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Female choice by electric pulse duration: attractiveness of the males' communication signal assessed by female bulldog fish, *Marcusenius pongolensis* (Mormyridae, Teleostei)

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SUMMARY

In adult males of the South African weakly electric bulldog fish, *Marcusenius pongolensis*, the duration of the electric organ discharge (EOD) increases with body size over lifetime. Although there is experimental support for intrasexual selection (male–male competition) having shaped the males' EOD pulse duration in evolution, nothing is known about intersexual selection, such as female choice. Playback of 25 natural male EODs of pulse duration varying from 320 μs (close to the average female value) to 716 μs, to eight female experimental subjects elicited approach, head butts and circling behaviour. The rate of head butts on the dipole electrode model increased significantly with stimulus pulse duration in seven out of eight experimental subjects. In ten experimental female subjects we contrasted the shortest playback pulse with simultaneous playback of one of four longer ones (424, 524, 628 and 716 μs). Pooled responses for all experimental subjects were stronger for the dipole playing back the longer pulse in a pulse pair. The difference in the number of head butts (Δhead butts) that were dealt the two dipoles per 60 s test session increased significantly with the difference in pulse duration (Δpulse duration). The increase followed a significant linear trend (*P*<0.0001). Similar results were obtained for Δassociation time, Δcircles with head butts, and Δcircles without head butts. These results suggest that a male's reproductive success is enhanced by longer, i.e. more attractive EODs, and that both intra- and intersexual selection must have played a significant role in shaping the EOD of male *M. pongolensis*.

Key words: electric organ discharge, sexual selection, sensory exploitation, sensory bias, playback stimulation, electrocommunication, handicap, predation pressure.

INTRODUCTION

Weakly electric fish generate electric organ discharges (EODs) for nocturnal electrolocation and electrocommunication (for reviews, see Kramer, 1990; Kramer, 1996; Moller, 1995; Bullock et al., 2005; Ladich et al., 2006). In mormyrid fish, individual EOD pulse waveform usually is characterised by great long-term stability (Kramer and Westby, 1985; Bratton and Kramer, 1988; Crawford, 1991). EOD waveform may convey information as to species, sex and individual identity. In addition to marked differentiation of EOD waveforms among species (Hopkins, 1988; Hopkins, 1999; Kramer, 1996), there is consistent (and usually more subtle) diversity among individuals, for example, in pulse duration of both South American gymnotiform fish (Westby and Kirschbaum, 1982; McGregor and Westby, 1992) and African mormyrid fish (Kramer and Westby, 1985; Bratton and Kramer, 1988). In mormyrid fish, conditioned discrimination tests have demonstrated an extraordinary sensory acuity for minute waveform differences on the microsecond scale, such as found among different species, and even among individuals of the same species. To the best of our knowledge, this sensory acuity is unparalleled in the animal kingdom (Graff and Kramer, 1992; Paintner and Kramer, 2003; Markowski et al., 2008).

In adult males of the South African bulldog fish, *Marcusenius pongolensis*, EOD pulse duration increases with standard length (*SL*; measured from tip of upper jaw to midbase of caudal fin) over lifetime (Kramer et al., 2007); this is not found in females. Male EOD pulse duration therefore seems to signal strength and fighting ability to conspecific contenders for territories or access to females, a conclusion supported by a study of male–male encounters in a territorial conflict setting, both with live neighbours and simulated ones by electrical playback stimulation (Hanika and Kramer, 2005) (Hanika and Kramer, in press). Furthermore, these studies suggested that the males recognised their territorial neighbours, or 'dear enemies' (Fisher, 1954) individually, discriminating them from strangers. Individual discrimination by EOD pulse waveform has also been demonstrated in a gymnotid electric fish (McGregor and Westby, 1992); however, unlike in the bulldog studies (including the present one), in the gymnotid EOD there was no clear gradient along any waveform character that was related to dominance or potential mating success. It is therefore reasonable to assume that in *M. pongolensis* intrasexual selection, such as male–male competition, has shaped the male EOD pulse and its duration (Hanika and Kramer, 2005). This does not, however, preclude the possibility of additional intersexual selection, such as female choice.

Females of certain gymnotiform species distinguished between conspecific female and male EODs (Kramer and Otto, 1988; Shumway and Zelick, 1988; Kramer, 1999), however, it was not clear whether the females discriminated among males, or even were attracted by males. This question was addressed in another gymnotiform species that had been shown to be sexually dimorphic in tail anatomy, size, and EOD characteristics (Hopkins et al., 1990). In this species, females showed a clear preference for larger males that had stronger EODs of longer duration (Curtis and Stoddard, 2003). Because the females had a choice between unaltered, live males it is not clear which male traits the females preferred; they need not even be electrical.

We therefore designed EOD playback experiments to examine the question of intersexual selection, and whether or not it has left its mark on the male bulldog EOD, in addition to intrasexual selection. We examined females' responses to male playback EODs of a wide variety of male pulse durations, and recorded female association time to stimulators, and other behavioural variables that have been observed in the reproductive behaviour of females.

M. pongolensis appeared to be a suitable study species because aggression, territorial and reproductive behaviour have been successfully studied in this species (Werneyer and Kramer, 2002; Werneyer and Kramer, 2005), and because it responds well to electric playback stimuli (Hanika and Kramer, 2005).

MATERIALS AND METHODS

Animals

All fish used in this study were considered to be *Marcusenius pongolensis* (Fowler, 1934), a recently resurrected species, the synonymisation of which with *M. macrolepidotus* (traditionally referred to as the bulldog fish) was recognised as unjustified (Kramer et al., 2007). All fish originate from the South African part of the Incomati River system in Mpumalanga Province that eventually drains into the Indian Ocean, or are first-generation descendants of these fish raised in the laboratory. Specific places of collections, all within the Incomati System, were: Two experimental subjects, E1 (*SL* 12.8 cm) and E4 (*SL* 18.2 cm) originated from the Sabie River (Kruger National Park, bridge near Lower Sabie Rest Camp, 25°07′14.5″S, 31°55′29.8″E, collected by F. H. van der Bank and B. Kramer, 29–30 March 1996), and another two experimental subjects, E2 (*SL* 16.5 cm) and E3 (*SL* 14.1 cm) originated from the Crocodile River (Stentor Estates near the community of Kaapmuiden, 25°30′35″S, 31°11′58″E, collected by F. H. van der Bank and J. Engelbrecht, 14 February 1997). The two localities are 84 km apart. Another seven experimental subjects were laboratory-raised descendants of fish sampled from the Crocodile River (E5–E11; *SL* 9.5–11.4 cm at the time of the tests; locality as given above).

For playback stimulation we used the EODs of 25 different, wild-caught males, seven of which had been recorded in the field near the site of their capture [collected by P. Skelton and B. Kramer, 23–25 September 1993: one male, Sabie-Sand River at Londolozi, 24°47′31″S, 31°31′32″E; five males, Sabie River above Hazyview, 25°02′S, 31°00′E; one male, Sabie River just above Hazyview, 25°01′48″S, 31°01′21″E; further details are given elsewhere (Kramer et al., 2007)]. Recordings from the other 18 males originating from the Crocodile River (see above) were made in the laboratory (see Kramer et al., 2007). The two inter-discharge interval (IDI) patterns used for driving the output of playback pulses were also recorded from two captive Crocodile River males.

Animal care

Keep aquaria of 360–760 l held two or three fish at 23–26°C, at a water conductivity of about 100 μS cm^{-1} and a photoperiod of 12 h:12 h L:D. Conditions for the experimental tank (250×50×50 cm, filled to 40 cm) were similar but controlled more narrowly (24.4±0.2°C, 100±3 μS cm^{-1}). Fish were fed with freshly defrosted *Chironomus* sp. larvae following test sessions (if in experiment) 5–7 times per week.

EOD recording and analysis

For EOD recordings, fish were placed head-to-tail between a pair of low-impedance carbon electrodes, at 25°C and 100 μS cm^{-1} water conductivity when in the laboratory, using a differential amplifier (1–100 000 Hz; custom-made by Electronics Workshop, Biology Department, University of Regensburg) and a digital oscilloscope (TDS 420, Tektronix Holland, NV, Heerenveen, The Netherlands; bandwidth 150 MHz, 1 MΩ impedance, sampling rate 250 kHz, vertical resolution 11 bit, record length 2500 points per single sweep). Conditions were similar for field-recorded EODs (Kramer, 1997).

All EODs were biphasic short pulses with a head-positive first phase followed by a negative one, as is common in the genus *Marcusenius,* and described in more detail elsewhere (Kramer et al., 2007). Custom-made routines programmed in the package FAMOS (imc, Berlin) measured EOD pulse duration using an amplitude criterion of ±5% of the peak amplitude P of an EOD. This was necessary because of the asymptotic start and termination of *M. pongolensis* EODs (Fig. 1). An EOD started when the rising edge of the head-positive first phase P crossed the +5% threshold of the peak amplitude, and an EOD was considered terminated when the rising edge of the negative phase crossed –5% of the peak amplitude P. A Fourier transformation routine provided by FAMOS generated amplitude spectra of single EOD pulses (centred on baseline, 2^{13} data points, 50 kHz sampling rate, 0.2 Hz frequency resolution).

The 25 male EODs recorded from 25 different males varied in pulse duration, ranging from a low value close to the female average, 320 μs, to up to 716 μs that is exclusively found in males (Fig. 1A) [and fig. 15 (Kramer et al., 2007)]. Field-measured EOD duration of 20 females (size range 9.1–20 cm *SL*) originating exclusively from the Incomati System (same sampling places) was 318±5.56 μs (mean ± s.e.m.); lowest and highest values were 277.6 μs and 359.1 μs, respectively. In the single-dipole test series, all 25 male EODs served for playback stimulation of each experimental subject, whereas in the double-dipole test series only five EODs that were about equally spaced in duration increase were used (320, 424, 524, 628 and 716 μs; Fig. 1B).

From maturity onwards, male EODs (but not female EODs) start to increase in duration such that there is a significant correlation with *SL* [see fig. 15 (Kramer et al., 2007)].

EOD playback

Amplitude-normalised EODs were resampled at 500 kHz and 8 bit vertical resolution using FAMOS to meet DAM requirements. From a host PC these files were transferred to one or both DAM machines (two in the case of the double-dipole test series) for generating the analogue output signal to be sent to the dipoles (Fig. 1C). The DAM is a microprocessor-controlled D/A converter with memory, custom-made by the Electronics Workshop, University of Regensburg (Kramer and Weymann, 1987). The output signal was bipolar and fed into the dipoles directly.

When a single dipole was used, the distance from the porous pot was 50 cm; in the double-dipole tests the distances were 30 cm from the porous pot in the middle (Fig. 2). Stimulus amplitude was determined as the mean amplitude generated by six fish. An experimental subject resting inside its porous pot (Fig. 2) received playback stimuli at 300 $\mu V_{p\text{-}p}$ cm^{-1} field strength at 30 cm distance from both dipoles in the double-dipole tests, and 110 $\mu V_{p\text{-}p}$ cm^{-1} at 50 cm distance in the single dipole tests. After having moved to one dipole in the double-dipole tests, an experimental subject still received the signal generated by the opposite dipole at 50 $\mu V_{p\text{-}p}$ cm^{-1} field strength (a 60 cm distance). Horizontal field strength was measured using a vertically held 1 cm measuring dipole made from glassy carbon electrodes (Sigradur G, HTW

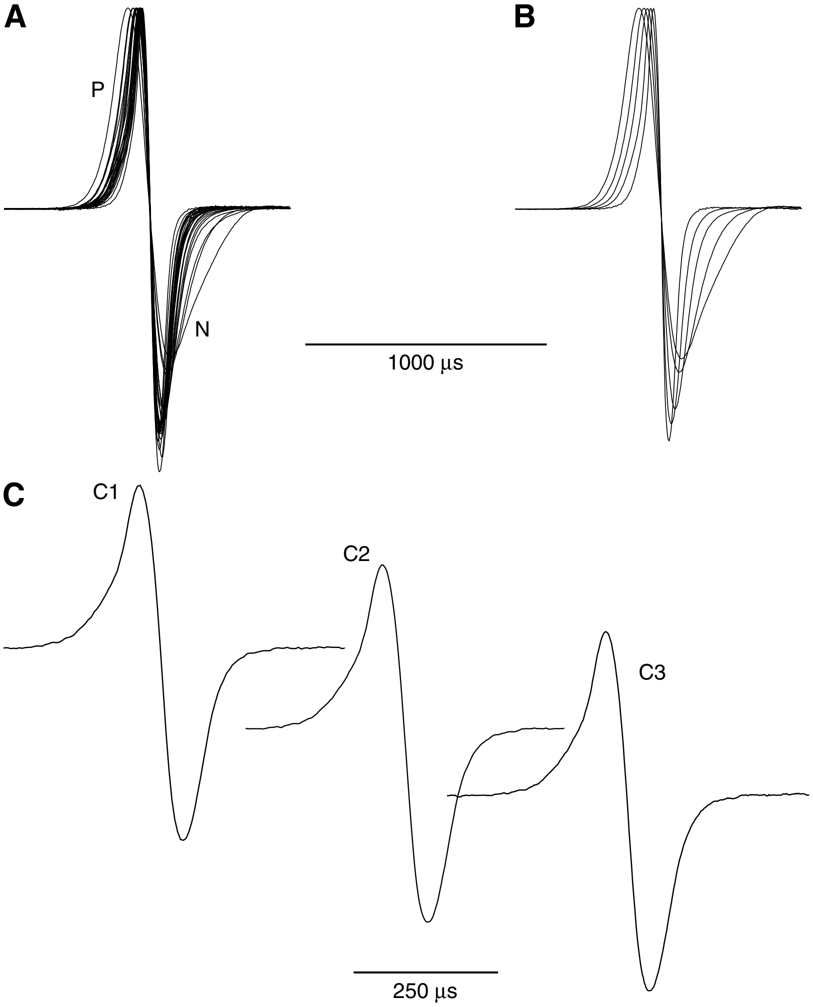

Fig. 1. (A) Twenty-five EODs recorded from 25 different *M. pongolensis* males, as used in single-dipole playback tests. Centred on zero-crossing point, superimposed, and normalised to peak amplitude P=1 V. P, head-positive first EOD phase; N, head-negative second phase. (B) Same as A but for the five EODs used in the double-dipole playback tests. (C) Comparison of playback model of 320 µs pulse duration with actual playback output. (C1) EOD as recorded from fish. (C2) Same as C1, but recorded from DAM output. (C3) Same as C1, but recorded from experimental aquarium.

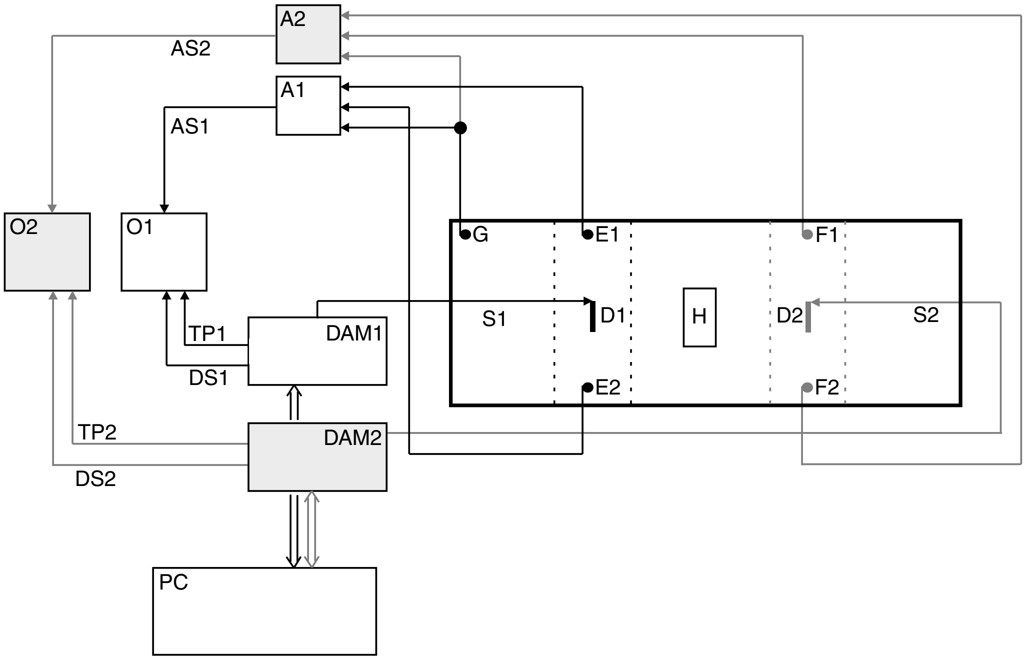

Fig. 2. Setup for playback experiments. Equipment for the single-dipole tests, white boxes connected by black lines; additional equipment for the double-dipole tests in grey. E1 and E2, F1 and F2, recording electrode pairs; G, grounding electrode; A1, A2, differential pre-amplifiers; AS1, AS2, amplified signals; O1, O2, oscilloscopes; DAM1, DAM2, digital-to-analogue converters; PC, personal computer; D1, D2, stimulus dipoles, with specified area ‘near’ around; H, porous pot. TP1, TP2, external trigger pulse; S1, S2, DS1, DS2, playback pulse output.

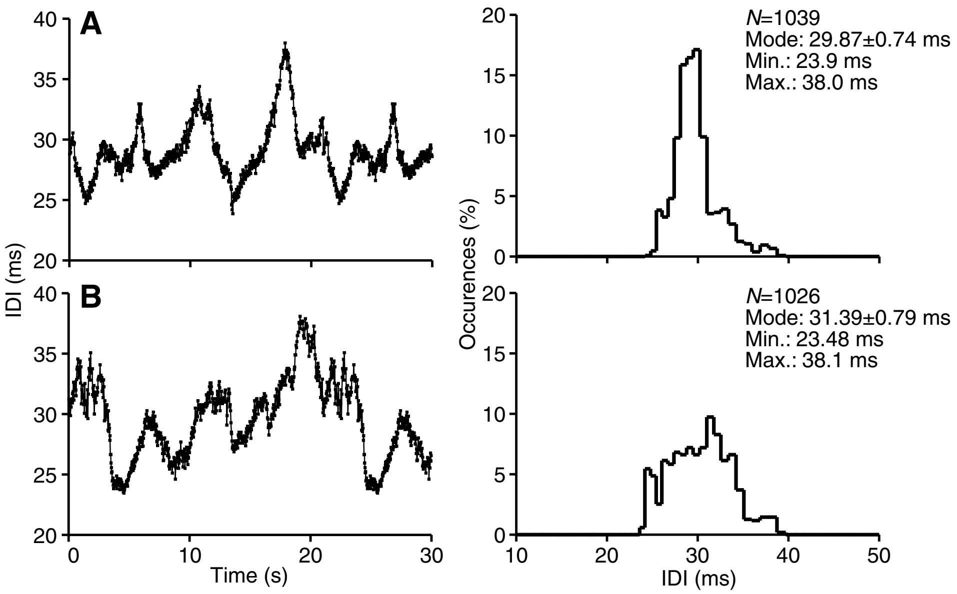

Fig. 3. (A,B) Inter-discharge interval (IDI) patterns recorded from two isolated *M. pongolensis* males, slowly swimming about their aquarium at night, and used for driving the output of playback pulses in the single-dipole tests. Left panels, inter-discharge interval (IDI; in ms) over time (s); right panels, histograms of patterns (together with statistical data). Mean pulse rates, 34.65 Hz (A), 34.22 Hz (B).

GmbH, 86672 Thierhaupten, Germany; diameter, 1 mm; length, 6 cm; insulated except for the lowest 3 mm; rod separation centre-to-centre, 1 cm). Maximum field strength was found by rotating the measuring dipole at the site. Stimulation dipoles consisted of a pair of vertically orientated carbon rods mounted on a horizontally orientated Plexiglas™ tube that was kept in position on the aquarium floor by suckers (Fig. 2). The carbon rods of 5 mm diameter and 10 mm height were 3 cm apart, and generated the electric stimulus field.

The choice of an inter-discharge interval (IDI) pattern for driving the output of playback pulses required pilot experiments that compared the suitability of IDI patterns recorded from bulldog males during different behavioural states: (i) diurnal inactivity state while hiding, (ii) nocturnal foraging, (iii) nocturnal agonistic interaction with conspecific, (iv) nocturnal locomotory activity of semi-isolated fish (conspecific in neighbouring compartment separated by mesh partition). As expected from earlier playback studies (e.g. Kramer, 1979), IDIs recorded from semi-isolated fish slowly swimming at night (without any abrupt movements) proved best. These IDI patterns were characterized by histograms with a single mode of about 30 ms, a narrow range of IDIs of between 24 and 38 ms, and a medium pulse rate near 35 Hz. There were neither abrupt discharge rate accelerations nor discharge breaks (as occurring during aggression and threatening behaviours) that might have excited and distracted listeners from attending to EOD waveform characteristics. Two such patterns recorded from two different males were selected for the present tests, data were transformed to the format required by DAM using a routine programmed in Quickbasic, and uploaded into DAM memory. The DAM, when configured, generated the pulse waveform at the natural IDI pattern preselected, on command by PC. IDI sections of 22 s were played back in a loop for the time period required. Care was taken to join end points of the IDI sequence such that there were smooth transitions in interval length and trend (Fig. 3).

For the double-dipole test series we used only one IDI pattern in order to limit the number of playback parameter combinations to a manageable size, but chose different starting points for the two IDI sequences such that pulse output of the two DAMs was asynchronous (Fig. 4).

Procedure and behaviour recorded

In the single-dipole test series, all 25 prerecorded male EODs served for playback stimulation to each of the eight female experimental subjects. We used only stimulus pulses from males that had been kept separate from experimental subjects for at least 6 months prior to the start of playback tests, in order to avoid potential effects associated with individual recognition (Hanika and Kramer, 2005). Each experimental subject was tested only once with a specific pair formed from playback pulse type (1 out of 25) and IDI pattern (1 out of 2). The sequence of presentation of specific pairs was determined using a randomized blocks design following table 15.7 (Cochran and Cox, 1957), never using a block twice. Under this regimen there were 50 test sessions per experimental subject. The average of the two repeats for a specific playback pulse entered statistical analysis.

The ten experimental subjects of the double-dipole test series received a pair of stimulus pulses played back concurrently, with pulse pairs formed from the shortest pulse (320 μs that was used in each pair) and one out of a list of four longer ones; that is, there were four pulse pair combinations. To guard against potential sidedness of experimental subjects, each pulse pair combination was used twice, with the roles of left and right dipoles reversed. Therefore, there were 8 pulse pair and side combinations per fish, the presentation sequence of which also followed table 15.7 (Cochran and Cox, 1957). We ran three complete rounds of this experiment, that is, 24 test sessions per fish. Behavioural scores for the six repeats of a specific pulse pair combination were averaged to yield a single value per fish that entered statistical tests.

An experimental subject underwent a maximum of eight individual playback tests per night, separated by rest periods of at least 10 min. An individual test session lasted for 30 s in the single-dipole series of playback experiments, and 60 s in the double-dipole series of playback experiments.

The behaviour of experimental subjects was video-taped (Panasonic model AG-7330, Hifi, S-VHS) using an infrared-sensitive camera (model FK 6990 B-IQ, Cohu, San Diego, CA, USA; lens, Pentax/Cosmicar 1.0: 8–48 mm, Tokyo, Japan), and infrared illumination with no visible light (model UF 500 FL, Derwent Systems, Cramlington, UK, $\lambda \geqslant 800$ nm). For precise

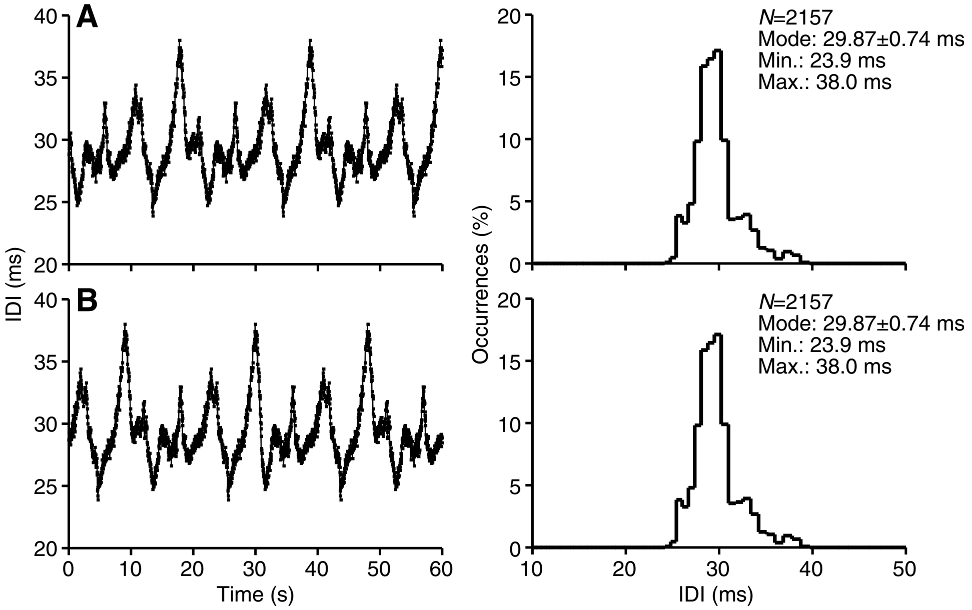

Fig. 4. (A,B) Similar to Fig. 3, but the longer pattern shown here was used for the double-dipole tests. Inter-discharge interval (IDI) pattern of 22 s duration recorded from one slowly swimming *M. pongolensis* male at night. Pattern duration of 1 min achieved by concatenation such that transitions were smooth. Lower and upper panels show same data, but starting points were different.

temporal offline analysis and correlation of overt behaviour with stimulus IDI list, each stimulus pulse triggered a pulse marker (electronics workshop, University of Regensburg) for blanking a short part of a video image line, visible as short white bar on the left margin of a frame. The vertical spacing of white bars recorded during successive frames (of 20 ms duration) corresponded to the IDI list for stimulus output.

List of behaviours observed and recorded:

(1) Head butt: (a) following an acceleration towards the dipole, frontal collision with dipole; (b) lateral head butt against dipole when swimming alongside.

(2) Circling without head butt: one circle was tallied for each 360° circle performed in close proximity to and just above the dipole.

(3) Circling with head butt: while circling, fish dealt the dipole lateral head butts.

(4) Association time: following stimulation onset, the cumulated time an experimental subject spent within a specified area centred on a stimulus dipole. This area was 20 cm wide (Fig. 2).

In *M. pongolensis* prespawning behaviour, the male is rather passive and stationary whereas the female is very active, visiting the male repeatedly while vigorously attacking him before she eventually spawns with that male. Therefore, visiting a male and unprovoked female aggression is a good indicator of readiness, especially when the male does not or only weakly counter-attack (Werneyer and Kramer, 2005).

For statistical analysis we used the software package Prism v4 with α=0.05 (two-sided).

RESULTS

In the present study, the 25 male EODs used for playback stimulation of female subjects ranged from 320 to 716 μs in duration (determined with ±5% amplitude criterion applied). Spectral analysis showed that the peak amplitude frequency for the shortest EOD was at 4.42 kHz, and the –10 dB cut-off frequency at 11.33 kHz. The longest EOD had its peak amplitude frequency at 1.47 kHz and showed a considerably smaller bandwidth, with the –10 dB cutoff at 3.9 kHz (Fig. 5).

Irrespective of the stimulus pulse that was played back to them, all subjects left their porous pot shelter and headed for an active dipole within a few seconds following onset of electrical stimulation. We then observed head-butt attacks and circling, behaviours that were aimed at the active dipoles although during in-between stimulation periods, when dipoles were silent, attacks or circling never occurred. These observations held for both single- and double-dipole tests.

Single-dipole playback tests

Head butts

In these tests a single playback pulse type at a time was presented *via* a single dipole. In seven of eight female experimental subjects, the attack rate, measured as head butts per 30 s, increased with stimulus pulse duration (linear regression: 'weakest' significant result obtained with experimental female E3: rate of head butts=0.0135×pulse duration+0.92, $F_{1,23}$=4.26, P=0.0489, r^2=0.1564). For all other individuals (except E3 and the nonsignificant E4) regression slopes were steeper, r^2 higher, and P values lower (<0.0001). Table 1 lists statistical results for the other experimental subjects, Fig. 6 shows linear regression graphs. Subject E4 later developed physical abnormalities and may have been unhealthy at the time of the test series (this wild-caught specimen was the biggest and probably oldest).

Association time

Although on stimulation onset all experimental subjects headed to a dipole rather promptly, not all of them remained in its vicinity for the whole stimulation period of 30 s (as delineated by markers on the aquarium bottom; Fig. 2). Some experimental subjects left and returned repeatedly, and it was the cumulative time within the area specified by markers that was used for statistical analyses in these cases. After playback had stopped, fish continued to swim about, neither avoiding the area around the dipole nor concentrating on it. An inactive dipole was ignored completely.

Association time, i.e. the time an experimental subject spent in the specified area around an active dipole, was independent of the duration of playback pulses for all eight experimental subjects (linear regression: $F_{1,23}\leq 1.55$, $P\geq 0.2251$, $r^2\leq 0.0633$; 'best' result obtained in fish E7).

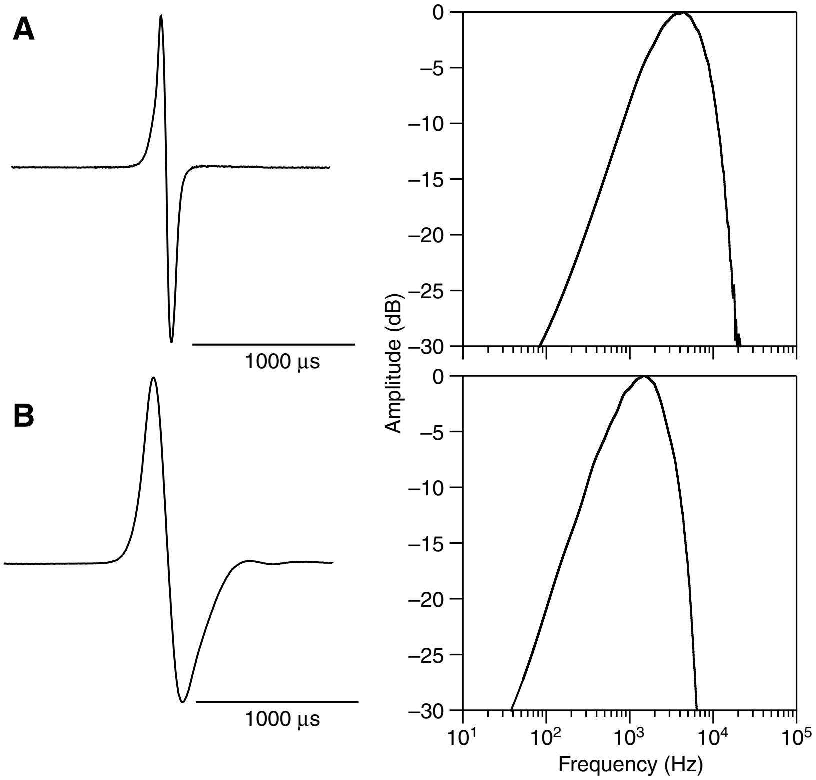

Fig. 5. (A) Shortest playback pulse of 320 µs duration. Left, EOD waveform (V by time); right, associated amplitude spectrum (amplitude in dB re: strongest amplitude=0 dB over frequency in Hz). (B) Same as A, but for longest pulse of 716 µs.

Circling without head butt

In contrast to stimulus-evoked head butts that were observed for all experimental subjects, circling around the dipole in close contact (with or without head butts) was common in some and rare in others. Only in one out of the eight experimental subjects, did the rate of circling without head butt increase significantly with playback pulse duration (linear regression for fish E8: circling without head butt=0.003×pulse duration –1.188; $F_{1,23}$=12.82, P=0.0016, r^2=0.3579). No significant dependency of any experimental subject was obtained for circling with head butt (linear regression: $F_{1,23}\leqslant 1.78$, $P\geqslant 0.1957$, $r^2\leqslant 0.0717$).

Double-dipole playback tests

In order to give experimental subjects an opportunity to choose between (or to show a preference for) playback pulses varying in duration, as may be expected to occur in nature, we designed a test series with simultaneous playback of two pulses differing in duration. Rather than using all 25 playback pulses, we only used five in these tests, from the shortest (320 µs) to the longest (716 µs), that were about equally spaced. A stimulus pulse pair consisted of the shortest pulse that was always used, and one of the longer ones, played back concurrently from an experimental subject's right and left (Fig. 2). We used 10 female experimental subjects including all previous experimental subjects except E4 that had fallen ill. The test series started 2 months after completion of the single-dipole series.

Rather than absolute behaviour scores as in the single-dipole tests, we determined the difference between behaviour scores for the two playback pulses (the dependent variables). The independent variable was the difference in pulse duration. Only the scores where there is a difference between the responses to the paired playback EODs are informative, that is, contribute to confirming or rejecting the null hypothesis. Because of the great homogeneity of the results in the single-dipole tests, and with the unhealthy subject E4 excluded, we felt pooling of results for all experimental subjects was justified.

Table 1. Linear regression of the dependency of the rate of head butts on stimulus EOD pulse duration in single-dipole tests

Subject	Slope ± s.e.m.	*y*-intercept ± s.e.m.	r^2	*F*	*P*
E1	0.0160±0.0032	4.550±1.554	0.6085	35.76	**<0.0001**
E2	0.0229±0.0028	–4.954±1.349	0.7542	70.56	**<0.0001**
E3	0.0135±0.0065	0.920±3.172	0.1564	4.26	**0.0489**
E4	0.0026±0.0027	7.546±1.312	0.0609	1.49	0.2346
E5	0.0191±0.0024	0.356±1.164	0.7175	58.43	**<0.0001**
E6	0.0223±0.0020	0.948±0.959	0.8940	193.90	**<0.0001**
E7	0.0181±0.0027	2.976±1.288	0.6656	45.78	**<0.0001**
E8	0.0181±0.0033	–1.139±1.581	0.5327	26.22	**<0.0001**

s.e.m., standard error of the mean; *P*, level of significance (α=0.05; significant values in bold type), d.f.=1,23.

Head butts

As had already been observed in the first test series, aggression was readily evoked in all experimental subjects, independent of whether they had served in the first series or not. Again, the longer playback pulses evoked more aggression than the short playback pulse; the greater the difference in pulse duration the more aggression. The difference in the rate of head butts (Δhead butts) increased with the difference in pulse duration (Δpulse duration; repeated-measures ANOVA: $F_{3,9}$=16.96, P<0.0001; *post-hoc* test for linear trend: slope=1.528, P<0.0001; Fig. 7A).

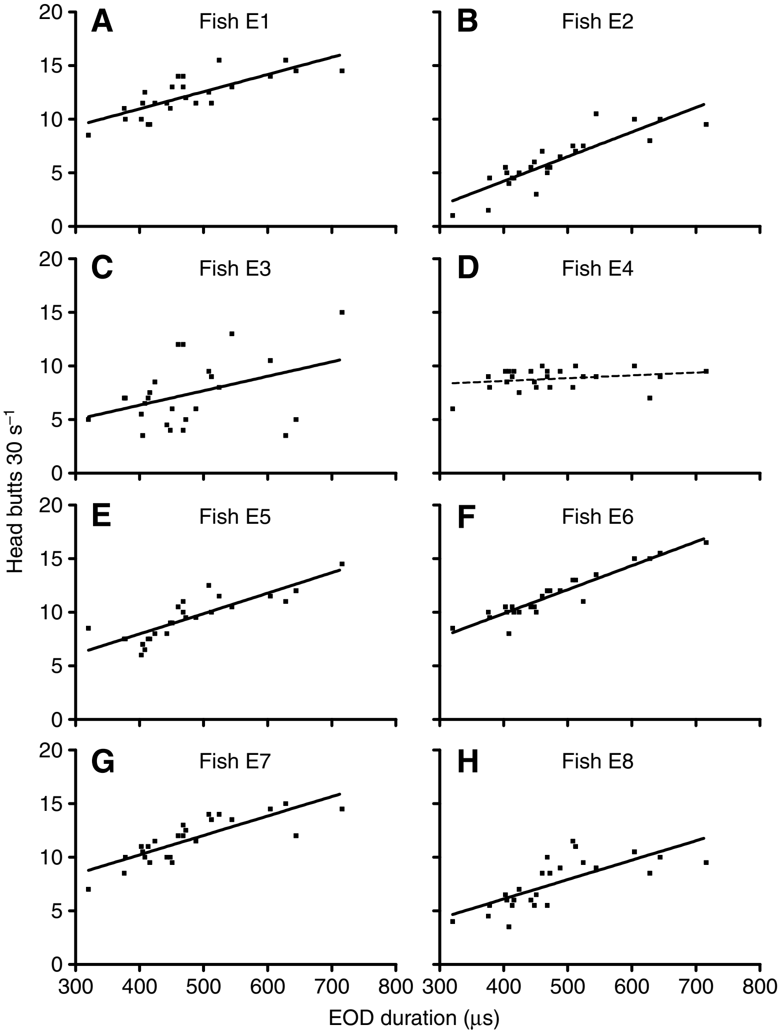

Fig. 6. Relationship of rate of head butts, evoked by single-dipole playback stimulation (ordinates), as related to playback pulse duration, in μs (abscissae). Each point is the average of two repeats. (A–H) Individual graphs for eight female experimental subjects. Least-squares regression lines significantly (P<0.05) differed from slope zero except where shown as a broken line (graph D, fish E4).

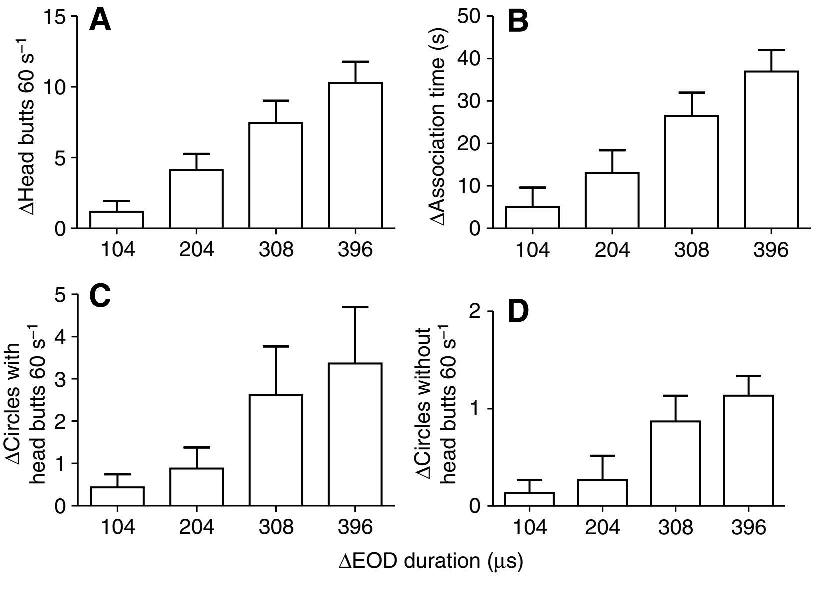

Fig. 7. Results of double-dipole tests. Behavioural scores of 10 experimental subjects (pooled) as related to difference in pulse pair duration (of longer pulse minus that of shortest pulse of 320 μs). Behavioural scores shown as difference between scores for longer pulse minus scores for shortest pulse. Difference scores are shown as means + 1 s.e.m. Note that for four behavioural variables the difference scores increased significantly with the difference in pulse pair duration (linear trend, significant).

Association time

All experimental subjects, after having left their porous pot hiding place on stimulus onset, headed for one of the two active dipoles. Some spent the whole stimulation period with the same dipole, others switched between the two. The tendency for switching was a characteristic of certain individuals. In switching individuals, association time was measured as the cumulative time for the association with a certain dipole.

Association time increased with pulse duration. ΔAssociation time thus increased with Δpulse duration (repeated-measures ANOVA: $F_{3,9}$=13.28, P<0.0001; *post-hoc* test for linear trend: slope=5.456, P<0.0001; Fig. 7B).

Circling

Both circling without head butts and circling with head butts occurred significantly more often the longer the playback pulse. Therefore, both Δcircling with head butts and Δcircling without head butts increased with Δpulse duration (Fig. 7C,D). Repeated measures ANOVA for Δcircling with head butts: $F_{3,9}$=4.997, P=0.0069; *post-hoc* test for linear trend: slope=0.5267, P<0.0008; Fig. 7C. Repeated-measures ANOVA for Δcircling without head butts: $F_{3,9}$=4.576, P=0.0102; *post-hoc* test for linear trend: slope=0.1800, P<0.0012; Fig. 7D.

DISCUSSION

This study has shown that playback of male EODs *via* a dipole decoy evoked fully fledged elements of aggression and courtship behaviour in female listeners: the longer the male playback pulse, the stronger the females' responses. When only a single stimulus dipole was present, the rate of head butts female experimental subjects dealt the dipole increased with playback pulse duration (effect significant in seven out of eight females). In the double-dipole tests, female experimental subjects could compare the shortest male pulse of the present study with one of four gradually longer ones simultaneously. Here, differential results included three additional behavioural elements: increasing with the difference in stimulus pulse pair duration were not only the differences in head butt scores but also those for association time, circling with head butts and circling without head butts, always with higher scores for the longer playback pulse. The reason why double dipole testing was more effective in showing discrimination in females may be the unnatural isolation under test conditions. An isolated bulldog fish will probably show an already maximal associating response when there is only a single conspecific (or its decoy) nearby, independent of sex. This is reminiscent of the intra- *vs* interspecific attraction response reported by Moller and Serrier (Moller and Serrier, 1986). A choice situation seems to be better suited for the female to show a clear preference for a sexually more attractive stimulus.

All these behaviours, associating with a male in his territory, head-butting him, circling with or without additional head butts, occur during courtship and spawning behaviour which in *M. pongolensis* is rather aggressive and dominant on the female side, whereas the male appears surprisingly passive and inactive (Werneyer and Kramer, 2005). Because of the females' clear preference for long male EODs we conclude that intersexual selection must have played a significant role in the evolution of the male EOD pulse, in addition to intrasexual selection that was demonstrated previously (Hanika and Kramer, 2005).

The ancestral EOD pulse waveform in mormyrids probably was similar to those found in strongly electric fish, such as the electric catfish or the electric eel: a simple monopolar muscle action potential (Bennett, 1971), as also found in the exceptional mormyrid *H. szaboi* (Kramer et al., 2004) and in one South American gymnotiform, *Hypopomus sp.* (Kramer et al., 1981). Why have so few weakly electric pulse fish retained the presumed ancestral condition and acquired a second head-negative phase that usually follows the first phase so fast it curbs peak-to-peak amplitude? One, and perhaps the major, reason is predation pressure from electrosensory catfish. A second phase of opposite polarity reduces the DC component of an EOD pulse, and its spectral low-frequency content in general (Stoddard, 1999). It is the long-duration EODs of male bulldog fish with their strong low-frequency content and narrow bandwidth that are detected by predatory sharptooth catfish at high sensitivity (Hanika and Kramer, 2000).

Therefore, a long EOD pulse in males corresponds well to Zahavi's hypothesis of a sexually selected ornament that is a 'handicap' for its owner (Zahavi, 1975): the handicap is an 'honest' one (Maynard Smith and Harper, 2003) because of the predation cost, as documented in the bulldog fish by Merron (Merron, 1993). A long EOD pulse is only found in males that have survived in spite of their handicap, thus revealing fighting potential and 'good genes' to choosy females.

What may be the reason for EODs of long pulse duration being selected for in males, apparently by both sexes in *M. pongolensis*, and in spite of severe catfish predation pressure? An initial female preference for long male EODs (starting a process in which males are selected for longer EODs that eventually reduce fitness) may be explained by the hypotheses of sensory bias and sensory exploitation (Ryan and Keddy-Hector, 1992; Alcock, 2005; Kappeler, 2006).

In *M. pongolensis*, the proximate mechanism for a sensory bias is not difficult to see: like all mormyrids and in fact all electroreceptive fish, *M. pongolensis* possesses ampullary electroreceptor organs tuned to low frequencies, in addition to their high-frequency-sensitive tuberous organs that are limited to mormyriforms and gymnotiforms. Ampullary organs in nature signal the presence of live objects such as food, but also respond weakly to the spectral low-frequency component of biphasic pulse EODs (Bell and Russell, 1978), especially long-duration ones, such as documented in Fig. 5B. During evolution, ampullary organs must have been acquired first by the ancestors of both mormyriforms and gymnotiforms, as indicated by electroreceptive representatives of the respective sister groups that do not possess tuberous organs: Xenomystinae for the Mormyriformes, Siluriformes for the Gymnotiformes (Finger, 1986; Braford, 1986; Jørgensen, 2005).

A mutant male bulldog fish with a long pulse EOD might benefit from sensory exploitation of a pre-existing sensory bias for low-frequency signals in females, because a long EOD pulse represents a stronger stimulus than a short EOD of female duration. (This is because in addition to the Knollenorgane, the tuberous receptor-type organ for communication in mormyrids, ampullary organs will also respond.) It is usually the stronger stimulus that is preferred by females if there is variation on the population level (Ryan and Keddy-Hector, 1992).

In *M. pongolensis* neither sex provides care for their offspring, and males do not build a nest. Thus all a female gains from her partner is his genes. This is a situation which is usually associated with a male handicap and female choice, to ensure that her eggs are fathered by a male with superior genes (Zahavi, 1975; Grafen, 1990).

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