small chirps are thought to be involved in intrasexual aggression and big chirps in courtship (Bastian et al., 2001; Engler et al., 2000; Engler and Zupanc, 2001) (reviewed by Zakon et al., 2002).

EODf, EOD waveform and EOD modulations also vary enormously across species (reviewed by Crampton and Albert, 2006). Stoddard convincingly suggested that the transition from simple, primitive monophasic signals to more complex biphasic signals observed among gymnotiforms serves as a mechanism to achieve greater crypsis from electrosensory predators (Stoddard, 1999; Stoddard, 2002). The interspecific variation in gymnotiform signals, however, goes far beyond the simple transition from monophasic to biphasic signals. For instance, the EODf of wave-type species can be as low as 25 Hz (*Sternopygus branco*) or as high as 2180 Hz (*Sternarchella schotti*) (Crampton and Albert, 2006). EOD waveforms are equally diverse, with some EODs looking like simple sine waves ('pure tone', e.g. female *Eigenmannia virescens*) while others are complex multiphasic waveforms with very strong harmonic composition (e.g. *Sternarchella* sp.). Similarly, EOD modulations (chirps and rises) of different species vary in their frequency excursion and duration (Turner et al., 2007). Because of this interspecific variation, EODf, EOD waveform and EOD modulations could all serve a species recognition function. Several authors have investigated species differences in electric signals between sympatric species of African mormyriformes and species recognition based on these differences (Arnegard et al., 2006; Feulner et al., 2009; Markowski et al., 2008). For the American gymnotiforms, however, most authors have focused on characterizing the divergence of signals across species without demonstrating the behavioral relevance of these differences. Some studies have shown species clustering in multivariate signal space based on EOD waveform or EOD modulations (Turner et al., 2007; Crampton et al., 2008), but it has yet to be shown that the fish pay attention to these differences.

We set out to verify whether the EODf and EOD waveform of wave-type gymnotiform fish are indeed implicated in species recognition. Because there is considerable intraspecific variation and interspecific overlap in EODf, the fundamental frequency of the signal alone may not be a precise enough cue for unambiguous species recognition for fish that live in large sympatric assemblages (Kramer et al., 1981; Moller, 1995). EOD waveforms could carry more reliable species-specific information as they vary in many more dimensions. It was previously shown that *E. virescens* can discriminate between stimuli differing only in waveform and not frequency but these experiments were done with non-biological stimuli or with sexually dimorphic stimuli from the same species (Kramer and Zupanc, 1986; Kramer and Otto, 1988). To our knowledge, the ability of wave-type gymnotiforms to discriminate between the EOD waveforms of different species has not been investigated.

We have measured in the laboratory and the field the signals of several species of wave fish and devised a landmark-based waveform quantification system to investigate whether signals are species specific with respect to their frequency and/or waveform. We used discriminant function analysis (DFA) to classify fish into species based on the frequency or waveform of the EOD. We hypothesized that waveform would differentiate species better than EODf because of the great overlap in EODf that is seen across species (Kramer et al., 1981; Turner et al., 2007). We complemented this approach with two types of playback experiments to demonstrate discrimination between signals of different species and to test whether this discrimination is based on the frequency or the waveform of the signal. A 'chirp test' (Dye, 1987) was used as an assay to see whether male *A. leptorhynchus* would react differently to stimuli of different waveforms. Because chirps are thought to be intraspecific communication signals, we hypothesized that male *A. leptorhynchus* would chirp more in response to stimuli with a species-typical waveform. It is known that the production of communication signals differs in restrained and free-swimming situations (Dunlap and Larkins-Ford, 2003); therefore, we also performed a series of experiments with free-swimming fish with frequency-matched, waveform-matched or unmatched stimuli of different species in which the approach and communication behavior of the fish were monitored. We again hypothesized that fish would show a preference towards species-typical stimuli and chirp more in response to such stimuli than to those with the waveform or frequency of another species.

MATERIALS AND METHODS

Animals

Fourteen *Apteronotus albifrons* Linnaeus 1766 and 6 of the 18 *Eigenmannia* cf. *lineata* Müller and Troschel 1849 used for signal quantification and stimulus design were obtained from local fish dealers and kept in tanks of various sizes in groups of 3–6 individuals. The other 12 *E.* cf. *lineata* were captured and measured in Ecuador by the authors at two locations, in the province of Succumbios on the North side of the Napo River and in the province of Orellana in the Parque Nacional Yasuní. Based on their external morphology and EOD similarity, all *Eigenmannia* were considered to be the same species (*E.* cf. *lineata*) and the data from the different populations were pooled for comparison with those of the other three species. The *Sternarchorhynchus* cf. *curvirostris* Boulenger 1887 used for species comparison and stimulus design were caught by the authors in the Ucayali region of Peru. The *A. leptorhynchus* Ellis 1912 used for species comparison, chirp testing and the free-swimming experiments were bought from local fish dealers and kept in tanks of various sizes in the laboratory in groups of 1–4 per tank. All fish in the laboratory were kept on a 12 h:12 h light:dark cycle and were fed live blackworms. Tank temperature varied from 25 to 28°C, conductivity from 120 to 300 μS and pH from 6.5 to 7.5. All tanks contained plastic plants and PVC tubes to provide shelter to the fish. All animal manipulations were approved by the animal care committee of McGill University.

Electric signal recording and analysis

Subjects were brought to a 60 cm×30 cm×25 cm (45 l) glass tank (laboratory) or a collapsible, 30 cm×30 cm×20 cm (18 l) plastic tank (field) filled with water from the home tank in our fish holding facility or the capture locality. The fish were placed in a tube of mesh screen and their signal was recorded *via* silver wire electrodes placed at both ends of the tube, near the fish's head and tail. The signal was amplified with a DAM 50 differential amplifier (World Precision Instruments, Sarasota, FL, USA) and digitized with a PCI-6259M data acquisition board (National Instruments, Austin, TX, USA) (laboratory) or a National Instruments USB-6211 data acquisition device (field) at a sampling rate of 40 kHz. We processed and analyzed signals with custom-written Matlab programs (The Mathworks, Natick, MA, USA). Signals were first processed by

subtracting the signal average from the voltage trace to remove potential DC offsets introduced by the laboratory or field recording equipment. We performed a fast Fourier transform on each recording to extract the fundamental frequency and relative amplitude of the first two harmonics of the signal. Values for fundamental frequency were converted to a standard temperature value of 27°C using a Q_{10} of 1.62 (Dunlap et al., 2000). We calculated the relative amplitude values of the first two harmonics (F2–F1 and F3–F1, F1 being the fundamental frequency) by subtracting the amplitude of the fundamental (in dB) from the amplitude of the respective harmonic (see Fig. 1C). Then, individual cycles were analyzed to obtain the relative duration of each phase of the EOD. The phase durations of the EOD were computed by calculating the duration, in ms, of the rise from 0 V to the maximum value of the discharge (P1), then from the maximum to the minimum (P2), and then from the minimum back to zero (P3) (see Fig. 1D). Because the absolute duration of these phases depends on the frequency of the discharge, relative values (duration of P*x* divided by the total duration of the EOD cycle) were extracted for comparison between species with different EODf. Ten EOD cycles were analyzed for each fish and the scores were averaged to get a single value for each signal parameter for each fish.

We wanted to compare species EODf and waveform and see whether the species identity of a fish could be predicted from either of the two or both combined. Because EOD waveform is multidimensional and expressed as a set of intercorrelated landmark variables, we first used principal component analysis (PCA) to reduce the dimensionality of the dataset. These components were calculated using either waveform measurements only or both waveform parameters and EODf. We then performed three DFA to investigate whether a model would perform best (discriminate best among species) using only EODf values, only waveform parameters, or both EODf and waveform parameters. For the waveform only and waveform plus EODf DFA analyses, the PCA scores calculated earlier were used instead of the raw values. We performed all DFA with a leave-one-out cross-validation procedure. The performance of the three DFA (the number of individuals classified in the correct species) was compared using contingency tables and chi-square tests.

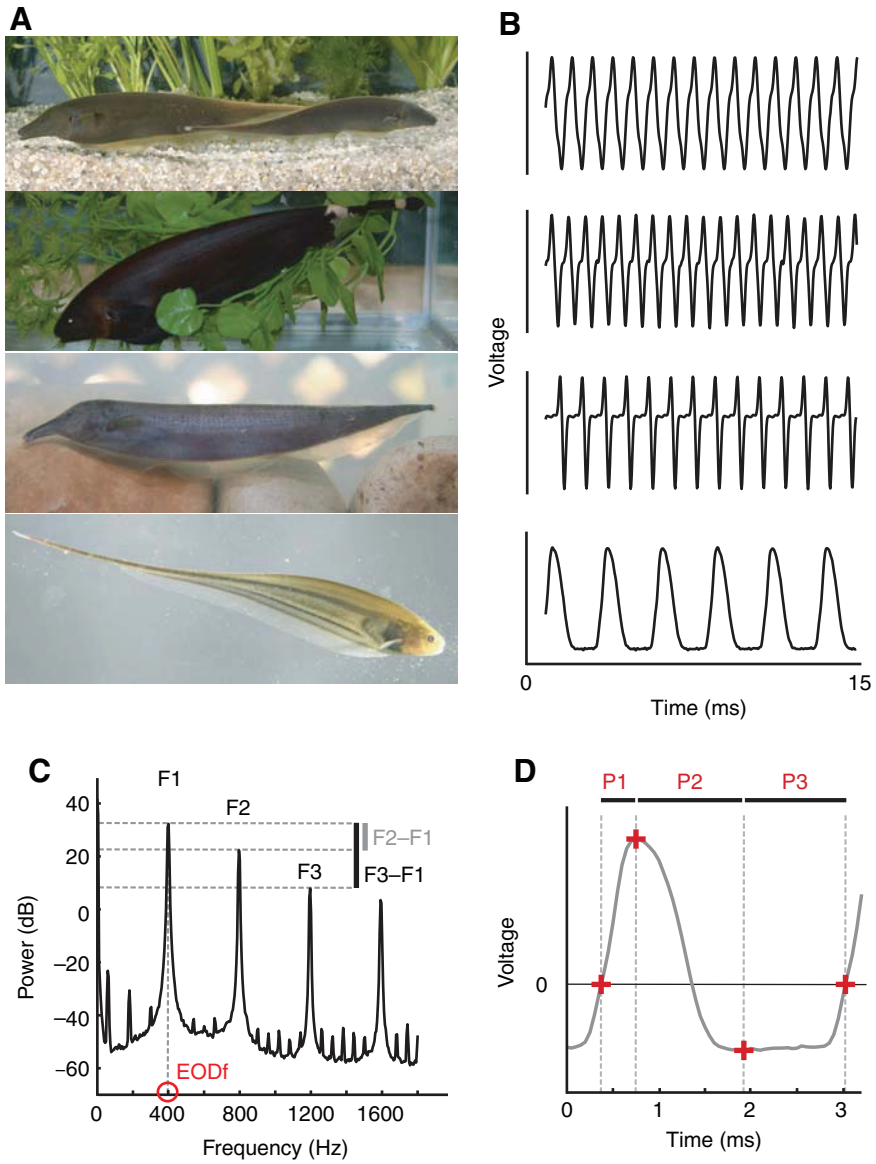

Fig. 1. (A) Photographs of the four species from which recordings were taken. From top to bottom: *Apteronotus leptorhynchus*, *Apteronotus albifrons*, *Sternarchorhynchus* cf. *curvirostris*, *Eigenmannia* cf. *lineata*. (B) Examples of signals from these four species. (C) Power spectrum of an *E.* cf. *lineata* recording illustrating the relative amplitude of the first two harmonics (F2 and F3) relative to the fundamental frequency (F1, or electric organ discharge frequency, EODf, on the frequency axis). (D) Subdivision of one electric organ discharge (EOD) cycle from *E.* cf. *lineata* in three phases. Photo credits: Guy l'Heureux (*A. leptorhynchus*), V.F. (*A. albifrons*), Angelika Meschede (*S.* cf. *curvirostris*, *E.* cf. *lineata*).

Chirp test

Only large mature male *A. leptorhynchus* were used for the chirp test (average size 22.4 cm, range 19–28.5 cm). We determined sex by the presence of a large snout as well as a high 'small-chirp' rate in response to stimuli whose fundamental frequency was 5 Hz below the fundamental frequency of the test fish's EOD (–5 Hz stimuli) (Dulka and Maler, 1994; Dunlap et al., 1998; Dye, 1987; Hagedorn, 1986; Zupanc and Maler, 1993). A test fish and water from its tank were brought to the experimental tank and the fish was given 20 min to acclimate before the experiment. The test fish was then placed in a PVC tube with mesh screen covering the endings and an opening (8 cm×3 cm) on each side of the tube for stimulus presentation. The lights were turned off and 10 2 min-long stimuli were presented, in random order, at a strength of 1 mV cm^{-1} on the fish's skin. The stimuli were presented from a pair of silver electrodes spaced 15 cm apart, perpendicular to the fish midline and arranged on one side of the fish (Kelly et al., 2008). Two electrodes located close to the fish's head and tail were used for recording. We included 4 min delays between successive stimulus presentations to prevent habituation. The stimuli included all possible combinations of five different waveforms×2 different frequencies relative to the fish's own EODf. The five waveforms included four different wave-fish species with increasing taxonomic distance from the test subjects (*A. leptorhynchus*, *A. albifrons*, *S.* cf. *curvirostris* and *E.* cf. *lineata*) as well as a computer-generated sinusoidal wave. The two frequency differences were –5 Hz and –200 Hz in order to elicit both small and big chirps. All stimuli were amplitude matched based on the root mean squared value of the signal. Custom-written Matlab programs were used for the online measurement of the fish EODf, stimulus preparation and presentation, and analysis of the recordings.

Chirps were detected and their frequency excursion was characterized using zero-crossing analysis of the recorded signals. All chirps fell within the same duration range (with an average of about 10 ms), but a clear bimodal distribution in the frequency excursion of the chirps was observed, with a peak around 80 Hz and a second peak around 580 Hz, and no chirps with a frequency excursion between 200 and 250 Hz. We classified chirps with a frequency excursion of less than 200 Hz as small chirps and those with a frequency excursion above 250 Hz as big chirps. The production of small chirps and big chirps at –5 Hz and –200 Hz was compared with paired *t*-tests by pooling the responses to all five stimuli presented at these frequencies. Because average chirp rate varied strongly across subjects, we compared the production of small chirps for the –5 Hz stimuli of different waveform by computing a proportion value for each stimulus for each fish: number of small chirps produced when that stimulus was playing relative to the total number of small chirps that the fish produced during the five –5 Hz stimuli. The proportion values were arcsin transformed to obtain normally distributed, normalized chirp rate scores that could be averaged across subjects and compared across stimuli using a one-way repeated measurements ANOVA with stimulus as the factor. The production of big chirps was compared in a similar manner using the five –200 Hz stimuli.

Free-swimming playback experiment

We used both male and female *A. leptorhynchus* in this series of experiments. Also, both fish that underwent breeding conditioning and fish that did not were used (Kirschbaum, 1984). Breeding conditioning consists of a gradual reduction in the tank water conductivity, which mimics rainy season conditions, induces hormonal changes in the fish and makes them enter a reproductive state (e.g. females become gravid). We hypothesized that breeding conditioning could unveil certain behavioral patterns only exhibited during the breeding season. However, contrary to this hypothesis, but consistent with a previous study on wild *E. virescens* (Hopkins, 1974b), no difference in the behavior of subjects could be noted between breeding and non-breeding fish or between males and females (i.e. although response magnitude varied across sex and reproductive state, most followed a similar pattern of response across stimuli). The data from all fish were therefore pooled.

A test fish and water from its tank were brought to the experimental tank (the 45 l tank described above). The fish could swim freely in the tank and a refuge (a plant shelter) was provided in one corner. Lights were turned off to ensure that the subjects would exit the refuge during the experiment. Stimulus electrodes were glued to the bottom of the tank at the opposite corner of the tank and a 15 cm×15 cm square 'area of interest' was drawn on the tank bottom around the stimulus electrodes, to objectively determine when the fish would swim close to the stimulus electrodes. An infra-red camera along with a USB video-capture device recorded the fish movement during the experiment. The electrical behavior of the fish was recorded *via* two electrodes placed at opposite corners of the tank. The fish was given 30 min to acclimate before the experiment.

Four 4 min long stimuli were presented, in random order, with a 10 min pause after each stimulus. All stimuli were amplitude matched (root mean square) and multiplied with low-pass filtered noise (<0.4 Hz) to create small low-frequency amplitude modulations in the stimulus, to mimic the amplitude modulations normally caused by fin and whole-body movements (R.K., unpublished). We also included a 1 s ramp at the beginning and end of the stimulus to simulate the effect of a real fish entering and leaving the experimental tank. Stimulus strength was adjusted so that the mean amplitude experienced by the fish would not exceed 3 mV cm^{-1} when closest to the stimulation electrodes (i.e. when the fish touched one of the electrodes). However, because the fish was allowed to swim freely across the tank, the subjects experienced a whole range of different stimulus amplitudes over the course of the experiment. We chose to control for stimulus amplitude in this way to mimic what the fish would experience if a real fish was in the corner of the tank and if the test fish was allowed to interact freely with it.

Three sets of four stimuli were tested separately (subjects never experienced more than one set of stimuli per day). In the first experiment (first set of stimuli), each stimulus had both the frequency and waveform typical of the species from which the recording used for playback was taken. The *A. leptorhynchus* stimulus was presented at 900 Hz, the *A. albifrons* signal at 1170 Hz, the *S.* cf. *curvirostris* signal at 950 Hz and the *E.* cf. *lineata* signal at 405 Hz. These frequencies correspond to those of the 'most average' individuals from each species, as calculated from the EOD measurements described earlier.

In the second experiment (second set of stimuli), we re-sampled the four recordings from the four different species so as to frequency match them at 900 Hz. The resulting stimuli differed in waveform (see Fig. 1B for an illustration of the four waveforms) but not in frequency (i.e. the low-frequency *E.* cf. *lineata* recording used for stimulus preparation was 'compressed' in time to increase its frequency to 900 Hz while keeping its waveform, whereas the higher frequency *A. albifrons* and *S.* cf. *curvirostris* recordings were 'stretched' in time to reduce their frequency, also to 900 Hz).

In the third experiment (third set of stimuli), the four stimuli were created by re-sampling the same *A. leptorhynchus* recording at the four different frequencies included in experiment 1 (405 Hz, 900 Hz, 950 Hz, 1170 Hz). The resulting stimuli had the same waveform but

differed in frequency. Some fish were used in more than one of the experiments. Custom-written Matlab programs were used for stimulus preparation and presentation and analysis of the recordings.

The amount of time that the fish spent within the area of interest described earlier ('time in') was extracted from the video recordings. The fish was considered 'in' when its head entered the area of interest. Time in was compared when the stimulus was on or off to verify that the subjects were responding. For stimulus comparisons, a proportion value was extracted for each stimulus as the number of seconds spent in the area of interest while that stimulus was playing out of the total number of seconds spent in that area during all four stimuli. The proportion values were arcsin transformed, averaged across fish to obtain a mean response to a specific stimulus, and the mean responses to the four stimuli were compared with a one-way repeated measurements ANOVA with stimulus as the factor and *post-hoc* paired one-tailed *t*-tests.

The electrical recordings were converted into spectrograms in Matlab and chirps were detected visually. Visual inspection of spectrograms does not allow for reliable discrimination of chirp types so we included in our analysis all chirps, regardless of their frequency excursion. Chirp production (number of chirps per minute) was compared when the stimulus was on or off, and stimuli were compared with the same procedure as the video data, by calculating proportions (number of chirps produced during a given stimulus divided by the total number of chirps produced by the fish during stimulation during the entire experiment), arscin transforming them and comparing group means with a one-way repeated measurements ANOVA with stimulus as the factor as well as *post-hoc* paired *t*-tests. All statistics were done in R (www.r-project.org). Subjects that did not respond well (time in of less than 10 s during the entire experiment or less than 5 chirps produced across all stimuli) were excluded from the analysis (approximately 15% of subjects tested). Some fish (mainly females) responded well in terms of approach but did not chirp: in such cases the subject was included in the analysis of time in but not in the analysis of chirping.

RESULTS

Signal analysis

We analyzed the EOD signals of four species of gymnotiform fish (Fig. 1). *Apteronotus leptorhynchus* and *A. albifrons* are from the same genus (*Apteronotus*), *S.* cf. *curvirostris* is from the same family (Apteronotidae) as the two *Apteronotus* species, and *E.* cf. *lineata* is farthest taxonomically from the other three, in a different family (Sternopygidae) (Albert and Crampton, 2005). EOD frequency was lowest in *E.* cf. *lineata*, highest in *A. albifrons* and intermediate and similar in *A. leptorhynchus* and *S.* cf. *curvirostris* (Table 1). All four species had a frequency range of about 250 Hz. The strength of the first and second harmonics was notably high in *S.* cf. *curvirostris*, with F2 often being stronger than the fundamental. *Sternarchorhynchus* cf. *curvirostris* also had a longer first phase than the other two apteronotid species, and within that family the duration of P1 correlated very well with the strength of the first harmonic (R^2=0.83, t_{43}=14.54, P<0.0001). *Eigenmannia* cf. *lineata* had a markedly different waveform; its EOD had a similar harmonic content to those of the two *Apteronotus* species but P1 was shorter and P2 and P3 were longer.

The signals from the four species form clusters when they are plotted according to EODf, PCA scores computed from waveform only and PCA scores computed from both waveform and EODf (Fig. 2). The first principal component explains most of the variance in the two PCA, confirming that the intervariable correlations are very strong. No clear pattern emerges from the component loadings, rotated or not; the first principal component correlates well with all variables but in different directions (Table 2). When considering EODf alone, there is considerable overlap across the three apteronotid species, and especially so between *A. leptorhynchus* and *S.* cf. *curvirostris*. Except for a few individuals, species cluster well when the waveform PCA scores are considered. Adding EODf to the PCA model does not improve the clustering. DFA results (Fig. 3) confirm these observations quantitatively. As predicted, the waveform DFA model classified more individuals accurately than did the EODf DFA model (χ^2_1=4.63, P=0.0314). The DFA model based on both EODf and waveform also performed better than the EODf DFA model (χ^2_1=9.33, P=0.0023) but not better than the waveform-only DFA model (χ^2_1=1.08, P=0.2994). From these results we conclude that waveform contains more species-specific information than EODf, and we hypothesize that fish use this information to recognize conspecifics and find mates.

Chirp test

The experimental setup used for the chirp test and examples of small and big chirps are shown in Fig. 4. In general, more small chirps (4097) were observed than big chirps (166). Many more small chirps were produced in response to –5 Hz stimuli than to –200 Hz stimuli (t_8=9.56, P<0.0001; Fig. 5A) and many more big chirps were produced in response to –200 Hz stimuli than to –5 Hz stimuli (t_6=6.41, P=0.0003; Fig. 5B). No significant difference was observed between the small-chirp responses of *A. leptorhynchus* males to stimuli of different waveform (*A. leptorhynchus*, *A. albifrons*, *S.* cf. *curvirostris*, *E.* cf. *lineata*, sine wave) that were presented at a frequency of 5 Hz below the respective test fish's EODf ($F_{4,32}$=1.94, P=0.1271; Fig. 5C). Similarly, no significant difference was noted between big-chirp responses to stimuli of different waveforms that were presented at 200 Hz below the test fish's EODf ($F_{4,24}$=0.93, P=0.4621; Fig. 5D). These results suggest that the production of chirps by male *A. leptorhynchus* is not influenced by the waveform of the stimulus, but, as reported previously (Engler and Zupanc,

Table 1. Species means (±s.d.) for the six signal parameters measured

	Apteronotus leptorhynchus (*N*=15)	*Apteronotus albifrons* (*N*=14)	*Sternarchorhynchus* cf. *curvirostris* (*N*=16)	*Eigenmannia* cf. *lineata* (*N*=18)
EODf (Hz)	867±70	1184±183	968±72	435±73
Waveform parameters				
F2–F1 (dB)	–14.49±3.43	–8.02±4.96	0.68±1.30	–10.86±3.40
F3–F1 (dB)	–13.87±2.13	–14.93±4.38	–1.83±1.91	–21.71±3.52
Relative duration of P1 (%)	35.37±4.14	43.29±5.66	70.54±3.74	15.23±2.93
Relative duration of P2 (%)	45.71±2.95	36.67±7.43	18.68±1.88	50.00±4.24
Relative duration of P3 (%)	18.93±2.64	20.06±4.23	10.79±2.39	34.77±5.68

EODf, electric organ discharge frequency. For other definitions, see text.

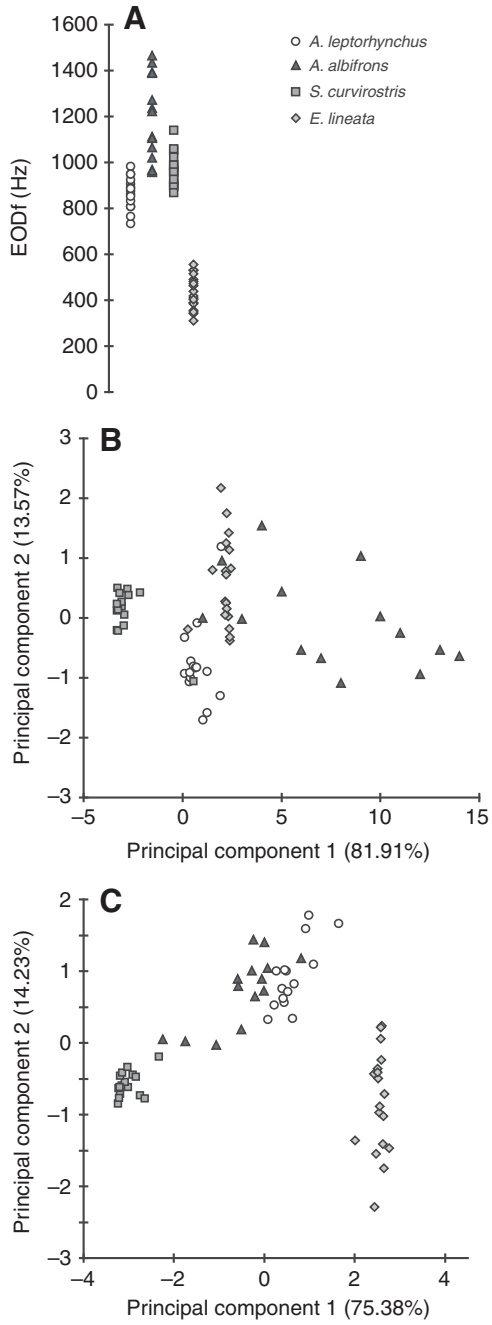

Fig. 2. (A) Frequency plot of all fish recorded, grouped by species. (B,C) Plot of subjects based on principal component analysis (PCA) scores calculated from waveform measurements only (B) and on PCA scores including the five waveform measurements and EODf (C). The numbers in parentheses indicate the variance explained by the respective principal component.

2001), the frequency of the stimulus greatly affects the number and type of chirps produced.

Free-swimming experiments

The setup used for the free-swimming experiments is shown in Fig. 6A and an example of a spectrogram of a fish chirping in response to a 900 Hz stimulus is shown in Fig. 6B. The fish spent significantly more time near the electrodes (time in) when the stimuli were on than when they were off (t_{11}=3.18, P=0.0044; Fig. 6C, left panel). The same applies to chirp production (t_{10}=2.17, P=0.0274; Fig. 6C, right panel). In the first set of stimuli tested, where stimuli differed both in waveform and frequency, time in was significantly affected by the type of stimulus ($F_{3,36}$=3.33, P=0.0301; Fig. 7A). Subjects spent significantly more time near the electrodes when the *A. leptorhynchus* stimulus was played than when the *A. albifrons* signal (t_{12}=2.76, P=0.0086) or when the *E.* cf. *lineata* signal (t_{12}=2.30, P=0.02) was presented, but not when the *S.* cf. *curvirostris* stimulus was played (t_{12}=0.75, P=0.2339). A similar pattern was observed for chirps ($F_{3,39}$=4.85, P=0.0058; *A. leptorhynchus vs A. albifrons*, t_{13}=3.80, P=0.0011; *A. leptorhynchus vs E.* cf. *lineata*, t_{13}=2.50, P=0.0133), but the difference in the responses to the *A. leptorhynchus* and *S.* cf. *curvirostris* stimuli was also significant (t_{13}=2.17, P=0.0246).

Table 2. Principal component loadings for the two principal component analyses with waveform parameters alone and with both waveform parameters and EODf

	PC1	PC2	PC3
Waveform parameters only			
F2–F1	–0.40158	0.676143	0.173461
F3–F1	–0.46126	–0.11082	–0.87748
P1 duration	–0.48676	–0.15154	0.256363
P2 duration	0.473834	–0.25874	–0.16292
P3 duration	0.405609	0.663808	–0.32815
Variance explained	81.91%	13.57%	3.11%
Waveform parameters and EODf			
EODf	–0.32959	0.590836	0.687517
F2–F1	–0.36484	–0.61823	0.318595
F3–F1	–0.43041	–0.09744	–0.41156
P1 duration	–0.46532	0.039719	–0.11675
P2 duration	0.446047	0.264044	–0.16382
P3 duration	0.396894	–0.4335	0.464725
Variance explained	75.39%	14.23%	7.15%

PC, principal component.

In the second experiment, frequency-matched stimuli (all at 900 Hz) that differed only in waveform were used (Fig. 7B). The subjects responded strongly to all stimuli but neither time in ($F_{3,36}$=0.56, P=0.648) nor chirping ($F_{3,30}$=0.59, P=0.6229) was influenced by stimulus waveform (that is, the subjects responded equally well to all stimuli despite the difference in waveform). In the last experiment, the fish were exposed to waveform-matched stimuli that differed only in frequency (Fig. 7C). The 900 Hz stimulus corresponds to the *A. leptorhynchus* frequency, the 1170 Hz stimulus to *A. albifrons*, the 950 Hz stimulus to *S.* cf. *curvirostris* and the 405 Hz stimulus to *E.* cf. *lineata*. The results closely mimic the results of the first experiment. Both time in ($F_{3,30}$=3.12, P=0.0405) and chirping ($F_{3,27}$=5.80, P=0.0034) were significantly affected by stimulus frequency. *Post-hoc* analysis reveals the exact same pattern as in experiment 1; time in differed between the 900 Hz stimulus and the 1170 Hz (t_{10}=2.03, P=0.0347) and 405 Hz stimulus (t_{10}=3.09, P=0.0057) but not the 950 Hz stimulus (t_{10}=0.23, P=0.4105). The chirp responses also followed the same pattern as in experiment 1, with a higher chirp production in response to the 900 Hz stimulus than to the other three stimuli (900 Hz *vs* 1170 Hz, t_9=2.76, P=0.0111; 900 Hz *vs* 405 Hz, t_9=3.52, P=0.0032; 900 Hz *vs* 950 Hz, t_9=1.89, P=0.0459). Taken together, these results suggest that the approach and chirping behavior of *A. leptorhynchus* are strongly impacted by stimulus frequency but not by stimulus waveform.

It is known from previous studies that chirp rate correlates negatively with the absolute difference in frequency between the

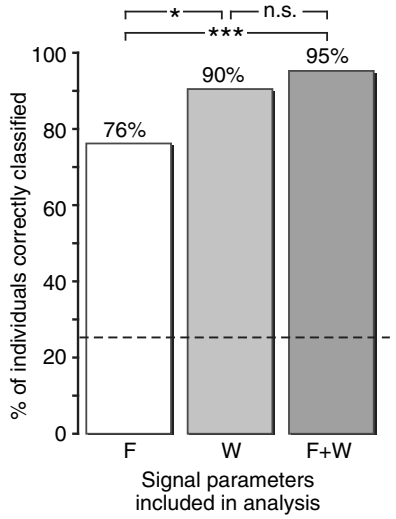

Fig. 3. Performance of the three discriminant function analyses (DFA) based on EODf only (F), waveform only (W) or both (F+W). The dashed line illustrates chance-level performance (25%). *P<0.05; ***P<0.005; n.s., non-significant.

stimulus and the fish's own EOD (hereafter referred to as Df) (Bastian et al., 2001; Engler and Zupanc, 2001; Hupé and Lewis, 2008; Kolodziejski et al., 2007; Zupanc et al., 2006). Fish chirp more in response to stimuli with a frequency close to their own. Stimulus intensity also influences chirp production: stronger stimuli, up to a certain maximum stimulus amplitude, elicit more chirps (Engler and Zupanc, 2001). To determine whether our results are consistent with these earlier findings, we examined our free-swimming data to verify whether there was a correlation between chirp rate and Df. We used the results from experiment 3, where all stimuli had the same waveform (and thus the same power distributed across the harmonics). Including the whole range of Dfs during all trials from all subjects, a significant negative correlation was found between chirp rate and Df (R^2=0.32, t_{30}=3.78, P=0.0007). This relationship is even stronger when one excludes the trials in which the Df was more than 300 Hz (R^2=0.55, t_{22}=5.21, P<0.0001). Those trials with a Df above 300 Hz happened for certain fish when they were presented with either the 1170 Hz or the 405 Hz stimuli. Although subjects chirped very little in response to the 1170 Hz stimulus, many fish chirped in response to the 405 Hz stimulus, which weakens the relationship between Df and chirp rate, and explains why the relationship is stronger when one only looks at the 0–300 Hz Df window. It seems likely that the subjects chirped in response to the 405 Hz stimulus because the first harmonic of the stimulus (at 810 Hz) creates a stimulation situation equivalent to a weaker stimulus played at a small Df. Had we used sine wave stimuli with no harmonics, as other groups have done, chirp rate in response to these high Dfs created by the 405 Hz stimulus would likely have been very low (Bastian et al., 2001; Engler and Zupanc, 2001). Time in, in contrast, did not correlate with Df (R^2=0.08, t_{30}=1.62, P=0.1167).

DISCUSSION

This study is the first to specifically look at the effect of stimulus waveforms recorded from different species on the approach and electrocommunication behavior of a wave-type gymnotiform fish. By quantifying the EOD frequency and waveform of fish from four gymnotiform species, we found that the EOD waveform carries more species-specific information than the EOD frequency and could therefore serve as a more reliable species recognition cue. However, in a chirp test and a series of experiments with free-swimming fish we found that the waveform of the stimulus had no impact on the chirping or approach behavior of the subjects whereas stimulus frequency had a profound effect.

We also found that chirp production was strongly correlated with the frequency difference between the fish EOD and the stimulus frequency but approach was not. This pattern could explain why chirp rate but not approach time was significantly higher in response to the *A. leptorhynchus* stimulus than the *S.* cf. *curvirostris* stimulus in the first free-swimming experiment and in response to the 900 Hz stimulus than the 950 Hz stimulus in the third free-swimming experiment. Indeed, the average Df between the subjects and the 900 Hz stimulus in experiment 3 was 60 Hz in contrast to 78 Hz for

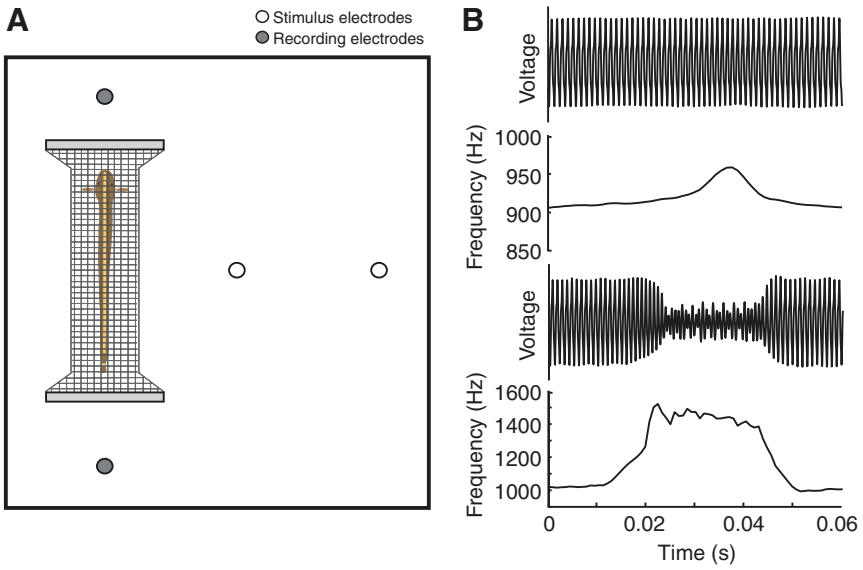

Fig. 4. (A) Setup for the chirp test. (B) Voltage trace and instantaneous frequency plot illustrating a small chirp (top) and a big chirp (bottom).

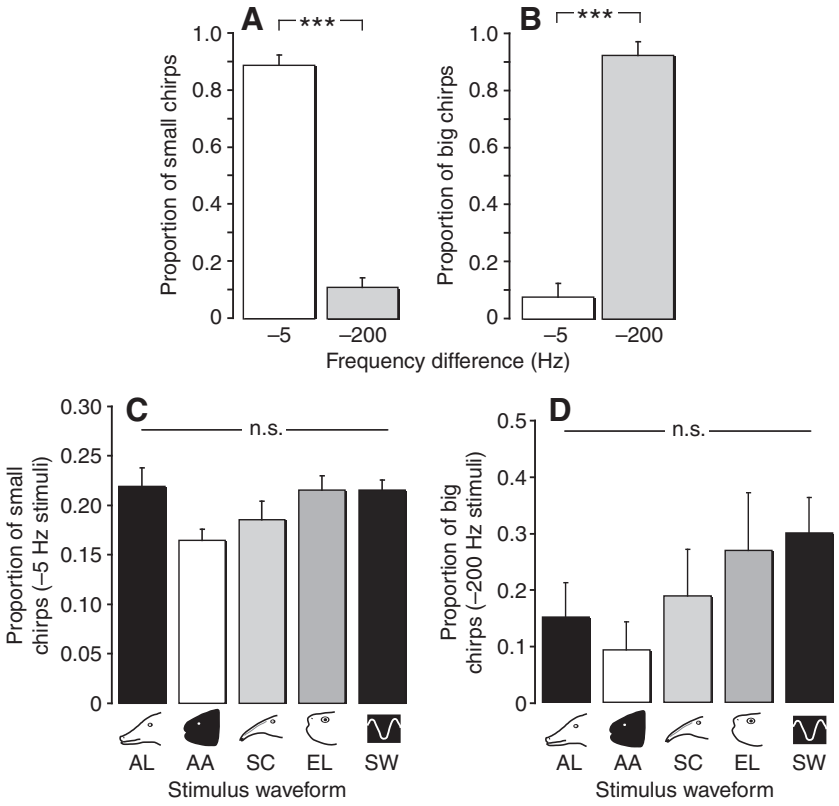

Fig. 5. (A) Proportion of small chirps produced in response to –5 Hz stimuli *vs* –200 Hz stimuli. (B) Proportion of big chirps produced in response to –5 Hz stimuli *vs* –200 Hz stimuli. (C) Proportion of small chirps produced in response to –5 Hz stimuli according to stimulus waveform. (D) Proportion of big chirps produced in response to –200 Hz stimuli according to stimulus waveform. The cartoon drawings at the bottom indicate the species to which the stimulus waveform corresponds (the four gymnotiform waveforms are those shown in Fig. 1B). AL, *A. leptorhynchus*; AA, *A. albifrons*; SC, *S.* cf. *curvirostris*; EL, *E.* cf. *lineata*; SW, computer-generated sine wave. Average number (±s.d.) of small and big chirps produced during the experiment: 455±348 small chirps and 23±17 big chirps. ***P<0.0005; n.s., non-significant.

the 950 Hz stimulus. If, on average, the Df was lower for the 900 Hz stimulus it is therefore expected that the fish would chirp more in response to this stimulus, which is what we observed. The same explanation could account for the difference in chirping in response to the *A. leptorhynchus* and *S.* cf. *curvirostris* stimuli in experiment 1. However, because the two stimuli also differed in waveform and because the *A. leptorhynchus* waveform has more power concentrated in the fundamental frequency than the *S.* cf. *curvirostris* waveform, an alternative explanation would be that the fish chirped more in response to the *A. leptorhynchus* stimulus because they perceived it as stronger (and stronger stimuli elicit more chirps) (Engler and Zupanc, 2001). Perceived stimulus strength is assumed to be increased in this case because the primary electrosensory afferents of wave-type gymnotiform fish have been shown to be tuned to the individual-specific EOD frequency (Hopkins, 1976). Approach, in contrast, does not depend on frequency difference. It seems that as long as the stimulus frequency falls within the right frequency range the fish will approach the stimulus electrodes. The fact that 950 Hz is still within the *A. leptorhynchus* frequency range could explain why there was no difference in response to the *A. leptorhynchus* and *S.* cf. *curvirostris* stimuli in experiment 1 and the 900 Hz and 950 Hz stimuli in experiment 3.

We hypothesize that fish are interested in any stimulus that is within the right frequency range but that chirping is then more

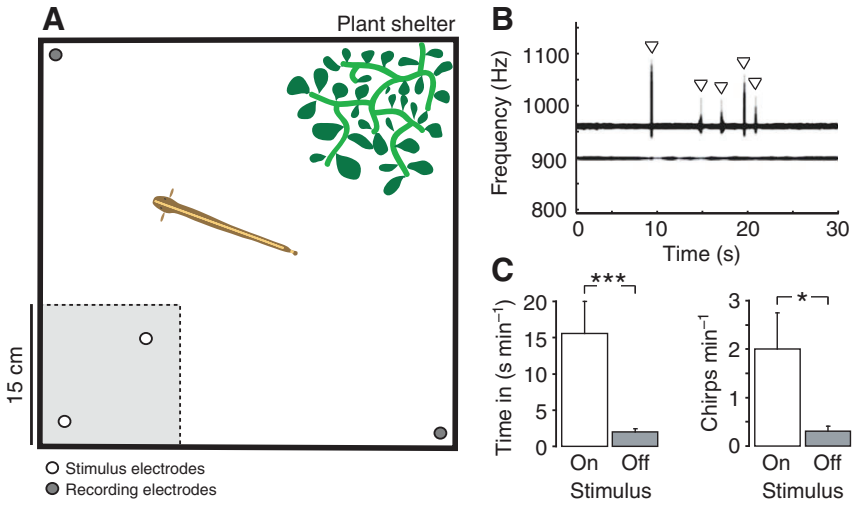

Fig. 6. (A) Experimental setup for the free-swimming experiment showing the 15 cm×15 cm area drawn around the stimulation electrodes. (B) Example of a spectrogram from a free-swimming experiment. The top trace is the fish EOD, at 957 Hz, and the bottom trace is the stimulus, at 900 Hz. During those 30 s, the fish produced 5 chirps, marked by arrowheads. (C) Left, average time that subjects spent in the stimulus electrode zone ('time in') when the stimulus was on or off. Right, average chirp rate of subjects when the stimulus was on or off. *P<0.05; ***P<0.005.

finely tuned to the stimulus/fish frequency difference. Experiments that address this hypothesis are currently underway in our laboratory.

Species specificity of gymnotiform EODs

A prerequisite for electric signals to serve a function in reproductive isolation is that the signals should be highly species specific in wild sympatric assemblages (Moller, 1995). Field investigations have produced mixed results. Early studies on small, multi-generic communities of pulse-type gymnotiforms reported that species could be distinguished unambiguously based on either the EOD rate or the peak power frequency of the discharge (Hopkins and Heiligenberg, 1978; Schwassman, 1978; Heiligenberg and Bastian, 1980). Kramer and colleagues studied a larger area of the Rio Solimoes in the Upper Amazon and found 43 sympatric species of gymnotiforms, both pulse-type and wave-type (Kramer et al., 1981). They observed overlapping discharge frequencies in both the wave-type and pulse-type species and questioned whether the EOD could really serve as an isolating mechanism in sympatric communities. Hagedorn suggested that EOD frequencies would be more species specific were we to look at syntopy and not only sympatry (Hagedorn, 1986); that is, some species may be found in the same geographical range but inhabit distinctive habitats, and it is within habitats that signal diversity should be examined. The study by Hopkins and Heiligenberg, however, was with such a small community inhabiting the same micro-habitat and although they could distinguish pulse-type species using the peak power frequency of the discharge, the EODf of two *Eigenmannia* cf. *virescens* types (identified as *E. virescens* A and *E. virescens* B by the authors) did not differ significantly (Hopkins and Heiligenberg, 1978). Hopkins found four species of wave-type fish in a small creek in Guyana (Hopkins, 1974a); two sternopygid species had characteristic EODf ranges but two apteronotid species had overlapping EODf. We also found groups of wave-type fish species living in the same streams, a few meters away from one another, that had considerable EODf overlap (*S.* cf. *curvirostris* and *A. albifrons* in Peru's Rio Llullapichis, and *E.* cf. *lineata* and *A.* cf. *albifrons* with unusually low EODf, in a small terra firme stream in Parque Nacional Yasuní, Ecuador) (V.F. and R.K., unpublished).

A few studies have reported waveform to be a distinctive characteristic of EODs. Westby, working in two creeks of French Guiana, found seven species of pulse-type gymnotiforms with overlapping interpulse intervals but qualitatively distinct pulse waveforms (Westby, 1988). Crampton and Albert described a community of six syntopic *Gymnotus* species living and breeding within the same floating meadow root mass (Crampton and Albert, 2006). EODs from these six species overlap considerably in peak power frequency of the discharge but they are perfectly distinct in multivariate signal space delimited by principal components extracted from a suite of waveform variables. With respect to wave-type gymnotiforms, they suggest that the less speciose Sternopygidae could be distinguished by EODf alone but among the Apteronotidae, where up to 35 species were found in syntopy in the Amazon river, near Téfé (Brazil), EOD waveform and potentially other cues would be necessary for unambiguous species recognition. Turner and colleagues used DFA to classify captive subjects from 13 apteronotid species using EOD parameters (Turner et al., 2007). The majority of subjects could be correctly classified using a combination of EODf and the relative strength of the first two harmonics. Although they lumped EODf and two measures of waveform in their DFA, it seems that the waveform parameters (the strength of F2 and F3) were more species specific than EODf as they explained 60% of the intersubject variance in contrast to 33% for EODf. Waveform is also very stable over time and environmental conditions, furthering its possible role as a recognition cue (Rasnow and

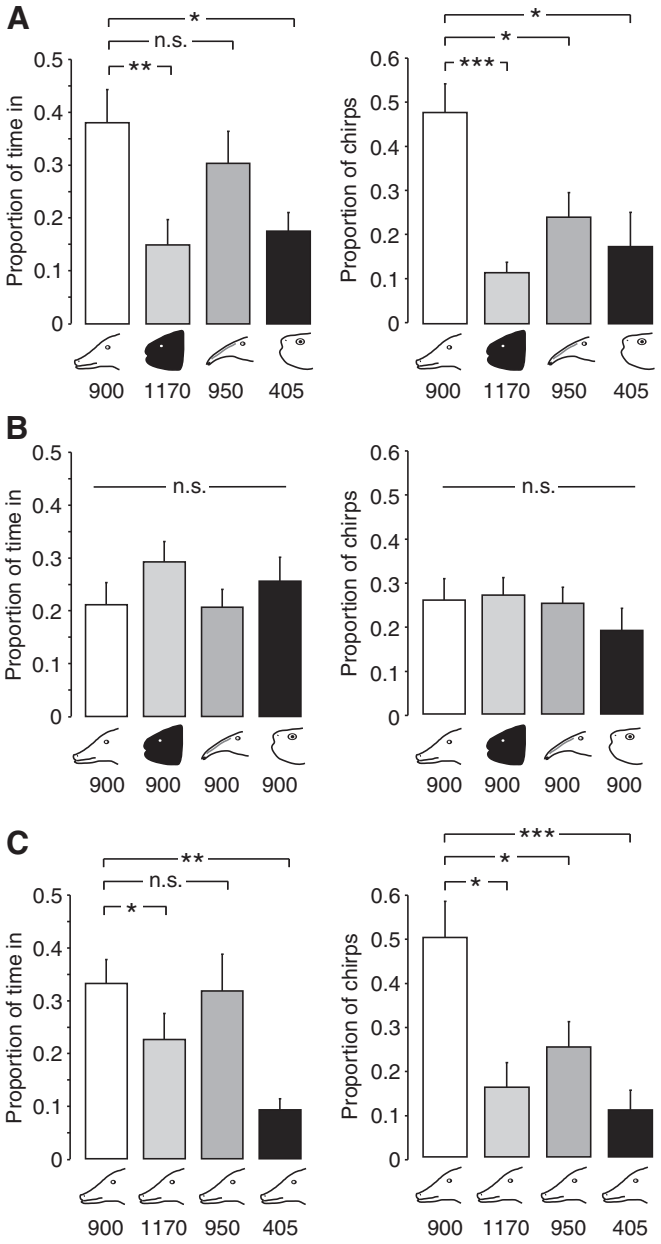

Fig. 7. Proportion of time spent in the stimulus electrode zone (left) and proportion of chirps produced by the stimulus (right). The cartoon drawing of the fish indicates the species to which the stimulus waveform corresponds (in A and B, from left to right, *A. leptorhynchus*, *A. albifrons*, *S.* cf. *curvirostris*, *E.* cf. *lineata*; in C, *A. leptorhynchus* only) below which the stimulus frequency is written (Hz). (A) Experiment 1 with unmatched stimuli. Average time in (for the 4 stimuli in total, ±s.d.)=235±239 s, average chirp production=69±153 chirps. (B) Experiment 2 with frequency-matched stimuli. Average time in=374±225 s, average chirp production=316±444 chirps. (C) Experiment 3 with waveform-matched stimuli. Average time in=293±126 s, average chirp production=155±223 chirps. **P*<0.05; ***P*<0.01; ****P*<0.005; n.s., non-significant.

Bower, 1996; Thomas et al., 1997) [see Baier (Baier, 2008) for a mormyrid example].

Our conclusion that waveform appears to be more species specific than EODf is consistent with previous studies. However, it seems that the question of whether EODs are species specific will remain unresolved until more field studies of signal diversity and behavioral responses are realized, with sympatric/syntopic assemblages, during the breeding season. Studies of wave-type gymnotiforms are especially lacking.

Effect of stimulus frequency and waveform on chirping and approach

The effect of stimulus frequency on chirping in *A. leptorhynchus* is well known. Many studies with fish in restrained conditions state that artificial sine waves presented at a frequency close to the fish's EODf (small Df) elicit many chirps (Dye, 1987; Zupanc and Maler, 1993; Dulka and Maler, 1994). When Df is systematically manipulated, subjects chirp more in response to stimuli with a lower Df (Bastian et al., 2001; Engler and Zupanc, 2001; Kolodziejski et al., 2007). Also, the frequency excursion of chirps is different for different Dfs, with small Dfs leading to the production of chirps with a small frequency excursion [termed 'type II' chirps, following Engler et al. (Engler et al., 2000); or what we called 'small chirps' in our chirp test] and larger Dfs leading to chirps with a big frequency excursion ('type I' chirps, or 'big chirps'). In experiments with two interacting fish, more chirps are produced in trials with subjects close in EODf than in trials with fish having more distant EODf (Zupanc et al., 2006; Hupé and Lewis, 2008). Our results are consistent with these previous findings. In our chirp test, type II chirps were produced in response to the small Df (–5 Hz stimuli; average chirp frequency excursion of 84 Hz) and type I chirps in response to the large Df (–200 Hz stimuli; average chirp frequency excursion of 589 Hz). Also, in both our free-swimming experiment and our chirp test, many more chirps were produced in response to small Dfs than large Dfs.

A similar pattern has been uncovered in other wave-type gymnotiform species. Hopkins realized a series of field playback experiments with *E. virescens* and *Sternopygus macrurus* during the breeding season (Hopkins, 1972; Hopkins, 1974a; Hopkins, 1974b). Playing sine wave stimuli from a broad range of frequencies revealed that the fish produced more EOD modulations ('rises' and 'interruptions') in response to stimuli of conspecific frequencies. When *E. virescens* was stimulated with a recording of any one of five sympatric species, subjects produced more EOD interruptions and attacked the stimulus electrodes more when the conspecific signal was played. Because these five stimuli differed in both waveform and frequency, this experiment is similar to our first free-swimming experiment, in which we found the same pattern for *A. leptorhynchus* (subjects chirped more and approached the electrodes more when the conspecific signal was played). Based on what we found in our second and third free-swimming experiment, we propose that both our results and Hopkins' (Hopkins, 1974b) results with unmatched stimuli of different species are explained by the frequency difference between the stimuli.

With respect to stimulus waveform, playback experiments with pulse-type gymnotiforms suggest that certain species analyze stimulus waveform and can use this information to assess the identity and relative dominance status of conspecifics (Heiligenberg and Altes, 1978; McGregor and Westby, 1992; Westby, 1974; Westby, 1975). With wave-type fish, only a few studies have specifically manipulated the waveform of the stimuli while keeping amplitude and frequency constant. Dunlap and Larkins-Ford (Dunlap and Larkins-Ford, 2003) report that male *A. leptorhynchus* chirp less in response to playbacks of a conspecific EOD than to an amplitude- and frequency-matched artificial sine wave. Because a sine wave has all of its power channeled into the fundamental frequency of the signal whereas the real EOD has power distributed across the harmonics of the fundamental, amplitude matching the two signals will result in the natural EOD having less power in the fundamental frequency than the sine wave. If the fish only pays attention to the fundamental frequency of the signal, it will perceive the EOD as a weaker stimulus than the sine wave, and so will chirp less in response to it (Engler and Zupanc, 2001). These results appear to conflict with the results of our chirp test as we did not find a difference in chirping in response to the sine wave and any of the other waveforms. The discrepancy probably arises from the different amplitude-matching methods used in the two studies. Dunlap and Larkins-Ford (Dunlap and Larkins-Ford, 2003) matched their stimuli using the peak-to-peak amplitude of the signal whereas we used root mean squared values. Peak-to-peak matching leads to larger amplitude of the sine wave stimulus than does root mean square matching. These behavioral results suggest that *A. leptorhynchus* evaluate conspecific EOD amplitude based mostly on the power of the fundamental frequency contained in the signal.

On a mechanistic level, the question arises whether these fish are just not able to perceive subtle differences in waveform, because the information may not reach the brain. It is indeed unlikely that waveform information can be extracted from individual EOD cycles. Of the two types of primary electrosensory afferents, the time-coding ones show exquisite phase locking to the EOD cycle and higher-level neurons have been shown to be sensitive to timing differences between different parts of the body at the microsecond level (Kawasaki et al., 1988). However, these timing differences are not waveform dependent. Similarly, while the other type of primary afferents, the amplitude-coding P-units, are very sensitive to modulations in EOD amplitude (Bastian, 1981), there is no evidence that they are sensitive to EOD waveform. Nevertheless, it appears likely that the information about details of the EOD waveform is available to the brain because, as a simple simulation of the involved signals shows, the modulation pattern of the beat that results from the summation of a fish's own signal with that of its neighbor is a time-expanded version of the original EOD waveforms with one beat period corresponding to one EOD cycle. Such amplitude modulations of the EOD have been shown by a number of studies to be reliably encoded by P-units (e.g. Wessel et al., 1996; Kreiman et al., 2000). Thus, waveform information is likely present in the electrosensory system, but it may not be used in the context of species recognition, at least under the conditions tested here.

Our study was the first to present wave-type gymnotiform fish with the waveforms of different species while controlling for stimulus amplitude and frequency. Our subjects did not seem to pay attention to these differences. However, it is still possible that intraspecific variance in waveform is used by the fish to assess the sex or dominance status of conspecifics, as was demonstrated in pulse-type fish (Westby, 1974; Westby, 1975). In a spontaneous preference test with *E. virescens* (Kramer and Otto, 1988), both male and female subjects spent more time in close association with a stimulus dipole playing a female *E. virescens* stimulus than one playing a male stimulus. These two stimuli were amplitude and frequency matched and differed only in waveform. This study confirms that at least one species of wave-type gymnotiform pays attention to the waveform of electrical stimuli and extracts relevant biological information from it. We also found a correlation between

the relative strength of the first harmonic (F2) and the size of individuals in a wild population of *S.* cf. *curvirostris* (V.F. and R.K., unpublished observation) and it is conceivable that within this species waveform carries information about the dominance status of individuals.

Future directions

One weakness of our playback experiments is that the recordings used for the experiments came from individuals of different species that were not captured in syntopy. However, all four species that we used are widely distributed in the Amazon basin (Albert and Crampton, 2005; Crampton and Albert, 2006) and should occur in sympatry and likely also in syntopy. We cannot exclude the possibility that local selection against hybridization favored waveform discrimination capacities in certain populations of *A. leptorhynchus* but not in others. For instance, *A. leptorhynchus* may be able to discriminate its EOD waveform from that of *A. albifrons* in areas where the two species co-occur but not in areas where they do not occur together. Such differences in signal discrimination and mate preference between populations in sympatry *vs* allopatry (reproductive character displacement) are well documented in many systems (e.g. Albert and Schluter, 2004; Jang and Gerhardt, 2006; Lemmon, 2009; Marshall and Cooley, 2000). Ideally, one would capture individuals from closely related species living in syntopy during the breeding season, play back recordings of the EODs of mature breeding males to mature breeding females and observe courtship and spawning behavior as direct measures of mate selection. Such experiments are challenging for several reasons. First, most wave-type gymnotiforms (and especially apteronotid species) occur in deep river channels (Crampton and Albert, 2006), which are not easily accessible for behavioral experiments; second, rainy season conditions make field work problematic in many Amazonian regions during the breeding season; third, the taxonomic status of many gymnotiforms is still uncertain (making it hard to compare species signals if species boundaries are not clear); and fourth, the reproductive ecology of most gymnotiforms remains unknown so it is still unclear what form courtship takes in most species. We are currently planning experiments in Central America where seasonal variations are less dramatic and where the gymnotiform fauna is less diverse and well described (Mago-Leccia, 1994).

It is conceivable that electrosensory species recognition involves not only frequency and waveform but also frequency modulation of the EOD. Turner and colleagues described chirp parameters from 13 apteronotid species and found that chirps are highly species specific (Turner et al., 2007). The potential role of chirps in species recognition could be tackled by performing playback experiments with stimuli that include chirps from various species. The stimuli would have a conspecific EOD frequency and waveform but this EOD would be modulated in frequency in a conspecific or heterospecific manner. Crampton and Albert hypothesized that the temporal rate of chirp production during courtship encounters could also be important (Crampton and Albert, 2006). Again, this could be tested with playback experiments in which the temporal pattern of the chirps contained within the stimuli is manipulated.

LIST OF SYMBOLS AND ABBREVIATIONS

Df	difference in frequency between a stimulus and the fish's own EOD frequency
DFA	discriminant function analysis
EOD	electric organ discharge
EODf	frequency of the electric organ discharge
F1	fundamental frequency of the discharge, same as EODf
F2	first harmonic of the discharge, equal to two times the fundamental frequency
F3	second harmonic of the discharge, equal to three times the fundamental frequency
P1	first phase of the EOD cycle
P2	second phase of the EOD cycle
P3	third phase of the EOD cycle
PCA	principal component analysis

ACKNOWLEDGEMENTS

We would like to thank Dr Eric Fortune (Johns Hopkins University), Erika Carrera-G. (Pontificia Universidad Católica del Ecuador), Isabel Gamboa and Dr Hernan Ortega (Universidad Nacional Mayor de San Marcos) for their help with the field component of the project. We would like to thank Jean-David Duguay and Martin Cuddy for help with Matlab programming and Dr Gerald Pollack for commenting on an earlier version of the manuscript. V.F. was supported by scholarships from NSERC and FQRNT and the research was funded by NSERC, CFI and a McGill startup grant to R.K.

REFERENCES

Albert, A. Y. and Schluter, D. (2004). Reproductive character displacement of male stickleback mate preference: reinforcement or direct selection? *Evolution* **58**, 1099-1107.

Albert, J. S. and Crampton, W. G. R. (2005). Diversity and phylogeny of neotropical electric fishes (Gymnotiformes). In *Electroreception* (ed. T. H. Bullock, C. D. Hopkins, A. N. Popper and R. R. Fay), pp. 360-409. New York: Springer.

Amorim, M. C. P., Simoes, J. M., Fonseca, P. J. and Turner, G. F. (2008). Species differences in courtship acoustic signals among five Lake Malawi cichlid species (*Pseudotropheus* spp.). *J. Fish Biol.* **72**, 1355-1368.

Arnegard, M. E., Jackson, B. S. and Hopkins, C. D. (2006). Time-domain signal divergence and discrimination without receptor modification in sympatric morphs of electric fishes. *J. Exp. Biol.* **209**, 2182-2198.

Baier, B. (2008). Effect of conductivity changes on the stability of electric signal waveforms in dwarf stonebashers (Mormyridae; *Pollimyrus castelnaui, P. marianne*). *J. Comp. Physiol. A* **194**, 915-919.

Bastian, J. (1981). Electrolocation. I. How the electroreceptors of *Apteronotus albifrons* code for moving objects and other electrical stimuli. *J. Comp. Physiol. A* **144**, 465-479.

Bastian, J., Schniederjan, S. and Nguyenkin, J. (2001). Arginine vasotocin modulates a sexually dimorphic communication behavior in the weakly electric fish *Apteronotus leptorhynchus*. *J. Exp. Biol.* **204**, 1909-1923.

Bell, C. C. and Maler, L. (2005). Central neuroanatomy of electrosensory systems in fish. In *Electroreception* (ed. T. H. Bullock, C. D. Hopkins, A. N. Popper and R. R. Fay), pp. 68-111. New York: Springer.

Bennett, M. V. L. (1971). Electric organs. In *Fish Physiology*, Vol. 5. (ed. W. S. Hoar, D. J. Randall and E. M. Donaldson), pp. 347-491. New York: Academic Press.

Crampton, W. G. R. and Albert, J. S. (2006). Evolution of electric signal diversity in gymnotiform fishes. In *Communication in Fishes*, Vol. 2 (ed. F. Ladich, S. P. Collin, P. Moller and B. G. Kapoor), pp. 647-731. Enfield, NH: Science Publishers.

Crampton, W. G. R., Davis, J. K., Lovejoy, N. R. and Pensky, M. (2008). Multivariate classification of animal communication signals: A simulation-based comparison of alternative signal processing procedures using electric fishes. *J. Physiol. Paris* **102**, 304-321.

Dulka, J. G. and Maler, L. (1994). Testosterone modulates female chirping behavior in the weakly electric fish, *Apteronotus leptorhynchus*. *J. Comp. Physiol. A* **174**, 331-343.

Dunlap, K. D. and Larkins-Ford, J. (2003). Production of aggressive electrocommunication signals to progressively realistic social stimuli in male *Apteronotus leptorhynchus*. *Ethology* **109**, 243-258.

Dunlap, K. D., Thomas, P. and Zakon, H. H. (1998). Diversity of sexual dimorphism in electrocommunication signals and its androgen regulation in a genus of electric fish, *Apteronotus*. *J. Comp. Physiol. A* **183**, 77-86.

Dunlap, K. D., Smith, G. T. and Yekta, A. (2000). Temperature dependence of electrocommunication signals and their underlying neural rhythms in the weakly electric fish, *Apteronotus leptorhynchus*. *Brain Behav. Evol.* **55**, 152-162.

Dye, J. (1987). Dynamics and stimulus-dependence of pacemaker control during behavioural modulations in the weakly electric fish, *Apteronotus*. *J. Comp. Physiol. A* **161**, 175-185.

Engler, G. and Zupanc, G. K. H. (2001). Differential production of chirping behavior evoked by electrical stimulation of the weakly electric fish, *Apteronotus leptorhynchus*. *J. Comp. Physiol. A* **187**, 747-756.

Engler, G., Fogarty, C. M., Banks, J. R. and Zupanc, G. K. H. (2000). Spontaneous modulations of the electric organ discharge in the weakly electric fish *Apteronotus leptorhynchus*: a biophysical and behavioral analysis. *J. Comp. Physiol. A* **186**, 645-660.

Feulner, P. G. D., Plath, M., Engelmann, J., Kirschbaum, F. and Tiedemann, R. (2009). Electrifying love: electric fish use of species-specific discharge for mate recognition. *Biol. Lett.* **5**, 225-228.

Hagedorn, M. (1986). The ecology, courtship and mating of gymnotiform electric fish. In *Electroreception* (ed. T. H. Bullock and W. Heiligenberg), pp. 497-526. New York: Wiley.

Hagedorn, M. and Heiligenberg, W. (1985). Court and spark: electric signals in the courtship and mating of gymnotoid fish. *Anim. Behav.* **38**, 520-525.

Heiligenberg, W. and Altes, R. A. (1978). Phase sensitivity in electroreception. *Science* **199**, 1001-1004.
Heiligenberg, W. and Bastian, J. (1980). Species specificity of electric organ discharges in sympatric gymnotoid fish of the Rio Negro. *Acta Biol. Venez.* **10**, 187-203.
Hopkins, C. D. (1972). Sex differences in electric signaling in an electric fish. *Science* **176**, 1035-1037.
Hopkins, C. D. (1974a). Electric communication in the reproductive behavior of *Sternopygus macrurus* (Gymnotoidei). *Z. Tierpsychol.* **35**, 518-535.
Hopkins, C. D. (1974b). Electric communication: functions in the social behavior of *Eigenmannia virescens*. *Behaviour* **50**, 270-305.
Hopkins, C. D. (1976). Stimulus filtering and electroreception: Tuberous electroreceptors in three species of gymnotoid fish. *J. Comp. Physiol.* **111**, 171-207.
Hopkins, C. D. (1988). Neuroethology of electric communication. *Ann. Rev. Neurosci.* **11**, 497-535.
Hopkins, C. D. and Heiligenberg, W. (1978). Evolutionary designs for electric signals and electroreceptors in Gymnotoid fishes of Surinam. *Behav. Ecol. Sociobiol.* **3**, 113-134.
Hupé, G. J. and Lewis, J. E. (2008). Electrocommunication signals in free swimming brown ghost knifefish, *Apteronotus leptorhynchus*. *J. Exp. Biol.* **211**, 1657-1667.
Jang, Y. and Gerhardt, H. C. (2006). Divergence in female calling song discrimination between sympatric and allopatric populations of the southern wood cricket *Gryllus fultoni* (Orthoptera: Gryllidae). *Behav. Ecol. Sociobiol.* **60**, 150-158.
Kawasaki, M. (2005). Physiology of tuberous electrosensory systems. In *Electroreception* (ed. T. H. Bullock, C. D. Hopkins, A. N. Popper and R. R. Fay), pp. 154-194. New York: Springer.
Kawasaki, M., Rose, G. and Heiligenberg, W. (1988). Temporal hyperacuity in single neurons of electric fish. *Nature* **336**, 173-176.
Kelly, M., Babineau, D., Longtin, A. and Lewis, J. E. (2008). Electric field interactions in pairs of electric fish: modeling and mimicking naturalistic inputs. *Biol. Cybern.* **98**, 479-490.
Kirschbaum, F. (1984). Reproduction of weakly electric teleosts: just another example of convergent development? *Environ. Biol. Fish* **10**, 3-14.
Kolodziejski, J. A., Sanford, S. A. and Smith, G. T. (2007). Stimulus frequency differentially affects chirping in two species of weakly electric fish: implications for the evolution of signal structure and function. *J. Exp. Biol.* **210**, 2501-2509.
Kramer, B. and Otto, B. (1988). Female discharges are more electrifying: spontaneous preference in the electric fish, *Eigenmannia* (Gymnotiformes, Teleostei). *Behav. Ecol. Sociobiol.* **23**, 55-60.
Kramer, B. and Zupanc, G. K. H. (1986). Conditioned discrimination of electric waves differing only in form and harmonic content in the electric fish, *Eigenmannia*. *Naturwissenschaften* **73**, 679-680.
Kramer, B., Kirschbaum, F. and Markl, H. (1981). Species specificity of electric organ discharges in a sympatric group of gymnotoid fish from Manaus (Amazonas). In *Advances in Physiological Sciences*, Vol. 31 (ed. T. Szabo and G. Czéh), pp. 195-219. Budapest: Pergamon Press.
Kreiman, G., Krahe, R., Metzner, W., Koch, C. and Gabbiani, F. (2000). Robustness and variability of neuronal coding by amplitude-sensitive afferents in the weakly electric fish *Eigenmannia*. *J. Neurophysiol.* **84**, 189-204.
Lemmon, E. M. (2009). Diversification of conspecific signals in sympatry: geographic overlap drives multidimensional reproductive character displacement in frogs. *Evolution* **63**, 1155-1170.
Macedonia, J. M. and Stamps, J. A. (1994). Species recognition in *Anolis grabami* (Sauria, Iguanidae): evidence from responses to video playbacks of conspecific and heterospecific displays. *Ethology* **98**, 246-264.
Mago-Leccia, F. (1994). *Electric Fishes Of The Continental Waters Of America*. Caracas: Clemente Editores, C.A.
Markowski, B., Baier, B. and Kramer, B. (2008). Differentiation in electrical pulse waveforms in a pair of sibling Dwarf Stonebashers, *Pollimyrus castelnaui* and *P. marianne*: possible mechanisms and functions (Mormyridae, Teleostei). *Behaviour* **145**, 115-135.
Marshall, D. C. and Cooley, J. R. (2000). Reproductive character displacement and speciation in periodical cicadas, with description of new species, 13-year *Magicicada neotredecem*. *Evolution* **54**, 1313-1325.
McGregor, P. and Westby, G. W. M. (1992). Discrimination of individually characteristic electric organ discharges by a weakly electric fish. *Anim. Behav.* **43**, 977-986.
Moller, P. (1995). *Electric fishes: History and behavior*, 584 pp. London: Chapman & Hall.
Rasnow, B. and Bower, J. M. (1996). The electric organ discharges of the gymnotiform fishes. 1. *Apteronotus leptorhynchus*. *J. Comp. Physiol. A* **178**, 383-396.
Ryan, M. J. and Rand, A. S. (1993). Species recognition and sexual selection as a unitary problem in animal communication. *Evolution* **47**, 647-657.
Schwassmann, H. O. (1978). Activity rhythms in gymnotoid electric fishes. In *Rhythmic Activity Of Fishes* (ed. J. E. Thorpe), pp. 235-241. London: Academic Press.
Stoddard, P. K. (1999). Predation enhances complexity in the evolution of electric fish signals. *Nature* **400**, 254-256.
Stoddard, P. K. (2002). The evolutionary origins of electric signal complexity. *J. Physiol. Paris* **96**, 485-491.
Stoddard, P. K., Zakon, H. H., Markham, M. R. and McAnelly, L. (2006). Regulation and modulation of electric waveforms in gymnotiform electric fish. *J. Comp. Physiol. A* **192**, 613-624.
Thomas, M., Florion, A. and Chrétien, D. (1997). The effect of pH and conductivity on the electric behaviour of *Apteronotus albifrons* (family apteronotidae) in a pollution monitoring system. *Environ. Tech.* **18**, 1069-1083.
Turner, C. R., Derylo, M., de Santana, C. D., Alves-Gomes, J. A. and Smith, T. G. (2007). Phylogenetic comparative analysis of electric communication signals in ghost knifefishes (Gymnotiformes: Apteronotidae). *J. Exp. Biol.* **210**, 4104-4122.
Wessel, R., Koch, C. and Gabbiani, F. (1996). Coding of time-varying electric field amplitude modulations in a wave-type electric fish. *J. Neurophysiol.* **75**, 2280-2293.
Westby, G. W. M. (1974). Assessment of the signal value of certain discharge patterns in the electric fish, *Gymnotus carapo*, by means of playback. *J. Comp. Physiol.* **92**, 327-341.
Westby, G. W. M. (1975). Further analysis of the individual discharge characteristics predicting social dominance in the electric fish, *Gymnotus carapo*. *Anim. Behav.* **23**, 249-260.
Westby, G. W. M. (1988). The ecology, discharge diversity and predatory behaviour of gymnotiform electric fish in the coastal streams of French Guiana. *Behav. Ecol. Sociobiol.* **22**, 341-354.
Zakon, H., Oestreich, J., Tallarovic, S. and Triefenbach, F. (2002). EOD modulations of brown ghost electric fish: JARs, chirps, rises, and dips. *J. Physiol. Paris* **96**, 451-458.
Zupanc, G. and Maler, L. (1993). Evoked chirping in the weakly electric fish, *Apteronotus leptorhynchus*: a biophysical and behavioral analysis. *Can. J. Zool.* **71**, 2301-2310.
Zupanc, G. K. H. and Bullock, T. H. (2005). From electrogenesis to electroreception: an overview. In *Electroreception* (ed. T. H. Bullock, C. D. Hopkins, A. N. Popper and R. R. Fay), pp. 5-46. New York: Springer.
Zupanc, G. K. H., Sîrbulescu, R. F., Nichols, A. and Ilies, I. (2006). Electric interactions through chirping behavior in the weakly electric fish, *Apetronotus leptorhynchus*. *J. Comp. Physiol. A* **192**, 159-173.