Corrigendum

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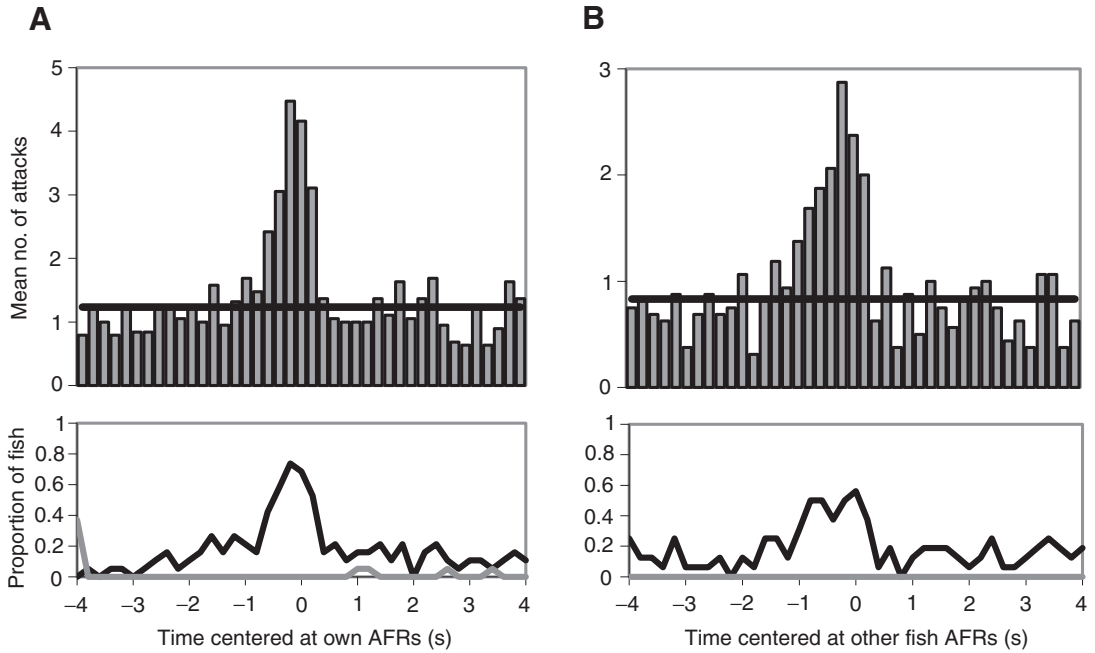
There was an error published in *J. Exp. Biol.* **211**, 1657-1667.

In Fig. 9B (top panel), an incorrect graph was published. Instead of the correct data, the data from Fig. 9A was inadvertently duplicated in Fig. 9B. The correct version of Fig. 9 is published below.



The authors apologise for any inconvenience caused and would like to reassure readers that this error does not influence any of the results or conclusions of the paper.

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Electrocommunication signals in free swimming brown ghost knifefish, *Apteronotus leptorhynchus*

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SUMMARY

Brown ghost knifefish, *Apteronotus leptorhynchus*, are a species of weakly electric fish that produce a continuous electric organ discharge (EOD) that is used in navigation, prey capture and communication. Stereotyped modulations of EOD frequency and amplitude are common in social situations and are thought to serve as communication signals. Of these modulations, the most commonly studied is the chirp. This study presents a quantitative analysis of chirp production in pairs of free-swimming, physically interacting male and female *A. leptorhynchus*. Under these conditions, we found that in addition to chirps, the fish commonly produce a second signal type, a type of frequency rise called abrupt frequency rises, AFRs. By quantifying the behaviours associated with signal production, we find that Type 2 chirps tend to be produced when the fish are apart, following periods of low aggression, whereas AFRs tend to be produced when the fish are aggressively attacking one another in close proximity. This study is the first to our knowledge that quantitatively describes both electrocommunication signalling and behavioural correlates on a subsecond time-scale in a wave-type weakly electric fish.

Key words: electrocommunication, weakly electric fish, chirps, abrupt frequency rises, behaviour.

INTRODUCTION

Many animals produce signals in a context-dependent fashion (Bradbury and Vehrencamp, 1998). A central goal of ethologists is to uncover why animals produce signals and reveal what functional significance they serve. We can examine the conditions under which an animal produces a given signal, or particular combination of signals, to shed light on factors that may influence or induce particular signalling behaviour (causes). In studying behaviours that may reflect signalling motivation, we can also observe any behavioural consequences of signal production in order to reveal the social relevance of different signal types (effects). Characterization of the actions associated with signal production has provided a means of evaluating whether communication occurs and has helped reveal the functions of signals in many animal systems (e.g. Hopkins, 1974; Crawford et al., 1986; Seyfarth and Cheney, 2003; Partan and Marler, 2005; Wong and Hopkins, 2007). Here, we take this approach to study electrocommunication signals in a species of weakly electric fish.

Brown ghost knifefish, *Apteronotus leptorhynchus*, are native to freshwater systems of South America and like all weakly electric fish, they both produce and detect electric signals (Moller, 1995). The strength of the generated electric signal is in the range of a few millivolts and is produced in a species-specific manner by specialized electrocytes that make up the electric organ. Because of its origin, the electric discharge produced by these fish is called the electric organ discharge, EOD. The EOD of *A. leptorhynchus* is emitted as a continuous quasi-sinusoidal wave. The EOD frequency (EODf) of *A. leptorhynchus* is sexually dimorphic and individually specific; males emit in the range of 800–1000 Hz, whereas females emit in the range of 600–800 Hz (Zakon et al., 2002; Zupanc, 2002; Dunlap and Larkins-Ford, 2003a). The fish are able to sense this self-generated signal and other electric signals in their environment *via* electroreceptors distributed over their skin surface. They use this combined motor-sensory system to navigate through their surroundings and find prey, a behaviour termed electrolocation (Nelson and MacIver, 1999; von der Emde, 2006). Weakly electric fish are also thought to use their electric sense for communication, specifically electrocommunication (Hagedorn and Heiligenberg, 1985; Zakon et al., 2002; Zupanc, 2002; Turner et al., 2007), the focus of this study.

Although the EOD is highly regular over time (Moortgat et al., 1998), stereotyped amplitude and frequency modulations are common in social situations. It is believed that these modulations serve as communication signals (Larimer and MacDonald, 1968). These modulations have classically been categorized into two broad categories: rises and chirps. Rises are characterized by an increase in the fish's EODf followed by an eventual decrease back to the baseline frequency, lasting from tens of milliseconds to minutes (Tallarovic and Zakon, 2002; Tallarovic and Zakon, 2005). Chirps are a second type of EOD modulation that tend to be shorter in duration (~20 ms), and are the most commonly studied signal type (Zakon et al., 2002).

Chirps have traditionally been defined as brief frequency excursions, and several subtypes have been identified. Although there remains controversy surrounding the categorization of chirps, we will be using the categorization scheme outlined by Zupanc and colleagues (Engler et al., 2000; Engler and Zupanc, 2001; Zupanc et al., 2006). Type 2 chirps are the most common type of chirp produced by *A. leptorhynchus*. They are 15–20 ms in duration and have frequency excursions of about 50–100 Hz. Type 1 chirps occur much less often than Type 2 chirps and they are characterized by a larger frequency excursion than Type 2 chirps, and a similarly short duration. Other longer duration chirp types have been described, called Types 3–6; however, these were found to be

produced at very low rates (Engler et al., 2000; Engler and Zupanc, 2001; Zupanc et al., 2006) and were not observed in the current study. Although chirps were first described in *A. leptorhynchus* by Larimer and MacDonald (Larimer and MacDonald, 1968), 40 years later very little is known about the social significance of these or other electrocommunication signals. Various experimental paradigms, involving both isolated fish ('chirp chamber' studies) and artificially interacting fish, have led to a number of conclusions regarding chirping behaviours (e.g. Zupanc and Maler, 1993; Triefenbach and Zakon, 2003; Kolodziejski et al., 2007). Although fundamental to our current understanding of chirping behaviour, there is a need to test if these behavioural patterns persist in naturally interacting fish (Dunlap and Larkins-Ford, 2003b).

The objective of the current study is to examine chirping in pairs of freely interacting male and female *A. leptorhynchus* and characterize the behaviours associated with the production of these signals. In addition to chirps, we observed that the fish commonly produce a type of frequency rise known as an abrupt frequency rise (AFR), a signal type that was first described by Engler and Zupanc (Engler and Zupanc, 2001) and also documented by Tallarovic and Zakon (Tallarovic and Zakon, 2005). This signal consists of a series of brief events, each with a frequency increase and subsequent decrease, produced in rapid succession, with variable duration and repetition number. Our observations of freely swimming fish allow us to analyze the behaviours that are associated with the production of different signal types. We characterized the behaviours associated with chirp and AFR production in *A. leptorhynchus* using three approaches. First, we examine the temporal sequence of signal production in order to reveal potential patterning, both within a fish and between fish. Second, we examine the relationship between signal production and aggressive encounters (attacks). Finally, we relate signalling with inter-fish distance to characterize further the behaviours associated with signal production in free-swimming *A. leptorhynchus*.

MATERIALS AND METHODS

The fish

We received our fish from a tropical fish supplier. A total of 13 mature *A. leptorhynchus* (Ellis, 1912) were assayed, including seven males and six females (mean length: 12.6±0.5 cm). Sex was determined by post-mortem gonadal inspection. The fish were housed individually or with up to three other tank mates prior to test trials. Tanks were kept at a temperature of 27–28°C and at a conductivity of 100–200 μS cm^{-1}. A light:dark cycle of 12 h:12 h was maintained. The fish were fed frozen bloodworms three times weekly. All experimental protocols were approved by the University of Ottawa Animal Care Committee (Protocol BL-192). Table 1 presents the individual characteristics of each of the fish tested and includes, for each fish, the sex, body length, mean EODf, the number of trials it was used in, and mean (±s.e.m.) Type 1 chirp, Type 2 chirp, AFR, and attack rates, averaged across all trials.

Experimental regime

We examined signal production in 21 different pairs of fish. For each trial only novel pairings were used; the two fish selected were housed in separate tanks and had not met following shipment. Table 2 presents, for each of the 21 trials, the identity of the two fish used, the difference in EODf between the interacting fish (the difference frequency, Df), the type of sex pairing, and Type 1 chirp, Type 2 chirp, AFR and attack counts for both of the fish examined.

Trials were performed in the dark in a 9.5 l tank measuring 30.0×17.0×13.5 cm. The water in the test tank was replaced with heated (26–27°C) water with a conductivity of 100–120 μS, between every trial or every second trial. To begin each trial, one fish ('fish 1' as listed in Table 2) was transferred from its home tank into the test tank. After 20 min a second fish ('fish 2') was added to the tank. Immediately upon introduction of the second fish into the test tank, 5 min electrical and video recordings of the interaction were taken. After 5 min of interaction both fish were returned to their respective home tanks. No effects of ordering on chirp or attack rates were found (paired *t*-test: P=0.72 and P=0.81, respectively). Fish were identified based upon their anatomical differences and EODf.

Video and electrical recordings

The EODs were recorded using two pairs of Teflon-coated silver-wire electrodes (diameter: 0.38 mm, insulated to the tip; WPI, Inc., Sarasota, FL, USA) positioned in opposite corners of the tank (Fig. 1A) and an AM Systems (Carlsborg, WA, USA) differential amplifier, model 1700 (amplified 10×, low frequency cut-off of 10 Hz, high frequency cut-off of 5 kHz). The signals were acquired at a sampling rate of 10 kHz using custom programs in Igor Pro (Wavemetrics, Inc., Portland, OR, USA). A grounded Teflon-coated silver-wire electrode (insulated to the tip) was attached to one corner of the test tank. The trials were recorded from above using a Sony video camera (model DCR-TRV 260) equipped with infrared illumination.

Table 1. Characteristics of individual fish used

Fish	Sex	Length (cm)	Mean EODf (Hz)	No. trials	Mean T1 chirp rate	Mean T2 chirp rate	Mean AFR rate	Mean attack rate
A	F	12.7	732±15.9	3	1.3±0.9	0.7±0.3	12.0±5.5	3.3±1.0
B	M	10.6	895±17.5	7	2.3±1.5	165.3±78.2	26.1±7.5	15±5.0
D	M	14.5	880±5.7	6	5.0±3.7	73.3±16.1	20.3±6.6	63.8±11
E	M	17.1	965±20.6	3	0	34.3±16.5	16.0±9.9	62.7±13.9
G	F	12.0	723±9.8	5	0	1.0±0.8	22.2±8.6	23.6±4.2
H	F	12.3	678	1	0	4	17	5
I	M	13.6	933	1	2	55	20	50
J	F	12.2	746±6.1	3	0.3±0.3	1.3±1.3	21.3±19.4	3.7±1.8
K	M	12.8	781±3.0	2	0	188.5±188	13.5±3.5	5.0±2.0
L	F	10.3	779	1	0	0	41	6
M	M	11.7	862±15.6	3	18±3.8	51.7±28.6	12.7±5.2	4.0±0.6
N	F	12.6	701±13.2	4	0	1.5±1.5	23.2±14.9	18.8±5.4
O	M	11.5	809±9.0	3	3.7±0.7	327.3±64.2	82.0±27.1	54±15.5

EODf, electric organ discharge frequency; AFR, abrupt frequency rise.

Signal characterization and identification of the signalling fish

For each of the 21 trials we created spectrograms using Matlab (window size 700 pts, NFFT 700, and overlap 600) and from these, by visual inspection, we recorded the times of all chirps and abrupt frequency rises (AFRs), and the fish producing each. In cases where the fundamental frequencies of the two fish were similar, the higher harmonics were used to identify the signalling fish.

Chirps were categorized as either Type 1 or Type 2 by visual inspection. A given fish produced at most two clearly distinct chirp types, which were distinguishable based upon their frequency excursion. Type 1 chirps had large frequency excursions whereas Type 2 chirps had notably smaller frequency excursions, comparable to the descriptions outlined by Zupanc and colleagues (Zupanc et al., 2006). All AFRs were lumped into one category and these were variable in terms of frequency excursion, repetition number, and duration. The electrical recordings from each trial were converted into an audio format. These audio files were played and chirps were counted (Dulka and Maler, 1994; Dunlap, 2002) and the timing of each recorded for each of the first five trials. These counts were compared to the chirp counts obtained from spectrogram analysis to assure the reliability of our method (regression: R^2=0.9998, P<0.001).

Table 2. Details of the 21 trials analyzed in this study

Trial	Fish 1	Fish 2	Df (Hz)	Pairing type	T1 chirps*	T2 chirps*	AFRs*	Attacks*
1	D	E	–78	MM	23, 0	20, 56	17, 0	16, 79
2	E	A	242	MF	0, 1	2, 2	14, 17	74, 2
3	B	D	74	MM	2, 0	71, 80	29, 29	1, 62
4	E	B	34	MM	0, 0	45, 34	34, 1	35, 1
5	D	A	163	MF	2, 3	73, 1	7, 18	91, 5
6	A	B	–199	FM	0, 1	0, 31	1, 11	3, 38
7	G	D	–143	FM	0, 1	4, 141	17, 11	9, 71
8	H	G	–46	FF	0, 0	4, 0	17, 31	5, 24
9	I	B	8	MM	2, 2	55, 620	20, 61	50, 6
10	G	J	–36	FF	0, 0	0, 0	7, 60	25, 7
11	K	L	5	MF	0, 0	376, 0	17, 41	7, 6
12	M	B	28	MM	11, 0	40, 126	21, 40	4, 29
13	D	N	208	MF	4, 0	56, 6	49, 15	90, 4
14	O	B	–33	MM	5, 0	455, 101	59, 19	46, 13
15	J	N	38	FF	1, 0	4, 0	4, 11	3, 22
16	J	O	–57	FM	0, 3	0, 275	0, 51	1, 32
17	M	N	145	MF	19, 0	9, 0	3, 0	3, 19
18	G	B	–139	FM	0, 11	0, 174	5, 22	35, 17
19	K	D	–107	MM	0, 0	1, 70	10, 9	3, 52
20	N	G	13	FF	0, 0	0, 1	67, 51	30, 25
21	O	M	–15	MM	3, 24	252, 106	136, 14	84, 5

Df, difference frequency (fish 1 – fish 2); AFR, abrupt frequency rise.
*(fish 1, fish 2).

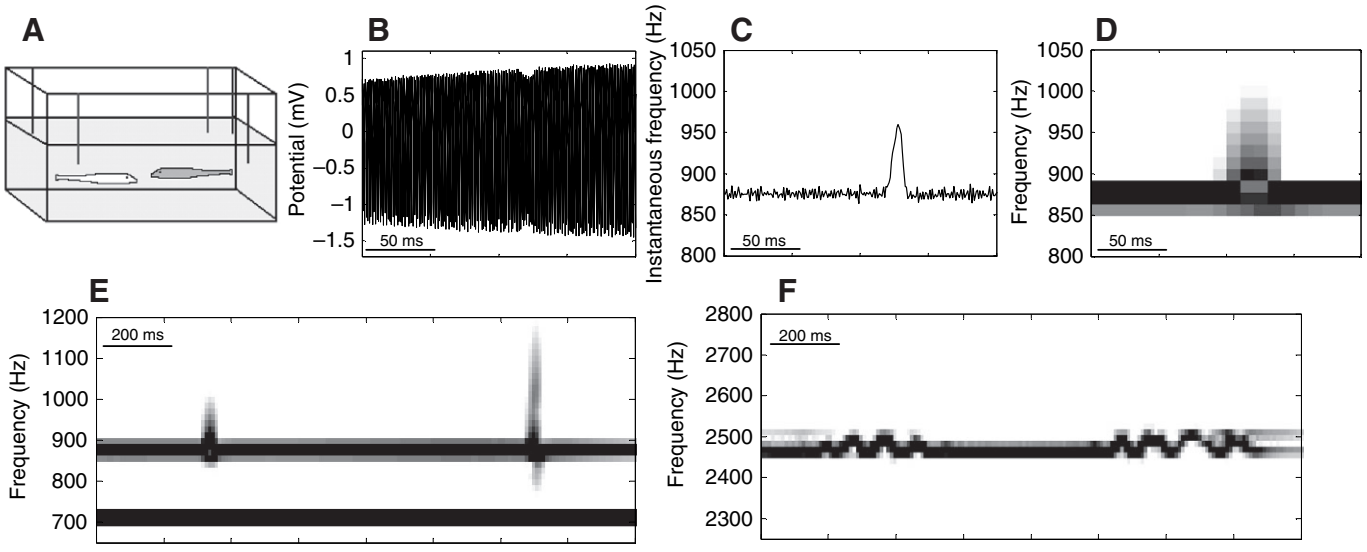

Fig. 1. (A) Experimental design; the short, vertical lines indicate the position of the recording electrodes in the 9.5 l tank, the additional short, vertical line in the back right corner indicates the position of the ground electrode. (B) Oscillogram of a representative Type 2 chirp recorded from an isolated fish. Note that its duration, indicated by the period of amplitude modulation, is 15–20 ms. (C) An instantaneous frequency plot corresponding to the same Type 2 chirp as illustrated in B. Instantaneous frequency values were derived by taking the inverse of the cycle length, calculated as the duration between consecutive downstroke zero-crossings. (D) A spectrogram displaying the same Type 2 chirp as illustrated in B,C). (E) A spectrogram showing a representative Type 2 chirp (on the left) and a Type 1 chirp (on the right) recorded during a dyadic interaction. The higher frequency of two fish (877 Hz) is modulating its electric organ discharge frequency (EODf) to produce these chirps, whereas the lower frequency fish (714 Hz) does not modulate its EODf during this segment of the interaction. Note that the Type 2 chirp is associated with a much smaller frequency excursion than is the Type 1 chirp. (F) Spectrogram showing two abrupt frequency rises (AFRs) produced in succession by the lower frequency of two interacting fish; the third harmonics are shown and during this segment of the recording the EOD of the higher frequency fish is relatively weak, allowing for a clearer representation of the EOD modulations of the other. Both of the AFRs shown consist of multiple distinct and consecutive small frequency rises. For display purposes, the low amplitude components of each spectrogram were removed and only the strongest (10–20%) amplitude components are shown.

Behavioural analysis

From the recorded videos of the interactions we noted the times of all observable attacks. Attacks included all open jawed biting behaviours and all high-speed lunges directed at a conspecific (Heiligenberg, 1973). For each trial we also tracked the position of each fish in the tank, again as viewed from above, using Videopoint software (Lenox, MA, USA). Frames of every 200 ms were analyzed. The distance from the head of one fish to the head of the other fish was used as a functional indication of the distance between the two fish, because most bites and lunges were initiated by, and largely directed at, this region.

Characterization of signal patterning

To characterize the temporal patterning of signal production we created correlograms relating the production of one signal with the production of a second signal type. For each trial, we created signal-centred histograms in which the counts pertaining to a second signal type were plotted in 200 ms bins, for 4 s prior to and following the production of the first reference signal. The histograms were then averaged over all trials to create correlograms for each comparison. Those that differed significantly from a flat distribution (the null distribution expected if signals are not temporally correlated), determined using repeated measures ANOVA (RM ANOVA) on ranks ($P<0.05$), were analyzed on a bin by bin basis (see Statistical analysis section). Trials in which fish produced less than 10 of a particular signal were omitted from the correlogram analysis. Although some fish were used in multiple trials, we are assuming independence between trials because all trials involve novel pairings.

To assure that the patterns we observed between two time series were not due to patterning within a given signal time series, we created 'reversed' time series for one of the two signals being considered for each correlogram, and recalculated the averaged correlograms in the same fashion as for the unmodified time series. To create reversed time series, we simply switched the first half of one trial (time=0–150 s) with the second half of the same trial (time=150–300 s). This reversed time series has the same autocorrelation function as the original, but is not temporally linked to the production of any other signal. In all cases, the reversed-time-series correlograms were not significantly different than the ones expected if all events occur randomly (RM ANOVA on ranks: $P>0.05$ for all cases), thus verifying that all patterns we observed in the correlograms were not artifacts of non-random patterning in the individual signals.

In a similar way, we created correlograms plotting the attack rate of one fish centered at a given signal type, for 4 s before and after, again in 200 ms bins. The same statistical analyses were performed to identify relationships between attack rate and signal production.

Statistical analyses

Summary data are expressed as means ± s.e.m., unless otherwise indicated. Linear regressions were used to characterize different parameters (EODf, Df and time) associated with average signal production rates. In all cases, an F-test was performed on the slope with $P<0.05$ as the threshold for statistical significance.

With respect to the correlograms, to determine significance on a bin-by-bin basis, we calculated a P value for each bin in each correlogram. To do this, for each correlogram we randomly shuffled one of the two signal (or attack) time series being considered and created a histogram relating this shuffled time series with the un-shuffled one. We repeated this procedure one thousand times and used the resulting distributions of counts in each bin to directly calculate a P value for any particular bin count in a given comparison. We then determined, for each bin, the fraction of all fish in all trials having significant P values (less than 0.05 or greater than 0.95) and plotted these below the corresponding averaged correlogram.

RESULTS

Description of signals

A total of 3409 chirps were recorded during the 21, 5 min trials. Chirps were classified as Type 1 (N=118) or Type 2 (N=3291) as described in the Materials and methods section. For illustrative purposes, we show a typical Type 2 chirp, recorded from an isolated fish, as an oscillogram (Fig. 1B), an instantaneous frequency plot (Fig. 1C) and a spectrogram (Fig. 1D). In addition, a typical Type 1 and Type 2 chirp, recorded during a dyadic interaction, are shown in Fig. 1E (a small Type 2 chirp first, followed by a larger Type 1 chirp; both produced by the higher frequency of the two interacting fish). Based upon the oscillograms and spectrograms of chirp recordings, we propose that these two signal types recorded from our free swimming males and females are the same as those defined by Engler and Zupanc (Engler and Zupanc, 2001). They show comparable amplitude decreases and frequency excursions. We did not observe any chirps with a duration greater than 15-20 ms. In the spectrogram, they may appear to last longer than this because of time smearing resulting from the spectrogram overlap parameters (compare for example the oscillogram, instantaneous frequency plot, and spectrogram of the Type 2 chirp shown in Fig. 1B,C,D); these parameters were optimized to provide sufficient resolution of both time and frequency.

In addition to these chirps, AFRs were produced in abundance by the fish under these experimental conditions. Two representative AFRs are shown in Fig. 1F; both are produced by the lower frequency of the two interacting fish (the third harmonics are shown on the spectrogram). At the time of the AFRs, the higher frequency fish's EOD signal is weak, allowing us to better illustrate the lower frequency fish's frequency modulations. The AFRs shown here consist of distinct and consecutive frequency rises produced in rapid succession. We recorded a total of 1046 AFRs during the dyadic interactions. They are of variable duration, lasting from tens to hundreds of milliseconds (mean duration: 404±4.3 ms). Although this signal type has been described as an AFR in only one study prior to this (Engler and Zupanc, 2001), we believe that it is the same signal type that Tallarovic and Zakon (Tallarovic and Zakon, 2005) describe as a short duration rise. We have found that AFRs are produced extensively during *A. leptorhynchus* social interactions. Furthermore, our results suggest that these signals are produced in a context-dependent fashion (see following sections), lending strength to the proposition that they are a distinct class of electrocommunication signals produced by *A. leptorhynchus*.

Long term changes in frequency, as well as frequency jamming behaviours (Tallarovic and Zakon, 2005) were observed infrequently, and were very variable in terms of frequency excursion and duration. We observed 48 frequency rises which, unlike AFRs, involved more gradual frequency modulations (over seconds and tens of seconds), and hence fit into the category of a gradual frequency rise [GFR (Tallarovic and Zakon, 2002; Serrano-Fernández, 2003; Tallarovic and Zakon, 2005)]. These longer, more gradual frequency rises, were produced infrequently during our studies and thus will not be discussed further.

Signalling rates as a function of gender, EODf and Df

Many researchers have pointed out that there is a need to compare the behaviour of fish observed under artificial experimental conditions with that of freely interacting fish (e.g. Dunlap and

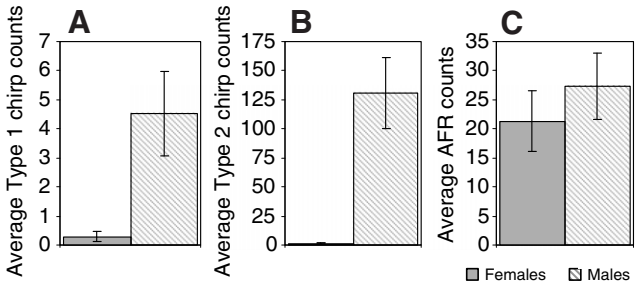

Fig. 2. Type 1 chirps (A) and Type 2 chirps (B) are produced at sexually dimorphic rates. AFRs (C) are not produced at sexually dimorphic rates. Values are means (± s.e.m.) for males and females, averaged across all trials (*N*=42).

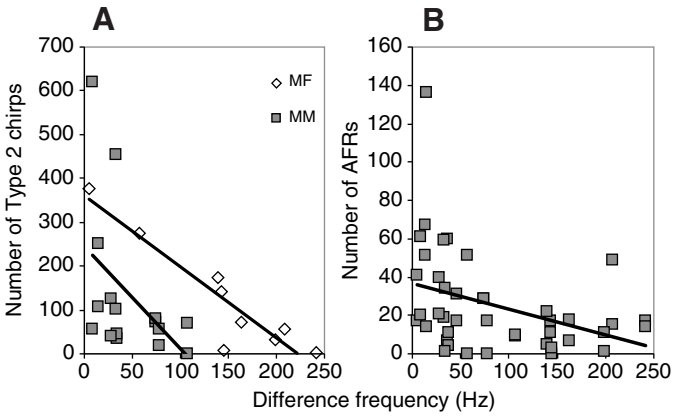

Fig. 3. The effect of the absolute difference frequency (Df) on signal production rates for Type 2 chirps (A) and AFRs (B). (A) Type 2 chirps are classified by sex pairing; the Type 2 chirp rates of males paired with a second male (squares; *N*=16, R^2=0.21), and of males paired with a female (diamonds; *N*=9, R^2=0.860). (B) AFRs in which all fish were pooled because there were no differences across the different sex pairings (*N*=42, R^2=0.14). Linear regressions are shown.

Larkins-Ford, 2003b). In this section, we compare the mean chirp rates of free swimming fish with those reported previously using chirp chambers and other experimental conditions. We examine how the chirp rates are influenced by gender, EODf and the difference in EODf of the interacting fish (the difference frequency, Df). For comparison, we analyze AFR production in the same manner.

Effect of gender

It is well established that chirping is a sexually dimorphic behaviour in *A. leptorhynchus* (Maler and Ellis, 1987; Dye, 1987; Dulka and Maler, 1994; Dulka et al., 1995; Dunlap and Larkins-Ford, 2003a; Kolodziejski et al., 2004). We found a similar pattern in free-swimming conditions: males produce significantly more chirps (Type 1 and Type 2 chirps) than females (Fig. 2A,B; Mann–Whitney rank sum test: T_{17}=262, *P*=0.01; and T_{17}=162.5, *P*<0.001, respectively). Furthermore, we found that under our conditions both genders predominantly produce Type 2 chirps, similar to previously reported behaviour in isolated fish (Engler et al., 2000), in chirp chamber studies (Engler and Zupanc, 2001) and in fish interacting electrically (Zupanc et al., 2006). Because Type 1 chirps were produced so infrequently, it is difficult to gain any further insight into the factors controlling their production. So, we will focus mainly on Type 2 chirps in the following sections, and restrict this to chirps produced by males because females chirped so infrequently.

Contrary to the sexual dimorphism seen with respect to chirping, we found no sexual dimorphism in the average AFR rates of males and females tested under these conditions (Fig. 2C; Mann–Whitney rank sum test: T_{17}=333.5, *P*=0.42).

Effect of EOD frequency and difference frequency

Previous studies have shown that chirp production rates in males are proportional to the EOD frequency (EODf) and inversely proportional to the difference frequency (Df) between a playback signal and EODf (Zupanc and Maler, 1993; Bastian et al., 2001; Dunlap, 2002; Dunlap and Larkins-Ford, 2003b; Kolodziejski et al., 2007). We found that Type 2 chirp rates were not significantly correlated with EODf in males (regression: R^2=0.10, *P*=0.12). However, we found that Type 2 chirp rates were negatively correlated with Df, independent of the sign of Df (Fig. 3A; regression: R^2=0.20, *P*=0.03). Interestingly, although males chirped with a comparable mean rate when paired with males or with females (137.8±42.5 *vs* 130.9±42.5 chirps per trial, respectively), the mean Df was smaller for trials in which males were paired with another male (47.1±8.4 Hz) than when paired with a female (144.6±24.7 Hz). From Fig. 3A we can see that for any given Df, males tend to chirp at lower rates when paired with a male than when paired with a female.

A similar analysis of AFR rates revealed no relationship with EODf (regression: R^2=0.03, *P*=0.32). However, when all fish are considered together we find that AFR rates are negatively correlated with the absolute Df (Fig. 3B; regression: R^2=0.14, *P*=0.01). In general, in agreement with previous studies (Zupanc and Maler, 1993; Bastian et al., 2001; Dunlap, 2002; Dunlap and Larkins-Ford, 2003b; Kolodziejski et al., 2007), our results suggest that fish tend to produce chirps and AFRs predominantly when interacting with a fish whose EODf is similar to its own.

Signalling rates over time: chirp rates increase with time

Many studies have reported that the chirp rates of male and female *A. leptorhynchus* tend to habituate over time, both in chirp chambers (Dunlap, 2002; Dunlap and Larkins-Ford, 2003b) and in fish communicating through a perforated barrier (Dunlap and Larkins-Ford, 2003b). Contrary to this, over a similar time period, we found that the Type 2 chirp rates of free-swimming male fish did not decrease; instead, they increased significantly as the trial progressed (Fig. 4A; regression: R^2=0.71, *P*=0.002). During this same time period, attack counts decreased significantly (Fig. 4B; regression: R^2=0.63, *P*=0.05); although the greatest decrease in attack rate occurred over the first minute of the interaction. This suggests a negative relationship between Type 2 chirp rates and attack rates; as chirp rates increase, attack rates decrease. We will investigate this relationship further in a following section. In contrast to Type 2 chirp rates, Type 1 chirp and AFR rates did not change significantly with time (regression: R^2=0.08, *P*=0.44; R^2=0.29, *P*=0.11, respectively).

Non-random signal production rates by individual fish

It has been reported that male *A. leptorhynchus* tested under a variety of conditions tend to produce chirps in a non-random, 'bursty' fashion (Bullock, 1969; Zupanc and Maler, 1993; Engler and Zupanc, 2001; Zupanc et al., 2006). Zupanc et al. (Zupanc et al., 2006) quantified this bursty chirping behaviour in male fish, interacting between adjacent PVC tubes, using inter-chirp interval histograms. They found that chirps tend to follow one another at a preferred latency of approximately 500 ms.

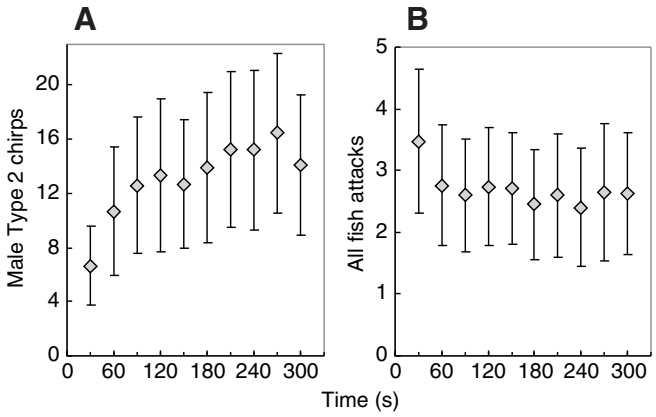

Fig. 4. Chirp (A) and attack (B) rates change with time. Mean (± s.e.m.) chirp and attack counts, binned into 30 s intervals, are shown. With respect to Type 2 chirps, only male counts were included (N=25); whereas for attacks, all fish were pooled (N=42).

We performed a similar analysis on the signals produced by male and female free-swimming interacting *A. leptorhynchus* in order to reveal patterns in the signalling of individual fishes. With respect to male Type 2 chirps, we found a pattern of 'burstiness' and a preferred latency period comparable to those reported by Zupanc and colleagues (Zupanc et al., 2006). Fig. 5 shows an interchirp interval histogram for all males across all trials. It suggests that males tend to produce chirps in a bursty fashion with a preferred latency of 400–600 ms. Fig. 6A (top panel) shows the averaged auto-correlogram corresponding to the male Type 2 chirp sequences (unlike the interchirp interval histogram, this analysis considers both first order and higher order inter-chirp latencies). Because the pattern in the auto-correlogram deviates from a flat (null or random) distribution, it clearly indicates that males chirp in a non-random fashion (RM ANOVA: χ^2_{40}=266.2, P<0.001). The lower panel shows the fraction of comparisons in which a given bin count (in the correlogram) is significantly greater than (black line P>0.95), or less than (grey line P<0.05) that expected by chance. In a large number of cases the chirp rates are much less than expected in the period immediately following a chirp event (grey line). This is followed by a period with an increased tendency to chirp, indicated by the peaks in the auto-correlogram and bottom panel (black line).

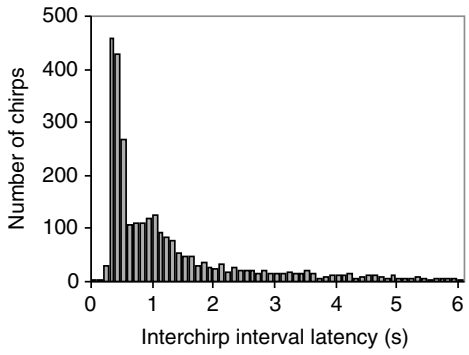

Fig. 5. Inter-chirp interval histogram with totals for all males tabulated over all trials (N=24, one male was excluded because he only produced one chirp). The preferred inter-chirp latency is 400–600 s.

AFRs are also produced in a non-random fashion. The AFR auto-correlogram and bottom panel (Fig. 6B) show that the probability of AFR production is reduced for a short time following the first AFR and increases to a peak at a preferred latency of 400–800 ms (RM ANOVA: χ^2_{40}=241.7, P<0.001). This suggests that AFRs, like chirps, tend to be produced in bursts, with a similar preferred latency.

Interestingly, not only are signals correlated in time with themselves, the patterns of two distinct signal types produced by a given fish are also correlated. The probability that an individual fish produced an AFR just before or after it produced a Type 2 chirp is significantly lower than chance (Fig. 6C; RM ANOVA: χ^2_{40}=169.1, P<0.001) and this trend is significant in about 40% of individuals (Fig. 6C lower panel, grey line). Overall, this patterning suggests that the fish tend to produce these different signals under different conditions, at different times during an interaction. In the following sections we show that there are different contextual factors associated with the production of the different signal types.

Signal production between two interacting fish is not independent

A previous study of *A. leptorhynchus* males interacting electrically but confined to separate tubes, has provided evidence for a so-called 'echo response' (Zupanc et al., 2006), such that chirps produced by one fish followed chirps produced by another with a preferred latency. We investigate the relationship between chirp rates in physically interacting fish by means of an inter-signal cross-correlogram, a histogram describing Type 2 chirp production in one fish at different times before and after another fish's Type 2 chirp. The cross-correlogram for male–male interactions is significantly different from that expected if the two fish chirped independently (Fig. 7A; RM ANOVA: χ^2_{40}=100.4, P<0.001). The fish tend not to chirp at the same time: the chirp rate of one fish at the time when the other is chirping (t=0) is much less than expected by chance in more than 70% of trials (Fig. 7A lower panel, grey line). In addition, the chirp rates in adjacent time bins are significantly greater than expected by chance (illustrated by the peaks in the black line, Fig. 7A lower panel), reflecting an 'echo response' at a 200–600 ms latency.

We performed similar analyses on AFRs. Cross-correlograms for AFR production of one fish relative to that of the other fish show that AFRs are not produced independently (Fig. 7B; RM ANOVA: χ^2_{40}=114.1, P<0.001), but rather, are produced concurrently. This suggests that either the two fish are responding at a very short latency with AFRs in response to their partner's AFRs, or that shared conditions or features of the interaction trigger AFR production in both fish at the same time (both fish may produce AFRs during aggressive situations for example).

In addition to interactions with signals of the same type, we also found a relationship between the patterning of AFRs of one fish relative to Type 2 chirps produced by the other fish. As indicated by the trend in the averaged correlogram (Fig. 7C; upper panel), one fish tends not to produce AFRs at the same time as the other fish is producing Type 2 chirps (RM ANOVA: χ^2_{40}=105.7, P<0.001). Although this trend is significant in a smaller proportional of fish considered, the rate of AFR production in one fish was consistently lower than expected at the time the other fish produces chirps.

Overall, this signal patterning analysis provides a quantitative measure of the temporal interactions in chirp behaviour. Further, it reinforces the idea that Type 2 chirps and AFRs are true

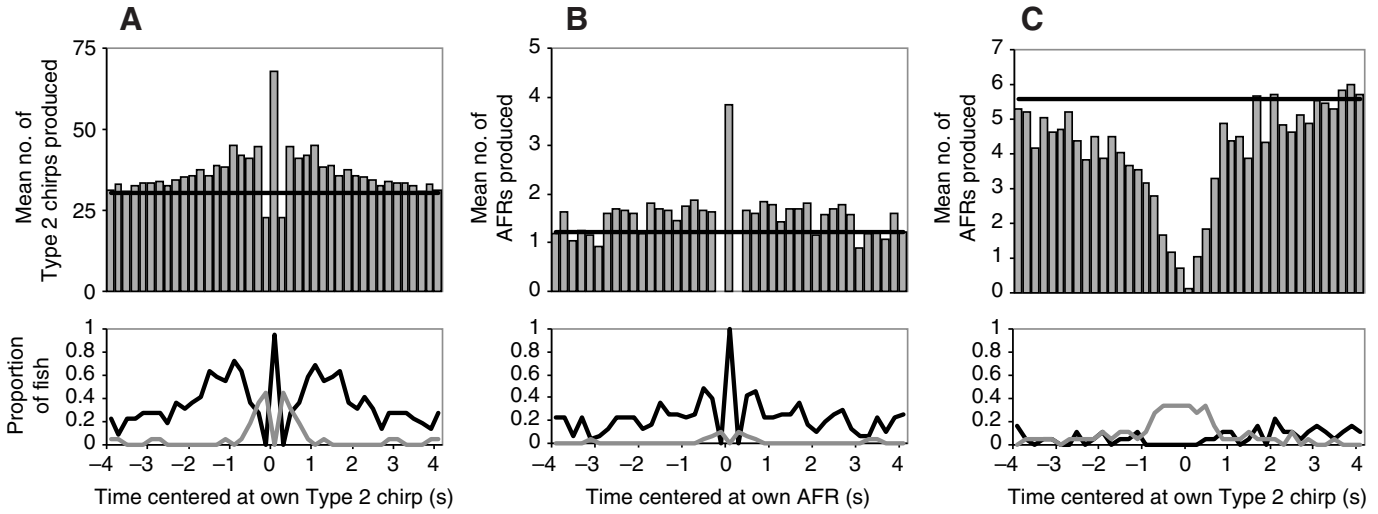

Fig. 6. Upper panels: auto-correlograms illustrate signal patterning of a single fish for (A) Type 2 chirps (*N*=22), (B) AFRs (*N*=31) and (C) Type 2 chirps and AFRs (*N*=18). Means for all fish for all trials in which fish produced 10 or more signals are plotted, except in the case of Type 2 chirps where only male counts are used in the analysis. The horizontal line denotes the null distribution: the distribution expected if signals are produced at random. Lower panels: bin-by-bin statistical analysis of the correlograms. The fraction of individual fish in which the corresponding bin height is either greater than expected by chance (P>0.95, black line) or less than expected by chance (P<0.05, grey line).

communication signals that are produced in bursty temporal patterns, influence the signalling behaviour of interacting conspecifics, and tend to be produced at different times during social interaction.

Attack rates are correlated with signalling

Many researchers have referred to chirps as aggressive signals (Bullock, 1969; Maler and Ellis, 1987; Dunlap and Larkins-Ford, 2003b; Triefenbach and Zakon, 2003). In order to investigate this possibility, we have quantified the temporal relationships between attack behaviours and signal production using cross-correlograms. Fig. 8A (top panel) shows that a fish's own attack rate is decreased near the time it produces a Type 2 chirp (RM ANOVA: χ^2_{40}=166.5, P<0.001). When we look at the bin-by-bin analysis (bottom panel), it is clear that in more than 40% of fish, attack rates are significantly lower than expected just prior to and following a Type 2 chirp (grey line).

When we consider interactions between fish, we see that chirping in one fish is associated with a decreased attack rate by the other fish (Fig. 8B; RM ANOVA: χ^2_{40}=76.8, P<0.001) and the trend is significant in over half of the trials considered. Fish tend to chirp after periods of low aggression, when both its own attack rate and that of the interacting conspecific are lower than expected.

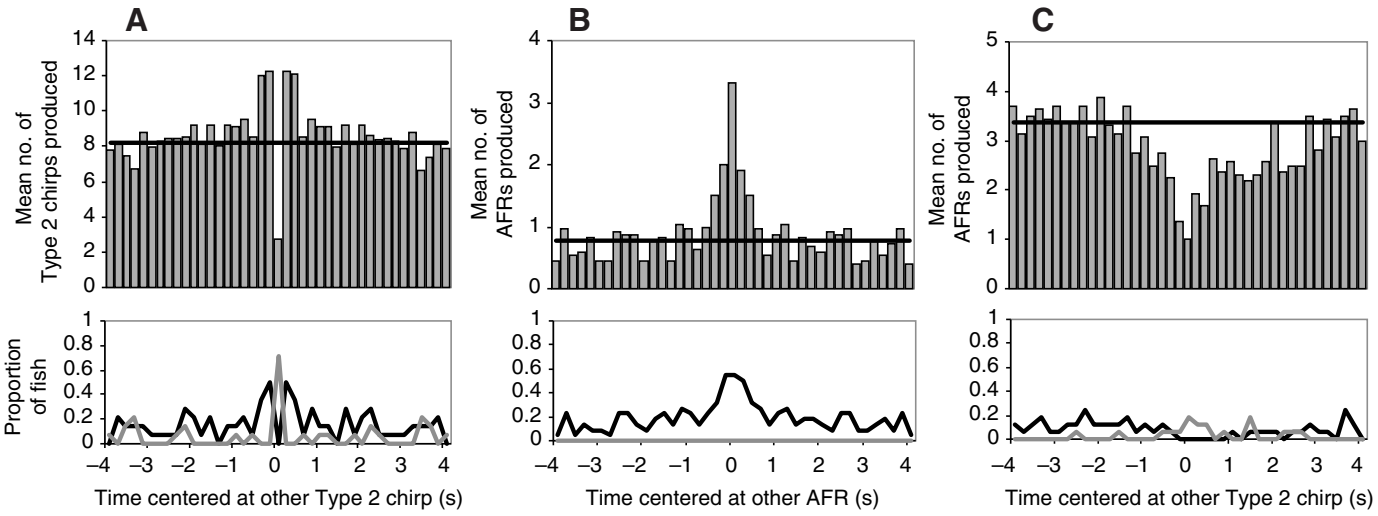

Fig. 7. Upper panels: cross-correlograms illustrate signal relationships between fish for (A) Type 2 chirps (*N*=14), (B) AFRs (*N*=22), and (C) Type 2 chirps and AFRs (*N*=16). Means for all fish for all trials in which fish produced 10 or more signals are plotted, except in the case of Type 2 chirps where only male counts are used in the analysis. Horizontal line denotes the null distribution. Lower panels: bin-by-bin statistical analysis of the correlograms. The fraction of individual fish in which the corresponding bin height is either greater than expected by chance (P>0.95, black line) or less than expected by chance (P<0.05, grey line).

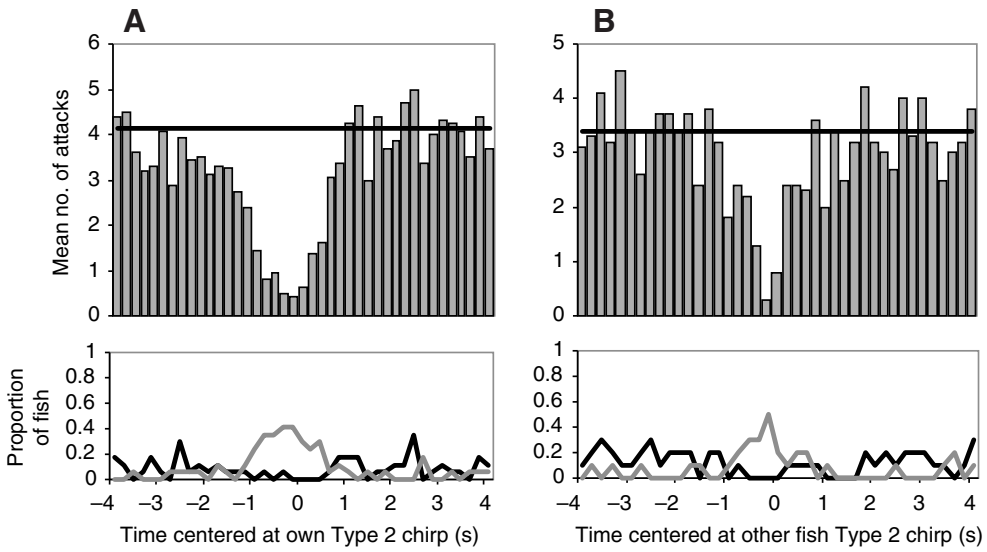

Fig. 8. Upper panel: cross-correlogram relating Type 2 chirp production with attack counts are given for (A) a single fish (*N*=16) and (B) between fish (*N*=10). Only male fish, and trials involving 10 or more chirps and attacks are used in the chirp-attack analysis. Horizontal line denotes the null distribution. Lower panels: bin-by-bin statistical analysis of the correlograms. The fraction of individual fish in which the corresponding bin height is either greater than expected by chance (*P*>0.95, black line) or less than expected by chance (*P*<0.05, grey line).

Contrary to the relationships observed for chirps, attack rate is highest at the time of AFR production in both the signalling fish (Fig. 9A; RM ANOVA: χ^2_{40}=138.1, *P*<0.001) and in the fish with which it was interacting (Fig. 9B; RM ANOVA: χ^2_{40}=115.4, *P*<0.001). In other words, a fish tends to produce AFRs at the same time that it attacks (significant in about three-quarters of fish considered, Fig. 9A; black line, lower panel), but also when it is being attacked (Fig. 9B), suggesting that AFRs may be aggressive signals used frequently during agonistic encounters.

Signal rates are correlated with inter-fish distances

As would be expected from our analysis of attack rates, the average distance separating the interacting fish also was related to the time of production of both Type 2 chirps and AFRs. The head-to-head distance increases prior to, and decreases immediately following, chirp production, with a maximum close to the time of the chirp (Fig. 10A). Alternatively, AFRs tend to be produced when the fish are nearest each other (Fig. 10B). These distance trends reinforce the conclusions of the attack correlogram analyses, and further suggest that chirps may be produced at a distance to deter aggressive behaviours whereas AFRs serve as proximity signals that accompany aggressive behaviours.

DISCUSSION

Neuroethological approaches have been very successfully applied towards the understanding of electrosensory-mediated behaviour in weakly electric fish (e.g. Heiligenberg, 1991). Because appropriate context is critical for the production of natural behaviours, such studies must be undertaken in the most natural context that is feasible under the given experimental constraints. In *A. leptorhynchus*, various experimental settings have been used to investigate putative electrocommunication signals, but none before the present study has provided a detailed temporal analysis of signalling between two freely interacting wave-type fish. Using spectrograms to separate the signals of each individual fish and cross-correlation analyses, we have provided further quantitative evidence for the existence of electrocommunication, as well as a first step towards understanding the meaning of the underlying signals. A complete neuroethological description of any physiological process requires both a neural and behavioural description of the phenomenon. Descriptions of the behavioural relevance of the electrocommunication signals produced by *A. leptorhynchus*, such as we are presenting here, will be necessary for elucidating the roles of neural circuits and modulators involved in electrosensory processing and signal production.

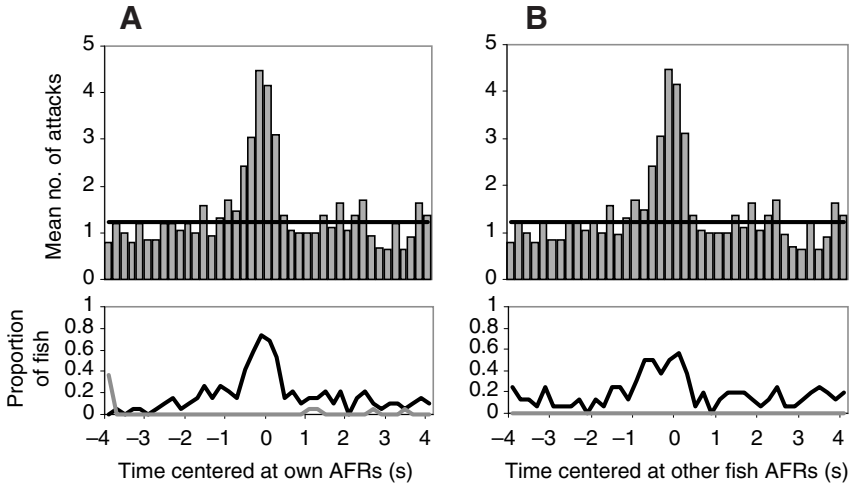

Fig. 9. Upper panel: cross-correlogram relating AFR production with attack counts are given for (A) a single fish (*N*=19) and (B) between fish (*N*=16) for all trials involving 10 or more AFRs and attacks. Horizontal line denotes the null distribution. Lower panels: bin-by-bin statistical analysis of the correlograms. The fraction of individual fish in which the corresponding bin height is either greater than expected by chance (*P*>0.95, black line) or less than expected by chance (*P*<0.05, grey line).

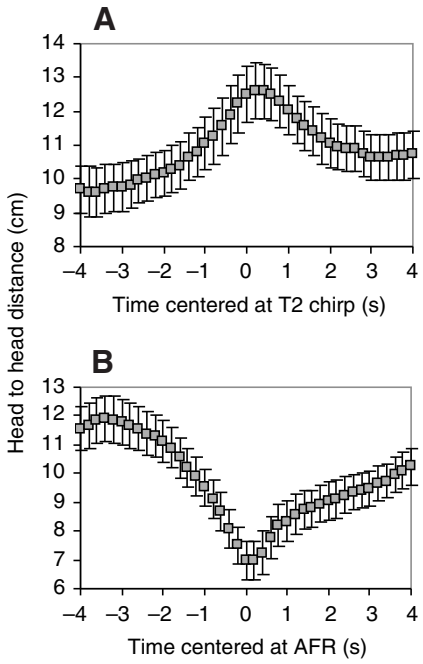

Fig. 10. Inter-fish distance centred at the time of signal production for (A) Type 2 chirps (*N*=25) and (B) AFRs (*N*=39). The inter-fish distances represent the distance from one fish's snout to the other fish's snout. Means ± s.e.m. are plotted for males only in the case of Type 2 chirps, and all fish in the case of AFRs.

Signalling behaviour: free-swimming *versus* constrained fish

Our study in free swimming *A. leptorhynchus* showed that some features of chirp behaviour are preserved between these and other experimental conditions. One feature of chirping that we confirm, and is consistent across many experimental regimes, is the sexual dimorphism in chirp rates (Maler and Ellis, 1987; Dye, 1987; Dulka and Maler, 1994; Dulka et al., 1995; Dunlap and Larkins-Ford, 2003a). Male chirp rates are much higher than female chirp rates (Fig. 2). A second relationship, previously described and confirmed in our study, is the effect of difference frequency, Df, on chirp rates (Maler and Zupanc, 1993; Bastian et al., 2001; Dunlap and Larkins-Ford, 2003b; Kolodziejski et al., 2007). There is an inverse relationship between Type 2 chirp rates of freely interacting male *A. leptorhynchus* and the difference in frequency between its own EODf and that of the fish with which it is paired (Fig. 3). This relationship persists when absolute Df is considered (Bastian et al., 2001). This implies that in any given pairing the lower and higher frequency fish tend to chirp at approximately the same rate; a rate influenced by the magnitude of the difference in the EODf of the two fish. This strong relationship between Df and Type 2 chirp rate can explain why the relationship between EODf and chirp rate is less apparent. Another point to consider with respect to the relationship between Df and chirp rates is how these signals are detected or perceived by conspecifics. Type 2 chirps and AFRs are produced most often when Dfs are small, and interestingly, this is also the range in which electroreceptor afferents most effectively encode small Type 2-like chirps (Benda et al., 2006). Our results clearly indicate that although fish chirp at the highest rate to fish whose EODf is similar to its own, they also respond to chirps when the Df is much greater. Thus there are likely to be additional mechanisms involved in the detection and encoding of chirps in cases when the Df is large (G.J.H., J.E.L. and J. Benda, manuscript in revision).

An important difference between the chirping behaviour of fish tested under previous experimental conditions and our results involves how chirp rates change with time. Fig. 4 shows that the rate of Type 2 chirp production increases significantly with time. This increase is contrary to what has been reported for chirp production in the past (Dunlap and Larkins-Ford, 2003b). It is possible that the decrease in chirp rates, reported in such studies, is at least in part a result of the fish habituating to unrealistic stimuli. In our experiments, the fish do not decrease chirp rates over time, presumably because multimodal sensory cues are present because of a dynamic interaction involving a real fish. Importantly, Dunlap and Larkins-Ford (Dunlap and Larkins-Ford, 2003b) report that chirp rates actually decrease in free-swimming fish. We suggest that this difference may be attributable to their use of a much larger tank in which the fish could separate themselves (Dunlap and Larkins-Ford, 2003b), whereas we used a relatively small tank which forced interaction.

Differences in chirping behaviour with that observed in previous studies suggest that simply being able to physically interact changes the chirping behaviour of *A. leptorhynchus*. Bullock (Bullock, 1969) used different stimulation techniques and fish models to evoke chirping in *A. leptorhynchus* and found that visual cues influenced chirping behaviour. Our study provides further evidence that interactions are an important aspect of shaping the natural signalling behaviour.

Perhaps the most important difference between the electrocommunication behaviour of freely interacting fish compared to those tested using previous experimental designs is the abundance of AFRs that we observed. It appears that features of natural, aggressive physical interactions are necessary to motivate AFR production in this species. Thus, these behavioural considerations will be necessary in future studies aimed at understanding the meaning of AFRs and the biophysical basis of their generation.

Behavioural correlates

Communication signals, by definition, must transfer some form of information from the sender to the receiver (Bradbury and Vehrencamp, 1998; Griffin, 2001). Evaluating this transfer of information is an obvious difficulty confronting ethologists because we are limited to drawing conclusions based upon an animal's observable behaviours. Thus, communication can be evaluated in terms of how an individual's behaviour is affected by signals emitted by another. It is important to ask what behaviours can be monitored in freely swimming fish that may also be related to signal production. As a step towards solving this problem, we have characterized signal production, attacks and inter-fish distance using correlation analyses.

Signal patterning

Our study has shown that in *A. leptorhynchus*, both chirps and AFRs are produced in a nonrandom, bursty fashion (Figs 5, 6). Also, through cross-correlation analysis, we provide evidence that the signals produced by one fish influence signal production in an interacting conspecific (Fig. 7). The echo response reported here has been observed in both pulse (Moller, 1995) and in wave-type weakly electric fish (Zupanc et al., 2006); in both cases, the fish tend to respond with a species-specific preferred latency. In *A. leptorhynchus*, the latency is much longer than processing by a reflexive sensory-to-motor neural pathway would suggest (Heiligenberg, 1991), so it is possible that higher level decision

making is involved in shaping this electrocommunicatory behaviour. In future studies, experimental design can be controlled more specifically to determine the nature of this decision-making process.

Signalling and attack behaviours

Additionally, our results suggest that different types of signals are produced under different contexts and hence probably serve distinct social roles. In the *A. leptorhynchus* literature, chirps are often referred to as aggressive signals. These assertions are based on experiments which show that males tend to chirp more in response to EOD mimics similar in frequency to their own (Maler and Ellis, 1987; Bastian et al., 2001), and thus more representative of male–male interactions (because of the sexual dimorphism in EODfs). Thus, because male–male interactions tend to be aggressive in nature, it has been suggested that Type 2 chirps are agonistic signals (Zupanc et al., 2002). Although chirps do occur during aggressive encounters, here we show that on a smaller time scale, chirps do not often occur during attack behaviours. Our analyses of attack rates (Figs 8, 9) suggest that Type 2 chirps are produced when fish are not attacking, and may be used at a distance by fish to deter aggressive behaviours.

Contrary to chirps, it appears that AFRs are aggressive signals, given that they are specifically produced during attacks when the fish are in close proximity. Aggressive signals are produced by a number of weakly electric fish species. For example, in 1974, Hopkins temporally correlated the observable behaviours of *Eigenmannia* (a related wave-type gymnotiform) with the patterns of electric signals they produce. He found that short duration interruptions were correlated with aggressive attack and threat behaviours. In this species, the number of interruptions contained in a bout is a reliable predictor of the likeliness that the animal will attack; the more interruptions produced the greater the probability of attack (Hopkins, 1974). Additionally, in a number of pulse species, transient pulse accelerations are associated with attacks and other aggressive behaviours (Carlson, 2002).

Our results agree with a previous study by Bullock (Bullock, 1969) who reported that *A. leptorhynchus* were often observed chirping in between bouts of attacks. In addition, a very recent study by Triefenbach and Zakon (Triefenbach and Zakon, 2008) has shown that gradual frequency rises and chirps tend to be produced under different contexts. When two fish are competing for a single tube shelter, they found that chirps tend to be produced when fish are not actively engaged, whereas gradual rises tend to be produced when fish are actively engaged in contact behaviours (Triefenbach and Zakon, 2008). Our results corroborate these and further show that these relationships are preserved at time scales as small as 200 ms.

Signalling and inter-fish distance

Fish confined to PVC tubes will only chirp in response to a conspecific when their PVC tubes are within 10–15 cm of each other (Zupanc et al., 2006). Further, they speculate that this limited communication distance is a consequence of a fish's limited ability to detect conspecific signals. Similarly, we found that freely interacting fish tend to chirp when they are on average 12.5 cm apart (head-to-head distance; Fig. 10A) but found many chirps are produced at distances even greater than this. It is not clear, however, that such a comparison is straight-forward because the distances reported under our conditions were dynamic whereas those of Zupanc et al. (Zupanc et al., 2006) were static. Nonetheless, these distances are within the range of those found to be behaviourally relevant for electrocommunication (Knudsen, 1975).

Conclusions

In this study, we found that allowing fish to freely interact changed their chirping behaviour, suggesting that many cues are involved in shaping electrocommunication signalling. It also allowed us to relate chirp production with features of social interaction, such as attack rates and interfish distances, not accessible using previous more-constrained methods. Furthermore, observing chirping in freely interacting fish revealed that AFRs, a relatively uncharacterized type of frequency rise, are produced in abundance. We found that both chirps and AFRs are produced in a non-random fashion and that production rates are influenced by the signalling behaviour of interacting conspecifics. Moreover, chirps and AFRs are also produced under different behavioural contexts: chirps tend to be produced in the time between attacks, whereas AFRs tend to be produced while the fish are in close proximity, during attacks. These results emphasize the importance of experimental context in studying communication signals. In addition, they provide more clues to guide future studies aimed at understanding the physiological mechanisms underlying the detection, interpretation and production of electrocommunication signals.

LIST OF ABBREVIATIONS

AFRs	abrupt frequency rises
Df	difference frequency
EOD	electric organ discharge
EODf	electric organ discharge frequency
GFR	gradual frequency rise
RM ANOVA	repeated measures analysis of variance

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