Intra-male variability of its communication signal in the weakly electric fish, *Marcusenius macrolepidotus* (South African form), and possible functions

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Summary

Mormyrid fish generate electric organ discharges (EODs) continuously for electrocommunication and electrolocation. EOD pulse waveforms are, within species limits, individually specific and usually stable over long periods of time. We conducted playback experiments with the African weakly electric snoutfish, *Marcusenius macrolepidotus* (South African form), or bulldog fish, originating from the Incomati River System in South Africa. Previous field observations had shown an increase of male, but not female, EOD pulse duration with body size. Therefore, we tested the hypothesis that EOD pulse duration might be of relevance in territorial male-male contests. We determined whether or not aggression scores recorded for males kept in adjacent aquarium compartments depended on playback EOD duration. Playback EODs had been pre-recorded either from familiar or from unfamiliar conspecific males, and varied naturally in duration.

As independently confirmed in all five experimental subjects, the attack rate on a dipole model playing back strangers' EODs from the territorial boundary increased significantly with stimulus EOD pulse duration (that varied from 329 μs to 975 μs in our field sample of playback EODs). This result was confirmed when one specific EOD, the duration of which was artificially decreased or increased, was used for playback, showing that in male *M. macrolepidotus* EODs of so long duration signal a greater threat than EODs of shorter duration. Therefore, EOD duration may be relevant for males in the assessment of their rivals' size and fighting potential. Moreover, experimental subjects confronted with familiar playback EODs showed a significantly lower rate of attack than expected for unfamiliar EODs of the same duration. An individual familiar neighbour's EOD evoked a significantly weaker attack rate when played back from the regular rather than from the opposite territorial boundary. These observations are compatible with the 'dear enemy' hypothesis.

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Introduction

Weakly electric mormyrid fish of Africa use their electric organ discharges (EODs) for electrolocation and electrocommunication (reviews: Kramer, 1990, 1996; Moller, 1995; Turner et al., 1999). The waveform of their EOD pulses carries sufficient information for species discrimination, it may even be individually specific and in certain species sexually dimorphic (Kramer, 1997a, b). In *Marcusenius macrolepidotus* (South African form), a mormyrid fish from the South African Incomati River system, a statistically significant difference between male and female EOD duration was found, and male, but not female, EOD duration increased with body length (Kramer et al., 1998, in prep.).

The identity of male communication signals may be relevant in various contexts. For females, male EODs may indicate the location of their mate, his reproductive status, or his fitness, but male EODs may also serve in intrasexual communication. Male communication signals in the use of assessment of rivals is a topic that has attracted far less attention than intersexual communication (ten Cate et al., 2002), even though the influence on the evolution of variable male signals may be great. Males could assess an opponent's body size and fighting ability (e.g., Davies & Halliday, 1978; Clutton-Brock & Albon, 1979) when the communication signals are correlated to fitness or size, the latter found in *M. macrolepidotus*' (South African form) EODs. Pilot experiments suggested a relationship between playback EOD duration and male aggression (that was directed at a dipole fish model). Therefore, we studied the dependency of the males' attack rate on playback EOD duration in more detail. We chose electrical playback to exclude all other cues, such as chemical, olfactory, acoustical or visual. We used *M. macrolepidotus* (South African form) because of their territorial behaviour in captivity (Werneyer & Kramer, 2002; SH, pers. obs.), the individually distinct EOD waveforms (Kramer et al., 1998, in prep.), and the readiness of male *M. macrolepidotus* to attack active dipoles playing back conspecifics' EODs.

Discrimination of conspecific communication signals may also function in kin recognition (review: Hepper, 1991), colony member recognition (review: Hare, 1994), and neighbour recognition (review: Temeles, 1994), as

documented in a wide variety of species. Individuals that recognise neighbours, resulting in more or less settled affairs, may be able to reduce their aggressive responses to these animals and would require less time and energy for territorial defence (Chellappa & Huntingford, 1989; Thorpe et al., 1995). This effect is called the 'dear enemy' phenomenon (Fisher, 1954; for reviews of neighbour-stranger discrimination see Ydenberg et al., 1988; Temeles, 1994). The 'dear enemy' phenomenon was observed in many different species, including cichlid fish (Peeke et al., 1971; Leiser & Itzkowitz, 1999), trout (Johnsson, 1997; Höjesjö et al., 1998), swordtails (Morris et al., 1995), and the gymnotiform *Gymnotus carapo* (McGregor & Westby, 1992).

Conditions necessary for neighbour recognition are (1) physical differences in signals used for communication, (2) sufficient sensory discrimination in the receiver, and (3) communication signals of sufficient constancy. All these conditions seem to be met in mormyrid fish: (1) in spite of EODs of very short duration (often < 1 ms), waveform parameters vary considerably between individuals (for example, Lücker & Kramer, 1981; Bratton & Kramer, 1988; Crawford, 1992; for *M. macrolepidotus* (Upper Zambezi), Kramer, 1997a). (2) The mormyrid species *Pollimyrus adspersus* discriminates between its short intraspecific (or synthetic) playback EODs, even when only slightly different (Graff & Kramer, 1992; Paintner & Kramer, 2003). The microsecond-resolution shown by these fish demonstrates a much higher sensitivity than required for species recognition, and makes sense only for the much more difficult task of intraspecific discrimination. (3) Whereas the intervals between EOD pulses are highly variable and specific for the social context (Scheffel & Kramer, 2000, in press; Werneyer & Kramer, 2002), EOD waveforms are usually constant over long periods of time (Kramer & Westby, 1985; Bratton & Kramer, 1988; Crawford, 1991).

Therefore, our second aim was to determine whether an individual mormyrid fish would reduce its aggression against neighbours compared to strangers on the basis of individual EOD waveform. According to theory, a fish is expected to respond less aggressively to a neighbour's than to a stranger's EODs.

Methods

Animals and animal care

All fish used in the present study originate from the Incomati River System (South Africa, Mpumalanga, 25° latitude South) with its two tributaries, the Sabie River (Kruger National Park; standard fish length 12-15 cm) and the Crocodile River (Stentor Estates near the town of Kaapmuiden; standard fish length 17-20 cm). According to present taxonomy these fish are *Marcusenius macrolepidotus* (Peters, 1852) because *Gnathonemus pongolensis* Fowler, 1934 (that became *M. pongolensis* (Fowler, 1934) by Taverne's (1971) revision of the genus *Gnathonemus*) was synonymized with that species. As there is controversy about the merit of this synonymization, we refer to our fish as *M. macrolepidotus* 'South African form' at present. EODs used for playback were recorded either in the field (B.K., Incomati River System; 'Stimulus' fish S1-S16) or in the laboratory (fish S17-S20) (no differences between field recorded and lab recorded EOD waveforms, B.K., unpubl. results). Fish S17-S20 had been housed separately from the experimental subjects from arrival in the lab on and were not used as experimental subjects. The experimental subjects (E1-E5) were kept in the same tank as their neighbours (N1-N10).

The artificially controlled light: dark period was 12:12 h, the water temperature 23°C and water conductivity 100 μS/cm. Fish were fed frozen bloodworms (chironomid larvae) five times per week, usually after the experiments. Experimental aquaria ($210 \times 60 \times 50$ cm high) were divided into three compartments of equal size using two plastic-mesh partitions that allowed electrical and limited visual interaction (Figure 1). In the centre of each compartment a porous pot was placed for shelter. The plastic mesh partitions prevented the fish from inflicting damage to each other. All males showed territorial behaviour. Especially at the beginning of the dark cycle, fish patrolled the boundaries of their territories and engaged in behaviour described as aggression acts by Werneyer & Kramer (2002): (1) the predominant behaviour was 'parallel swimming' of both neighbours along their common plastic mesh boundary, (2) but also 'antiparallel display' (remaining closely together in a head-to-tail position; however, circling — as would occur in free water — was impossible because of the plastic mesh partition), or (3) trying to attack each other through the plastic mesh.
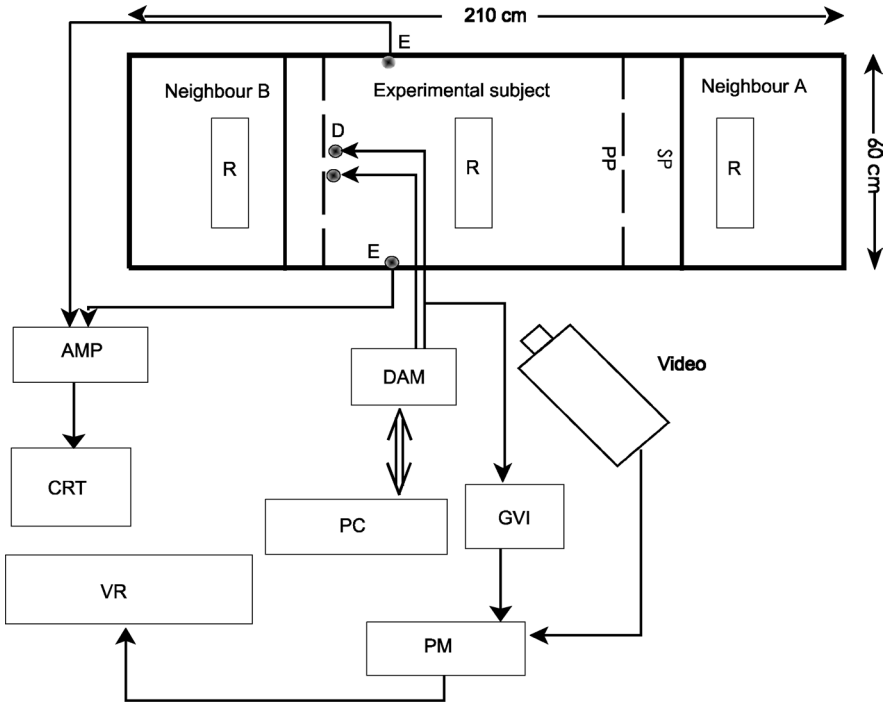
Figure 1. Set-up used for playback experiments with *Marcusenius macrolepidotus*. AMP, differential amplifier; CRT, oscilloscope; D, stimulus dipole; DAM, digital-to-analog converter with memory; E, electrode; GVI, full-way rectifying and summing amplifier with Schmitt trigger; PC, computer; R, resting position of fish; PP, plastic-mesh partition; SP, solid plastic partition; Video, videocamera; PM, pulse marker; VR, videotape recorder.

One out of six males available as experimental subjects did not engage in aggressive encounters with its neighbours and never responded to playback of EODs in any noticeable way ($N = 24$ trials). Therefore, we excluded this fish from further study.

Experiments were performed at reduced light intensity during daytime (8 lux, measured under water at the aquarium bottom) because fish became less active at higher light intensities. During all experiments the experimental subjects were electrically and visually shielded from both neighbours by two solid plastic partitions, in addition to the plastic-mesh partitions. The solid plastic partition attenuated electric field intensity, as measured with a 1-cm dipole, below detection level (measurement of EOD field potentials, see below). No behavioural responses of neighbours thus isolated to playback EODs from an experimental subject's territory were observed. However,

on removal of the solid plastic partition both fish immediately displayed vigorous parallel swimming at the plastic-mesh partition for a few minutes.

Stimuli

Given the high stability of EOD waveforms (Kramer & Westby, 1985; Bratton & Kramer, 1988), a single EOD represents all EODs of an individual mormyrid fish at a constant water temperature and conductivity (Bratton & Kramer, 1988; Kramer & Kuhn, 1993). In the laboratory, EODs were recorded at 23°C water temperature and 100 μS/cm conductivity, using low-impedance carbon electrodes. EODs were differentially amplified (10×; 0.2 Hz-100 kHz), digitised (Tektronix TDS oscilloscope 420, sampling rate 500 kHz, 13 bit vertical resolution) and stored on computer disk. Conditions of EOD recording in the field were similar (details: Kramer & Van der Bank, 2000). EOD files were downloaded onto a microprocessor-controlled digital-to-analog converter with memory and differential output amplifier (Figure 1, DAM; Kramer & Weymann, 1987). The device had an amplitude resolution of 8 bit at each amplification step and 2048 points per trace at a sampling rate of 500 kHz.

Field potentials generated by the stimulus dipole were measured at the experimental subject's resting position by a pair of glassy carbon electrodes (Sigradur G electrodes, rod diameter 1 mm, rod length 60 mm, insulated except for 10 mm at the tips) with an inter-electrode separation of 1 cm. The peak-to-peak amplitude of DAM-generated EODs, as measured at the resting position of the experimental subject, corresponded to the EOD amplitude generated by fish N1 (SL approx. 14 cm; 2.5 mV$_{P-P}$/cm) measured at the same position, and was used in all experiments. The other neighbour fish used in these experiments generated similar EOD amplitudes.

As already described (Kramer et al., 1998, in prep.; Werneyer & Kramer, 2002), EOD waveforms of all *Marcusenius macrolepidotus* specimens from South Africa used in the present study were biphasic, with a head-positive phase followed by a negative one (Figure 2). EOD duration was estimated using the software package Famos (IMC, Berlin). We used a 2% threshold criterion relative to the P-phase peak-amplitude for estimating onset and termination of an EOD waveform.

For playback, we varied the duration of one male's EOD by artifically increasing or decreasing the sampling rate using FAMOS (fish S20).
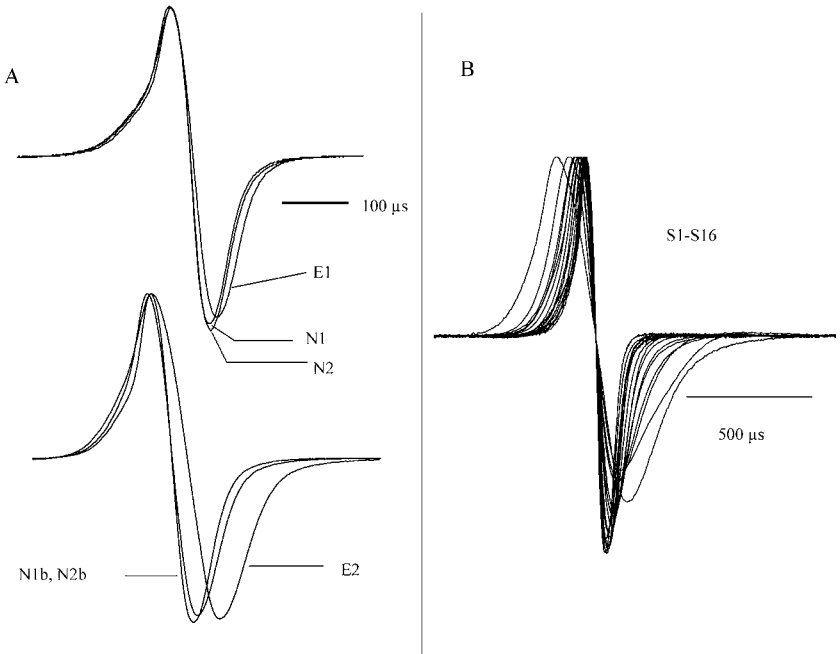
Figure 2.   Electric organ discharge (EOD) waveforms recorded from (A) the experimental subjects (E1, E2), their neighbours (N1, N2, N1b, N2b), and (B) 'stranger' fish (S1-S16). Superimposed EODs are from fish kept in the same tank. The EOD waveforms of N1 and N2 had increased in duration after a 5 months' break and were then termed N1b and N2b, respectively. Voltage over time (baseline = 0 V, head positivity is upwards), waveforms generated by the DAM. All experimental subjects and neighbours were male. Time in μs as indicated by time bars.

Stimulus dipole

EODs chosen for playback were presented using a stimulus dipole consisting of a pair of carbon rod electrodes (5 mm diameter, insulated except for 1 cm at the tips) that were orientated vertically and separated by 30 mm. Attacks directed at an electrically active dipole are well-known in mormyrids, even though the dipole bears no visual, mechanical or olfactory resemblance to an electric fish (Kramer, 1979; Schluger & Hopkins, 1987). The dipole electrode tips were about 5 cm above the aquarium bottom, and the dipole was placed in parallel and right next to the boundary shared with a neighbour (Figure 1).
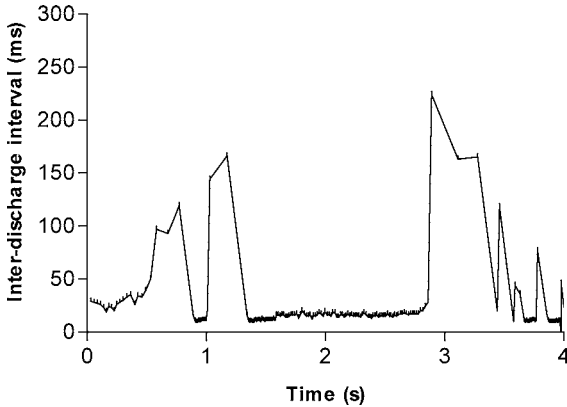
Figure 3. Inter-discharge interval (IDI) pattern of a male *M. macrolepidotus* over time. Points connected by lines to better show trends. IDI recorded of a male engaging in a high-intensity agonistic behaviour (parallel swimming).

Inter-discharge interval (IDI)

Whereas EOD waveforms of individual mormyrid fish vary little over time, inter-discharge intervals vary greatly from moment to moment, and show strong correlation with social context (e.g., Bauer & Kramer, 1974; Kramer, 1978; review Carlson, 2002). The behavioural response of a mormyrid fish to playback of stimulus pulses may depend on the pattern of inter-discharge intervals used for playback (Kramer, 1979; Teyssèdre & Serrier, 1986). Stimulus EODs presented at an IDI pattern that was recorded from a resting fish evoked only weak responses from the experimental subject, whereas the high-discharge rate pattern recorded from a fish displaying parallel swimming often evoked vigorous attacks on the dipole model (up to 65 butts/30 s). For our playback experiments a single IDI pattern was used, a pattern recorded during an aggressive male's parallel swimming (Figure 3). This is a typical behaviour for a pair of male fish at their common territory boundary (Werneyer & Kramer, 2002). The IDI sequence used lasted 4 s and was repeated in an 'infinite loop' during the 30-s stimulation period. The IDI pattern of Fig. 3 starts with intervals of 20-40 ms, followed by shorter intervals (10-20 ms) that are interrupted for about 4 times by long intervals (Figure 3). Stimulus EODs presented at this IDI pattern evoked vigorous attacks on the dipole fish model, especially at the beginning of a test series.

Protocol

Operated by the experimenter, a host computer gave the instructions necessary to select an EOD waveform from DAM memory, and to start and stop the stimulation. A stimulus presentation lasted 30 s, followed by a 2 min period of rest. The sequence of presentation of 2 to 5 different stimulus EOD waveforms used for a particular experiment (hereafter test) was determined using a randomised blocks design (Cochran & Cox, 1968: Table 15.7). Responses of territorial *M. macrolepidotus* to the playback of EODs were observed before, during and after playback. We videotaped the whole test for subsequent analysis. Every stimulus pulse generated by the DAM was recorded on the audiotrack of the videotape and also as a black line on the left side of the video image.

Attack rate was measured as the number of butts directed at the dipole during the 30-s stimulation period. Other kinds of evoked responses, such as the total time spent near the territory boundary during playback, or the latency from stimulus onset to an experimental subject's arrival at the dipole, were not related to EOD waveform: fish usually swam to the dipole immediately and usually remained there for the whole stimulation period.

Neighbour-stranger playbacks

These experiments were designed to determine whether or not resident males discriminate between EODs of familiar neighbours and unfamiliar strangers. All specimens of *M. macrolepidotus* used as 'strangers' had been kept in separate communal tanks from arrival in the laboratory. For neighbour-stranger comparison tests, a stimulus dipole was positioned just 'inside' and close to the regular shared boundary of an experimental subject, a position that should evoke responses from the territory holder both for strangers' and neighbours' stimulus EODs. To confirm the influence of EOD duration on attack rate we also used EODs of artificially increased or reduced duration. The original EOD had a duration of 498 μs which was either reduced or increased within the limits of 213 to 765 μs. An increase in duration was achieved by slowing down the sampling rate of the A/D converter; duration decrease by resampling of the EOD waveform using FAMOS, generating an EOD with fewer points. The waveform parameter ratios — such as the P/N ratio and ratio of areas — remained constant for all modified EODs. In an additional experiment, the dipole position was changed. Neighbours'

EODs were played back either from the regular ('correct') or the opposite ('incorrect') boundary. Experiments were performed with fish that had been neighbours for 1-4 weeks and were repeated after 2-5 months.

Results

Dependency of attack rate on stimulus EOD duration

During all experiments of this study, the experimental subjects were electrically shielded from both neighbours' EODs by solid plastic partitions. All five experimental subjects ignored the stimulus dipole unless a signal was on the electrodes. Upon playback of EODs at the territorial boundary, all fish left their shelter, swam to the dipole at the territory boundary and attacked it. We never observed attacks on the dipole in the 2-min rest period between tests, but especially at the beginning of a test series, fish remained at the territory boundary even after stimulation had stopped. This latter behaviour occurred more often when stimulus EODs had evoked vigorous attacks than when low rates of attacks had been observed.

Attack rate increased significantly with the duration of strangers' EODs (all unmodified) in all three experimental subjects E1-E3 (Figure 4 A-C; Table 1). We repeated this part of the study with two additional experimental subjects, E4 and E5, using the full range of stimulus EOD durations found in this species (Figure 4 D and E). A significant increase of attack rate with EOD duration was also confirmed in these fish ($p < 0.01$ for each individual separately, with at least 45% of the variation explained by EOD duration;

Figure 4. (A-E). Rates of attack on a dipole model evoked in the experimental subjects E1 to E5. For EODs of strangers (■), attack rate (butts/30 s) increased with EOD duration (least-squares regression line, means with ± 95% confidence intervals). A familiar neighbour's EOD (△) of a specified EOD duration evoked weaker responses than expected for an unfamiliar EOD of the same duration (□, extrapolation necessary for fish E3). $N = 55$ tests per fish and data point (A-E). (F) As (A-E), but attack rates shown by fish E1-E3 increased with duration of playback EOD (that had been recorded from the unfamiliar fish S20 and played back unmodified as well as artificially modified in duration). Attack rates for natural, unmodified playback EOD, white symbols; for artificially modified EODs, black symbols. Natural EOD duration, 498 μs; artificially modified EOD duration values, 213, 300, 550, 765 μs. For clarity, attack rates for the latter were slightly displaced horizontally to avoid the clutter caused by overlapping standard error ranges. $N = 45$ tests per fish per data point (see Protocol).
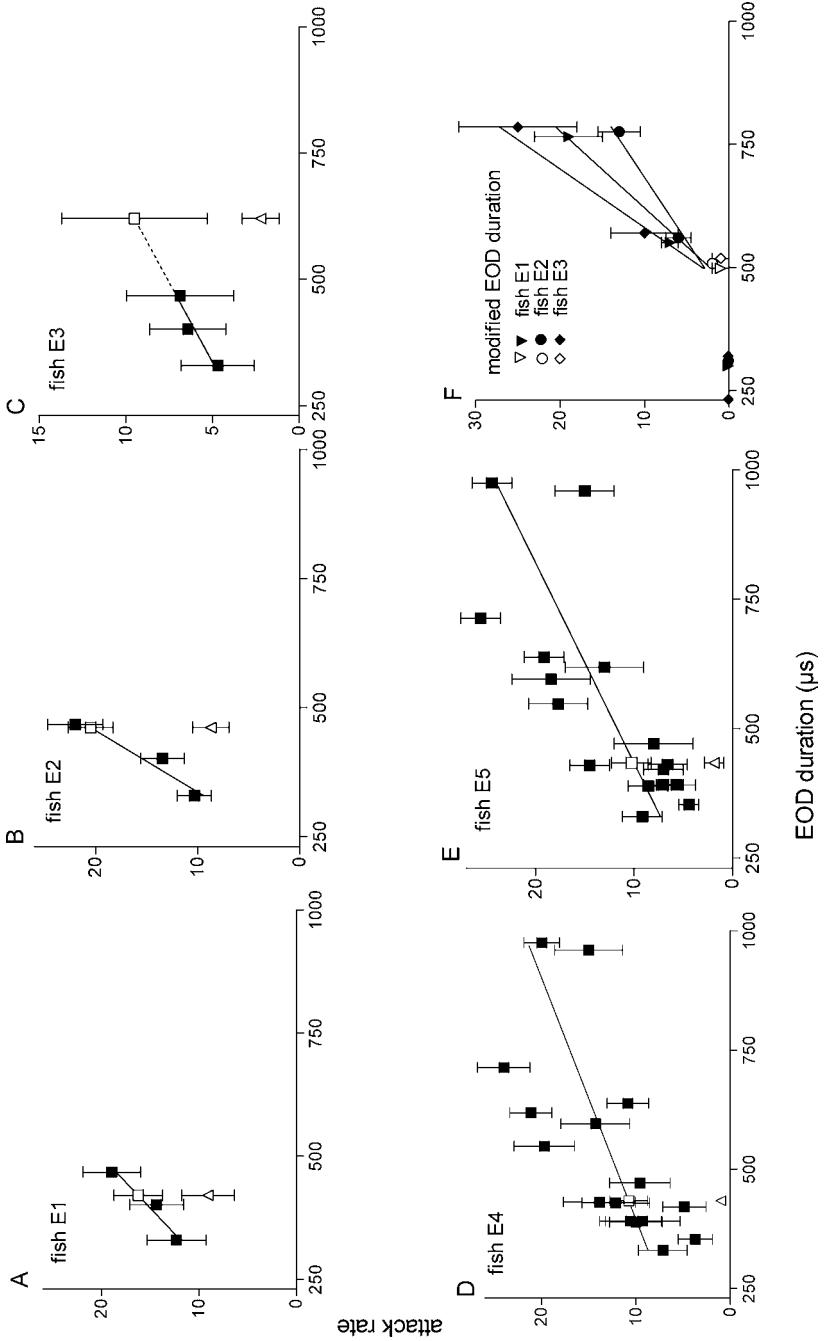
Table 1. Linear regression of the dependency of attack rate on stimulus EOD pulse duration

Exp. subject	Slope ± SD	Y-intercept ± SD	r^2	F	df	p
E1	0.045 ± 0.01	−2.1 ± 6.8	0.45	7.1	163	0.0085
E2	0.01970 ± 0.005	−11 ± 5.2	0.45	14	163	0.003
E3	0.086 ± 0.011	−19 ± 4.6	0.27	56.8	163	0.001
E4	0.025 ± 0.006	−1.2 ± 3.3	0.59	20.12	110	0.0005
E5	0.019 ± 0.005	−2.3 ± 3.3	0.45	11.46	110	0.0044
E1 (A)	0.064 ± 0.0095	−29.7 ± 5.9	0.9780	44.5	163	0.0089
E2 (A)	0.039 ± 0.007	−16.4 ± 4.4	0.9670	29.3	163	0.0035
E3 (A)	0.084 ± 0.016	−38.7 ± 10.2	0.9623	25.5	163	0.0150

(A) Playback experiments with natural as well as artificially modified EOD duration, linear regression line without EODs that evoked no reaction; r^2, variance; p, probability of mistakenly rejecting the null hypothesis of a regression line slope equalling zero.

Table 1). Attack rates varied individually between 7 ± 3.1 for experimental subject E3 and 22 ± 2.7 bites /30 s for E2 at the same stimulus EOD pulse duration (470 μs). However, independent of the individual aggression level, in every experimental subject aggression increased with increasing EOD duration (Table 1).

Attack rate evoked by EODs of artificially modified duration

The relationship between stimulus EOD duration and aggression detailed above also held for a single, pre-recorded EOD the duration of which was artificially varied (213 μs, 300 μs, 550 μs, 765 μs; Figure 4F). The original EOD (of 498 μs that was also used unmodified for stimulation) had been recorded from a male *M. macrolepidotus* that was unfamiliar to all experimental subjects.

None of the three experimental subjects responded to an EOD of artificially reduced duration (213 μs and 300 μs). Whereas the unmodified EOD of 498 μs evoked weak responses in all three fish, the EODs of increased duration, especially the longest one, evoked very strong responses. Compared to the original EOD, and depending on the experimental subject, attack rate increased from 6- to 25 fold when stimulus EOD duration was 765 μs (53% longer than that of the original EOD of 498 μs duration). Starting from the unmodified EOD, aggression increased with EOD duration in similar fashion as shown for a range of natural, unmodified EODs ($p < 0.01$; Table 1).

Neighbour-stranger comparisons

According to 'dear enemy' theory, a fish is expected to respond less aggressively to a familiar neighbour's than to a stranger's EODs. Our null hypothesis deals with each fish separately; it predicts that an individual fish's response to familiar and unfamiliar EODs will be the same (no difference). In this series of experiments, stimulus EODs pre-recorded from an experimental subject's familiar neighbours were contrasted with EODs from stranger fish. We tested whether attack rates evoked by an active dipole playing back EODs differed between EODs used: either familiar neighbours' or strangers' EODs.

In all five experimental subjects, playback of a familiar neighbour's EOD evoked significantly lower attack rates than expected for an unfamiliar EOD of the same duration (Table 2). Non-overlapping 95% confidence intervals for familiar playback EODs, compared with expected intervals for unfamiliar EODs, confirmed this result in each fish independently (Figure 4A-E). In experimental subject E3, the long duration of its neighbour's EOD made extrapolation necessary (Figure 4C). The extrapolation to an EOD duration of 620 μs appears acceptable in view of Figure 4D and E, where natural stimulus EODs of still greater duration were used. Pooling of the data for all fish was not permitted because the level of aggression evoked differed widely between individuals (as can hardly be expected otherwise). We therefore used each individual as its own control.

Location of playback dipole

We asked whether an experimental subject's attack rate evoked by playback of its neighbour's EODs depended on from which side, 'correct' or 'incorrect', it was stimulated. Had the experimental subjects and their neighbours been kept in the same tank for 1-4 weeks only, two out of three experimental subjects did not show any difference in attack rate ($p > 0.1$, two-tailed unpaired t-test, $N = 55$; Figure 5), whereas one did ($p \leqslant 0.036$, two-tailed unpaired t-test, $N = 55$). However, this fish's stronger response to neighbour B's switch of position was offset by the opposite result to neighbour A's position change.

When the experimental subjects and their neighbours had been kept in the same tank for two months or more, playback from a neighbour's opposite, 'incorrect' boundary evoked consistently stronger attack rates than from the
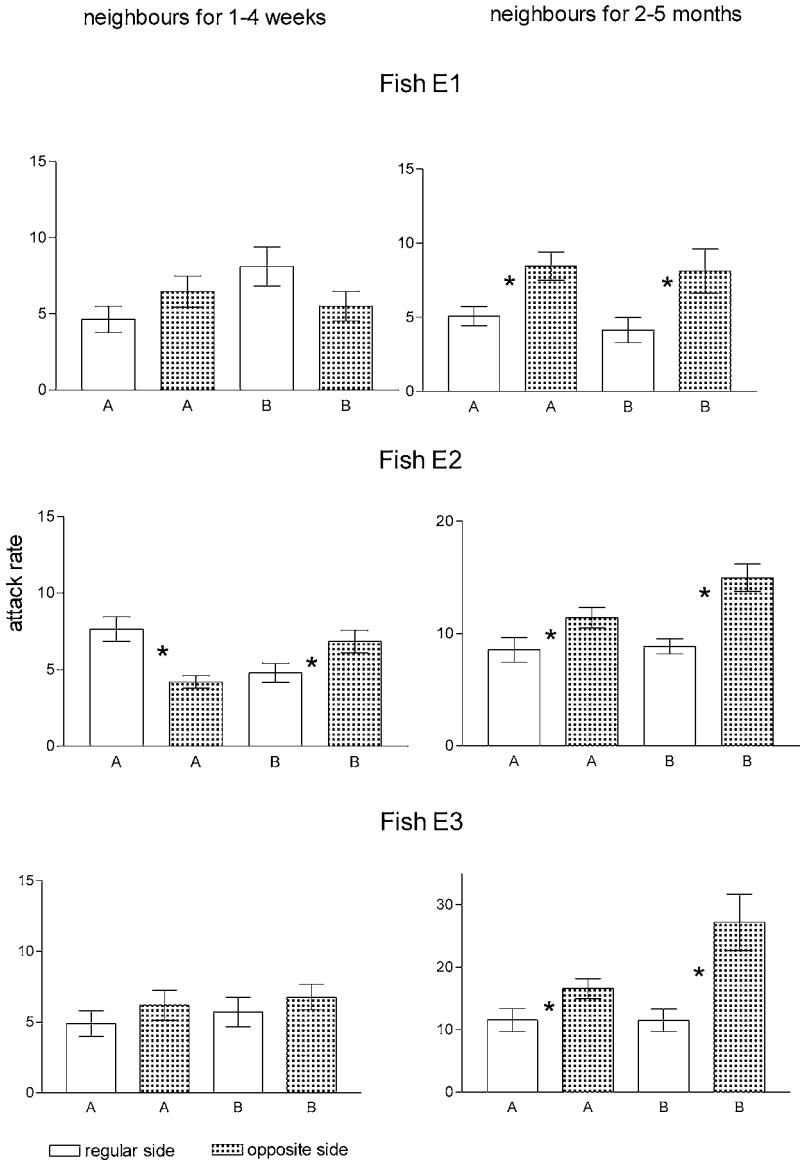
Figure 5. Rates of attack (butts/30 s) on a dipole model of experimental subjects E1, E2, and E3 evoked by the playback of EODs recorded from neighbouring males (A and B) that were presented either from the 'regular', common territorial boundary (white columns), or from the opposite territorial boundary (filled columns). Fish had been in the same tank for either one to four weeks (left panel), or two to five months (right panel). Means with $\pm$ 1 standard errors. Asterisks indicate significant differences at $p < 0.05$; $N = 55$ tests per fish and data point.

Table 2. Comparison of observed attack rates, evoked by familiar EODs, with expected attack rates for unfamiliar EODs of the same duration, for experimental subjects E1-E5

Exp. subject	E1	E2	E3	E4	E5
Observed (familiar)	9.1 ± 2.7	8.7 ± 2.5	2.2 ± 1.1	1.0 ± 0.49	1.86 ± 0.98
Expected (unfamiliar)	16.2 ± 2.5	20.51 ± 2.1	9.5 ± 4.2	10.7 ± 2	10.3 ± 2.0
t^1	8.688	14.35	7.516	12.55	9.975
df	38	38	38	12	12
p	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[1] unpaired t-test (two-sided).

$N = 20$ per playback EOD (experimental subjects E1-E3).

$N = 7$ per playback EOD (E4 and E5).

regular, 'correct' boundary ($p < 0.05$, two-tailed unpaired t-test, $N = 55$; Figure 5). Males showed differential behaviour (in the expected direction) even when the playback EODs were so similar that their negative phases differed by only 9 μs (see EOD waveforms for neighbours N1 and N2; Figure 2).

Discussion

A high degree of intraspecific variability in male EOD waveform seems to be typical for the genus *Marcusenius*, as confirmed in *Marcusenius senegalensis* (Scheffel & Kramer, 1997), in the South African form of *M. macrolepidotus* that was used in the present study (Kramer et al., 1998, in prep.), and still more so in the Upper Zambezi form of *M. macrolepidotus* (Kramer, 1997a, b). Our study suggests two functions for EOD variability in males: (1) a wide range of EOD durations may facilitate intrasexual competition between males, and (2) allow discrimination between neighbours and strangers. Additionally, variability in male traits in a genus or a family seems to facilitate the evolution of a sexual dimorphism in some of its members (Burkhardt & de la Motte, 1987). The sexually dimorphic Upper Zambezi form of *M. macrolepidotus*, and the less advanced South African form studied in the present paper, are examples for this rule (Kramer, 1997a, b).

Dependency of attack rate on EOD duration

An EOD of long pulse duration may indicate a high fighting potential in a male fish, because in the South African form of *M. macrolepidotus* studied in the present paper, EOD pulse duration increased weakly, but significantly with size in males (Kramer et al., 1998, in prep.). Therefore, playback EODs of long duration should represent a greater threat to a territory owner, and evoke more intense aggression, than EODs of short duration. Our experimental subjects behaved as expected, and attack rate increased with EOD duration over the full range of our field-recorded EODs (up to 975 μs duration). This result suggests that the high variability of male EODs in our fish is related to intrasexual selection.

Male communication signals are often used in the assessment of a rival. In the croaking gourami *Trichopsis vittata* the vocalisations generated by winners differed from those of losers by a higher sound pressure level and a lower dominant frequency, both of which were correlated with body weight (Ladich, 1998). Also in frogs, toads (Davies & Halliday, 1978; Robertson, 1986; Wagner, 1989) and red deer (Clutton-Brock & Albon, 1979), physical features of threatening calls (Reby & McComb, 2003) are used by rivals to avoid useless fighting.

The reason why our experimental fish subjects seemed prepared for escalating so readily, independent of their own size, may be related to the protection from the plastic mesh barrier so that none of the experimental subjects had the experience of loosing a fight.

A high inter-individual variability in communication signals may serve different functions even in the same species. In damselfish, for example, males discriminate territorial rivals by their individually distinct vocalisations (Myrberg & Riggio, 1985) whereas females use these signals so select mates (Myrberg et al., 1993). In *M. macrolepidotus* it is unknown whether there is female choice based on EOD waveform.

'Dear enemy' phenomenon

Playback EODs from neighbours and strangers evoked differential responses both in the South American gymnotiform *Gymnotus carapo* (McGregor & Westby, 1992), and in all experimental subjects of the African mormyrid, *M. macrolepidotus*, that were studied in the present paper. In both fish, strangers'

EODs evoked a stronger rate of aggressive responses than familiar neighbours' EODs. In both studies, cues from the individually distinct EOD pulse waveform alone were sufficient to evoke a change in behaviour. In the present study an additional relationship, not seen in the gymnotiform study, emerged: attack rate increased with EOD pulse duration. Our results in *M. macrolepidotus* are consistent with the high discrimination performance demonstrated among intraspecific natural and artificial EODs in *Pollimyrus adspersus* using the 'conditioned discrimination' method (Graff & Kramer, 1992; Paintner & Kramer, 2003).

As the 'dear enemy' phenomenon is thought to be based upon the relative threat presented by neighbours versus strangers (Temeles, 1994), our results suggest territoriality in male *M. macrolepidotus*. At least temporal territoriality in this species was suggested by intense male-male interactions observed in large aquaria (Werneyer & Kramer, 2002). Further support comes from reproduction behaviour observed in aquarium. For spawning, the female visits the male's territory repeatedly over many hours in one night (Werneyer & Kramer, in press). This is similar to the only other mormyrid species whose reproductive behaviour has been studied in detail, *Pollimyrus adspersus* (Crawford et al., 1986; Kirschbaum, 1987; Bratton & Kramer, 1989). Therefore we can state that in the context of a reliable neighbourhood *Marcusenius macrolepidotus* males are able to establish a 'dear enemy' relationship.

Our results suggest that male *M. macrolepidotus* identify their neighbours individually by EOD waveform, however, we can neither exclude additional EOD cues, such as inter-discharge interval patterns, nor chemical, acoustical, or visual cues in the natural situation. Natural EODs provide sufficient intraspecific variability for making possible the discrimination between large numbers of individuals (Bratton & Kramer, 1988; Paintner & Kramer, 2003). Conditions remaining constant, long- and short-term stability of EOD waveform has been demonstrated (Kramer & Westby, 1985; Bratton & Kramer, 1988; Crawford, 1991), supported by a low Q_{10} minimising temperature effects (Kramer & Westby, 1985), and by the capacity of rapid impedance matching of the electric organ even when water conductivity changes drastically (Kramer & Kuhn, 1993). The long time it took in our experiments for a differential response to a neighbours' EODs to evolve may indicate a very slow increase in readiness for reproduction, perhaps brought about by mutual electrical, visual, acoustic, or tactile stimulation, activating the pituitary-gonad axis.

Conclusion

Intraspecific variability in male EOD waveform seems to have at least two functions in intrasexual selection in *M. macrolepidotus* (South African form): (1) to signal fighting potential, since the level of aggression evoked increased with duration of playback EODs; (2) to facilitate the discrimination between neighbours and strangers, which reduces useless fights.

The comments of ten Cate et al. (2002) on evolution of bird song largely apply also here. According to these authors, four criteria should be met for evolution of a communication signal, such as EOD, to be influenced by male competition so that they are related to quality: (i) interindividual variation in EOD waveform should be present, (ii) the variation should correlate to competitive ability (e.g., size), (iii) some variants of the signal can only be produced by males with the highest competitive ability, and (iv) receivers, in this case males, should differentiate their responses according to the rivals' communication signals. All these preconditions are met in *M. macrolepidotus* (South African form) — except perhaps (iii), if variants are meant to be qualitative. Therefore, we conclude that evolution of EOD duration is greatly influenced by intrasexual selection.

We do not yet know whether or not male EOD variability is also supported by female choice in *M. macrolepidotus* (South African form). In any case, 'runaway' selection for EODs of still longer duration appears to be limited by electroreceptive predators like the sharptooth catfish, *Clarias gariepinus*. This fish detects long-duration EODs (>2 ms), as generated by male *M. macrolepidotus* from the Upper Zambezi (Hanika & Kramer, 1999, 2000). Accordingly, bulldogs from the Okavango/Upper Zambezi River system are heavily preyed upon by *C. gariepinus* and other catfish (Merron, 1993; Winemiller & Kelso-Winemiller, 1994). In contrast, bulldogs from South Africa with male EODs of much shorter duration (up to 880 μs in our study, which is below the detection threshold of *C. gariepinus*) have not been found to form part of *C. garipienus'* diet (Bruton, 1979). Consequently, South African bulldogs seem to have found a way to use their communication signals in male-male competition without alerting predatory catfish, unlike their cousins from the Upper Zambezi.

Acknowledgements

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