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Group behaviour of a nocturnal weakly electric mormyrid fish: investigations using an electro-communicating dummy fish

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ABSTRACT

Background: Tapir fish possess organs that emit weak electric discharges. *Mormyrus rume proboscirostris* has been observed to show group behaviour in captivity and electrical signalling is believed to play a crucial role in group communication and coherence.

Organism: *Mormyrus rume proboscirostris* (tapir fish).

Questions: (1) What role does electro-communication play in the formation and coherence of groups of three freely swimming weakly electric fish? (2) What happens to the group when a dummy fish that emits electric discharges is substituted for one of the fish?

Hypotheses: (1) Individuals will show specific patterns of electro-communication and motor behaviours when at different positions in a natural group. (2) When the fish swimming at the front is replaced by a dummy fish emitting the same series of electric signals, the structure of the group will persist and the remaining fish will behave to some extent as they would in a natural group.

Methods: We recorded and analysed the natural motor and electrical behaviours of tapir fish in groups of three freely swimming fish. We focused on their electric discharge patterns, interactive communication, and group coherence. Then we replaced the first fish with an artificial dummy fish that mimicked the electrical behaviour of the substituted individual. We analysed the coherence of the group, as well as the electrical signalling behaviour of the two remaining fish.

Results: Fish behaved similarly in the presence of an electro-communicating dummy fish as when following the first fish in a natural group. When the dummy came to a standstill but continued to emit electric signals, the fish remained significantly closer to the dummy than to a real fish. The electrical behaviour (regularization, double-pulses) of fish in mixed groups was similar to that of fish in natural groups. The interactive electro-communication patterns of the fish were directed towards the dummy in a similar way as to real fish. They were even more abundant in mixed groups, presumably because the dummy could not respond dynamically to the fish.

Keywords: electro-communication, group behaviour, robotic fish, weakly electric fish.

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INTRODUCTION

Inhabiting a dark environment is challenging for many vertebrates, since vision cannot be used for orientation and intraspecific communication. As a solution, nocturnally active weakly electric fish in Africa (Mormyridae) evolved an active electric sense (Moller *et al.*, 1979; von der Emde, 1999), which not only allows the fish to orientate and navigate in complete darkness (Cain *et al.*, 1994) but also to communicate with conspecifics (Moller, 1995). The electric organ is located in the caudal peduncle and is composed of modified muscle cells that have lost the ability to contract (Paul *et al.*, 2015). A discharge by this organ (electric organ discharge, EOD) builds up a three-dimensional electric dipole field around the fish (Bennett, 1971). Mormyromast electro-receptor organs, which are distributed over almost the entire skin surface of the fish, respond to the locally occurring EODs and to distortions induced by objects in the electric field. This enables the fish to probe their immediate environment and thus perform *active electrolocation* (Harder, 1968; von der Emde, 1999; Hollmann *et al.*, 2008). As a result, mormyrids are not only able to detect nearby objects, but can also distinguish between objects differing in size, shape, and material composition (Bell *et al.*, 1989; von der Emde, 2006; von der Emde *et al.*, 2010). Furthermore, weakly electric fish employ active electrolocation to find and identify prey items when foraging at night (von der Emde and Bleckmann, 1998).

With a second type of electro-receptor organs, the Knollenorgans (Derbin and Szabo, 1968), weakly electric mormyrids can perceive the EODs of other electric fish, especially conspecifics (Baker *et al.* 2013). Knollenorgans function as time coders and fire a single spike upon the occurrence of a foreign EOD (Bennett, 1965). Mormyridae are African pulse-type fish (Kramer, 1990), in that they emit brief pulse-type electric signals with a rather constant, species-specific, and sometimes sex-specific waveform but highly variable inter-discharge intervals (IDIs). The EOD waveform changes only slowly depending on age, reproductive state, and sometimes dominance relationships of the sender (Zakon, 1987; Hopkins, 1999; Carlson *et al.*, 2000; Terleph, 2003). In contrast to the EOD waveform, individuals can rapidly vary the IDIs between single EODs. For example, some mormyrids can be silent for several seconds and then suddenly start pulsing at frequencies of up to 100 Hz (Kramer, 1974; Grant *et al.*, 1999). These variable temporal patterns of IDIs convey information about the current motivational and behavioural state of the sender in different behavioural situations (foraging, resting, etc.) and during social interactions (aggression, reproduction, group interaction, etc.) (Moller 1970; Carlson and Hopkins, 2004; Baier and Kramer, 2007). Because weakly electric fish use their EODs for both electro-communication and for active electrolocation, it is sometimes difficult to determine which of these two functions controls the current emission pattern of electric signals of a fish.

Similar to flocks of birds that use specific vocal signals during foraging or migration to cohere the group and exchange social information (Marler, 2004), weakly electric fish are considered to use electro-communication when living in groups (Moller, 1976; Arnegard and Carlson, 2005). In the present study, we used the African mormyrid *Mormyrus rume proboscirostris* (hereafter referred to as *M. rume*), because in earlier studies this species showed social group behaviour in captivity that was accompanied by electro-communication (Gebhardt *et al.*, 2012b; Worm *et al.*, 2014). *Mormyrus rume* emits specific IDI patterns that could be linked to distinct behavioural conditions, such as swimming, aggression, and foraging (Gebhardt *et al.*, 2012b). In addition, it has been suggested that some IDI patterns might play a crucial role in the formation and coherence of groups. So-called 'double-pulses', for example, compromise a signalling pattern, which previously was suggested to function as a territorial courtship

(Baier and Kramer, 2007) or an aggressive signal (Carlson, 2002), but was later also described to occur during non-aggressive interactions in groups of *M. rume* (Gebhardt, 2012). By using an artificial electro-communicating dummy fish, Donati *et al.* (2016) and Worm *et al.* (2017, 2018a) were able to show that the emission of EODs by the dummy was crucial for the recruitment of single individuals or small groups of *M. rume* from a sheltered area into an open-field sector of an experimental tank. In the experiments conducted by Worm *et al.* (2017), neither the specific IDI pattern nor the overall EOD frequency played a role in recruitment, but double-pulses and discharge regularizations of the fish varied depending on the distinct playback patterns emitted by the dummy.

In addition to double-pulses, several other communication patterns produced by weakly electric fish have been suggested to play a role during electro-communication. For example, 'discharge accelerations' have been described as an aggressive signal in several mormyrid species (Bell *et al.*, 1974; Kramer, 1976; Carlson, 2002), while short or long 'pauses' of EOD production have been suggested to play an aggressive or submissive signalling role (Moller *et al.*, 1989; Baier and Kramer, 2007). So-called 'regularizations' of IDI duration have been reported to occur during swimming in *M. rume* (Gebhardt, 2012) and are often associated with an increase in discharge frequency, leading to a higher spatiotemporal resolution during active electrolocation because of the constant adaptation state of the electroreceptors (von der Emde, 1992; Hofmann *et al.*, 2013). However, regularizations have also been observed in social contexts and might have a communicative function as well (Bell *et al.*, 1974; Moller *et al.*, 1989; Terleph, 2004).

Electro-communication in mormyrids also involves interactive electrical discharge behaviours. For example, during the 'echo-response', a fish responds to an EOD of a conspecific by emitting a signal of its own at a fixed 'preferred latency' of several milliseconds (Kramer 1974; Russell *et al.*, 1974). In *M. rume*, echo-latencies range from 15 to 25 ms (Carlson, 2002; Gebhardt *et al.*, 2012a; Worm *et al.*, 2017). The function of this behaviour is debated in the literature (Heiligenberg, 1976; Lücker and Kramer, 1981; Carlson, 2002; Arnegard and Carlson, 2005). Continuous echo-responses during a social encounter result in a 'synchronization' of the discharges of two individuals (Gebhardt *et al.*, 2012a). In groups of several fish, individuals were found to switch synchronization partners, resulting in dynamic interactive patterns, which were suggested to lead to better group coherence (Arnegard and Carlson, 2005; Gebhardt *et al.*, 2012a).

In the present study, we addressed some basic questions relating to the group behaviour of *M. rume*. First, we aimed to shed some light on the role of electro-communication for the formation and coherence of social groups of three freely swimming fish. Since earlier studies had shown that the emission of electric playback signals by artificial group members was essential for social interactions and group behaviour to occur (Donati *et al.*, 2016; Worm *et al.*, 2017, 2018a), we hypothesized that individuals would show specific patterns of electro-communication and motor behaviours when occupying different positions in a group. In additional experiments, we substituted the individual swimming at the head of a group with an EOD-emitting artificial dummy fish. We predicted that if the dummy mimicked the real fish's electrical behaviour, the structure of the group would persist and the remaining fish would behave in a similar way to that in a natural group setting.

MATERIALS AND METHODS

Fish and husbandry

In this study, we used 24 *Mormyrus rume* individuals of approximately 2 years of age. The fish were bred in the laboratory at the Humboldt University Berlin (Schugardt and Kirschbaum, 2004). The standard length (SL = tip of the snout to the end of the vertebral column or posterior edge of the hypural plate) of individuals ranged from 6.6 cm to 11.6 cm, while their sex could not be determined owing to their small body size (Moller *et al.*, 2004).

In the home tanks, although several *M. rume* were housed together, individuals were kept apart by a water-permeable barrier. Thus, the fish could not interact physically but were still able to perceive the EODs of their neighbours. Direct neighbours were never tested together in the experiments to avoid any bias due to familiarization. In addition, this type of husbandry allowed individuals to be identified before and after trials. Fish were kept under a 12 hour light/12 hour dark cycle and water temperature was maintained at 25 ± 1°C. The fish were fed defrosted bloodworms each weekday. When used in experiments, fish were not fed prior to testing.

The experiments were approved by the state authority (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, LANUV, reference #84-02.04.2015.A444). All experiments were carried out in accordance with the guidelines of German law, the animal welfare regulations of the University of Bonn, and the 'Guidelines for the treatment of animals in behavioural research and teaching' (Association for the Study of Animal Behaviour, 2012).

Experimental set-up

Eight groups of three *M. rume* individuals ($N = 24$) were tested in this study. The groups were assembled based on the relative sizes of the individuals: each group consisted of one small, one medium, and one large fish with a minimum size difference of 0.4 cm and a maximum size difference of 1.5 cm. This group structure allowed us to distinguish between individuals in the video recordings. As one individual died at the beginning of the experimental sessions, one of the remaining 23 individuals (fish #18) was assigned to two groups (groups #4 and #7). To avoid interference, these two groups were tested 3 weeks apart.

The experimental programme consisted of two parts. In the first, *natural* groups of three fish were tested; in the second, the fish swimming at the front of each natural group was replaced by an artificial dummy fish resulting in a *mixed* group of two fish and a dummy. All experiments were conducted in a test tank (200 cm long × 50 cm wide × 50 cm high; Fig. 1), the floor of which was covered with white sand. The temperature of the water had a mean value of 27.3 ± 0.3°C, and the electrical conductivity was 100.8 ± 3.0 $\mu S \cdot cm^{-1}$. The experimental tank was divided into three areas. A segregated inaccessible area (87 cm) at one end contained the heater and the drainpipes. The living area (30 cm long) and testing area (83 cm long) were separated from each other by a plastic wall with a gate (9 cm wide × 16 cm high) that was permanently open. The 'finish line' (FL) at the end of the testing area was defined as the virtual line at the back end of the dummy at its stop position (Fig. 1). This line had to be crossed by all fish in natural and mixed groups at specific times after the trial started (see below). For video recordings, two cameras (Imaging Source DBK 21AF04, The Imaging Source Europe GmbH, Bremen, Germany) were placed centrally above the living and the testing areas (frame rate = 15 frames per second).

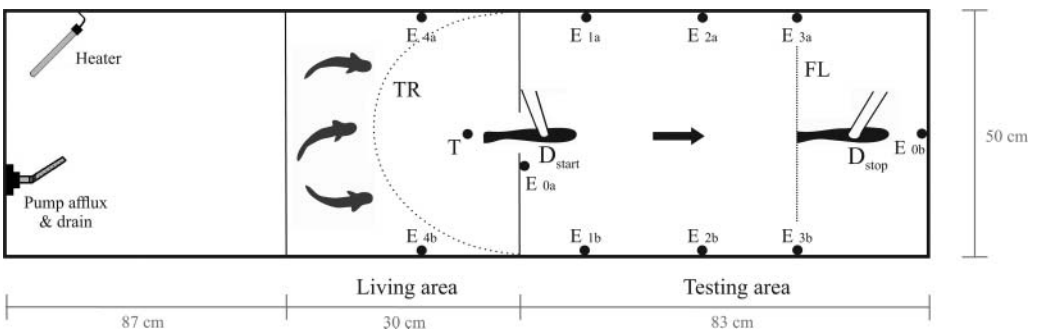

Fig. 1. Schematic representation of the experimental tank seen from above. T = trigger electrode, D_{start} = start position of the dummy, FL = finish line and end position of the dummy, E_{xa}–E_{xb} = electrode pairs. The dotted line (TR) in the living area shows the estimated threshold radius of the trigger electrode, in which the EOD of a fish entering could trigger the beginning of a trial. Modified after Worm *et al.* (2017).

For recording the EODs of the fish, five pairs of electrodes were placed in the tank: four pairs in the testing area (Fig. 1, E0–E3) and one pair in the living area (Fig. 1, E4). Each electrode consisted of a water-exposed graphite tip connected to a shielded coaxial cable. The electrodes of each pair were connected to an amplifier (Brownlee Precision Model 440, NeuroPhase LLC, Santa Clara, CA). Recorded signals were A/D-converted (CED Power 1401, Cambridge Electronic Design, Cambridge, UK) and recorded at a sampling rate of 50 kHz using the software Spike2 v.5.12a (Cambridge Electronic Design, Cambridge, UK). A trigger electrode (T) was buried in the sand of the living area in front of the gate (Fig. 1). When a fish emitted an EOD nearby, it started a trial by emitting a trigger signal through an oscilloscope (Yokogawa DL1620, Yokogawa Electric Corp., Tokyo, Japan). When the trigger was emitted, recordings of the electric discharges of the fish and the video were begun simultaneously.

The dummy fish used in the mixed groups was made from a black, 10 cm long fishing bait (Kopyto Relax). The mean size of fish used in this study was 8.7 cm. For emitting electrical playback signals (pre-recorded EOD of a *M. rume*), two graphite electrodes were inserted into both ends of the dummy. The resulting electrical field produced by the dummy resembled the electrical field of a real fish both in amplitude and shape.

Experiments

At the beginning of an experiment, the three fish of a natural group were placed in the living area of the tank. Over the next 120 minutes, the fish were observed while they swam freely in the tank. The recordings of EODs and video for each trial lasted 15 seconds. A trial was considered 'successful' if all three fish swam into the testing area, and all individuals crossed the finish line (Fig. 1, FL) within a specific time: the first fish within 7 seconds, the second and third fish within 15 seconds (the end of a recording). For all natural groups, at least one successful event was recorded. One successful trial per group was chosen at random and used for subsequent analysis. In Spike2, all recorded EODs of a trial were assigned to the emitting individual (Gebhardt *et al.*, 2012b) and transcribed into a time-series for further analysis.

Each natural group was tested 2 days later as a mixed group with the fish at the front being replaced by the artificial dummy fish. In mixed-group trials, the EOD time-series of the first fish of the respective natural group was transcribed into a playback which the dummy emitted. For that, it was passed on by Spike2 as a waveform timing output to the CED D/A converter, followed by a dB attenuator and an analogue stimulus isolator (model 2200, A-M Systems Inc., Carlsborg, WA). The attenuator was needed to adjust the amplitude of the emitted signals of the dummy to those of a live fish.

The experimental procedure used for mixed groups was similar to that used with natural groups. Before the trials started, the dummy was placed in the middle of the gate between the living and testing areas (Fig. 1, D_{start}). The two remaining fish of the natural group were put into the living area, and the experiments were begun without any acclimatization. The dummy did not move or emit electrical signals until a trial was started by a real fish, which set off the trigger by approaching the trigger electrode. This trigger signal started recordings of the EODs and the video. Simultaneously, the dummy started to move and emit playback-EODs for a period of 15 seconds. The dummy moved at a velocity of 0.11 $m \cdot s^{-1}$ in a straight line through the testing area for 7 seconds. Movement was induced by a DC motor pulling a slide above the tank, which was connected to the dummy by a plastic rod (Worm *et al.*, 2017). After 7 seconds, the dummy stopped at the finish line (Fig. 1) but continued to emit EODs for another 8 seconds.

A trial was considered 'successful' if both live fish followed the dummy through the testing area and crossed the finish line within 15 seconds. For each mixed group, trials were recorded until four successful trials had occurred, one of which was chosen at random for further analysis. An experimental period of 2 hours, as for natural groups, was not necessary because successful events were more common in all mixed groups and no more than seven trials were necessary to record four successful ones in a maximum of 20 minutes. Thus, almost all trials that commenced owing to the passing of the trigger's threshold by the fish was a successful trial. Further analysis of successful trials in mixed groups could not be performed as unsuccessful trials were not recorded.

Both arms of the experiment (natural groups and mixed groups) were conducted in dim light of approximately 11 lux using two LED spots. The spots were directed towards the walls so that the illumination was indirect and uniform in the test tank, creating 'dusky' light conditions.

Video analysis

On the recorded videos, individuals were identified according to their size using the program ImageJ v.1.50i (National Institutes of Health, Bethesda, MD). For tracking of the fish, we used the software Ctrax (Branson *et al.*, 2009). The centre distances between all group members in natural and mixed groups were determined for each frame of the trial. As not all three fish entered the testing area at the same time, the calculation of mean centre distance per frame for all test groups was based on different numbers of fish during the first few seconds of a trial only. To correct for mistakes in individual tracking and for further analysis of tracking data, we used MATLAB (R2016a, The MathWorks Inc. Natick, MA) and the toolboxes provided with Ctrax.

Electric discharge analysis

The electric discharge behaviour of each fish in a group was analysed by identifying specific discharge patterns (i.e. double-pulses, pauses, regularizations), as well as interactive patterns of all possible communication partners (i.e. echo-responses, synchronizations) (Worm *et al.*, 2017, 2018b). IDI patterns were analysed with regard to the positions of the individual within the group (first, second or third swimming fish) and, when possible, averaged for the eight groups tested. For the analyses of all IDI patterns, we used custom written MATLAB scripts (Gebhardt *et al.*, 2012a, 2012b; Worm *et al.*, 2017, 2018a, 2018b).

We defined *double-pulses* as IDI sequences of at least five consecutive alternating short (≤50 ms) and long (≥60 ms) IDIs. We calculated the mean number of double-pulses per trial for each individual in both natural and mixed groups and subsequently the mean for test groups. Discharge *pauses* were defined as IDIs longer than 450 ms and their number per trial, as well as the longest pause for each individual, were determined.

Echo-responses were quantified by measuring the latencies at which each group member responded to all other fish of a group (including the dummy). Echo-responses were analysed using the EOD-sequence of a potential communication partner as a reference and calculating the latencies with which the fish generated EODs in response to the partner's EOD. Also, we inverted the relative cumulative histogram of each reference IDI distribution to obtain the expected latency distribution if the IDI sequences of the two fish were independent. Echoing behaviour was then quantified according to Kramer (1974) by calculating the 'echo-quotient', i.e. the ratio of observed relative latencies at the mode of the latency distribution relative to the number expected at that latency. The modes used for this calculation were in the range of 14–20 ms following previous research in which echo-responses of *M. rume* were described to occur at similar latencies (Gebhardt, 2012b; Worm *et al.*, 2017, 2018b).

While the echo-response histograms and echo-quotients provide information about how common each 'echo' was during the 15 second duration of a trial, the temporal course of interactive signalling is omitted. Echo-responses may lead to the *synchronization* of electric discharges of the communication partners if produced continuously over a distinct period. Thus, the adaptive cross-correlation coefficients were determined, according to the procedure described by Gebhardt *et al.* (2012a, 2012b) and Worm *et al.* (2017, 2018b). In detail, the IDI sequences of each possible communication pair were transformed to high-resolution time-series by exponential filtering. Pearson's correlation coefficients were then determined for time lags (tau) of up to 200 ms, for a 'response time' of ±100 ms between the two time-series of two individuals (both ways separately) and over the trial duration of 15 seconds. For further details on the calculation of cross-correlation coefficients, see Gebhardt *et al.* (2012b).

Subsequently, the maximum cross-correlation coefficient of each high-resolution time point was extracted and colour-coded in a contour plot for each test group displaying the correlation lag of one fish towards its communication partner as positive 'response time' and the correlation lag of the partner fish as negative 'response time'. In these plots, the time course of synchronization and its reciprocity between individuals become apparent. For statistical analysis, we averaged the maximum cross-correlation values of each individual to all possible communication partners over the complete trial duration of 15 seconds and then calculated the median of the mean maximum cross-correlation over the eight test groups. As a result, we obtained an overall synchronization value of all communication partners to the other two group members.

As an indicator of the *regularization* of a fish's discharge, we calculated the maximum autocorrelation coefficients of each individual with its own discharge in a time frame of ±100 ms by applying the same exponential filter as for calculating the maximum cross-correlation coefficients. A contour plot displays the degree of regularization over a trial's duration with high autocorrelation coefficients indicating a high degree of regularized discharges, i.e. periods of IDIs with similar duration. Again, the maximum autocorrelation coefficients were averaged for all first, second, and third swimming fish over the duration of each trial, and subsequently the median over all eight test groups of those means was calculated.

The statistical analyses were performed in IBM SPSS Statistics for Windows v.22.0 (IBM Corp., Armonk, NY). We used the Shapiro-Wilk test to determine whether data were normally distributed and the respective parametric (ANOVA for repeated measures, paired-samples *t*-test) or non-parametric tests (Friedman's two-way ANOVA and Wilcoxon signed-rank test) were subsequently applied. Statistical significance was accepted at an α level of 0.05.

RESULTS

Group dynamics

In natural groups, during the overall observation period of 120 minutes, all events were recorded for when a single fish, pairs of fish or groups of three fish swam into the testing area. In most cases, just one test fish swam alone into the testing area. Somewhat less common were group sizes of three or two fish per group. Only in group 4 did fish swim most often as a group of three into the testing area (Table 1).

In 50% of trials the small fish and in the other 50% of trials the medium fish swam at the front of a group, never the large individual (Fig. 2A). Second position was most often occupied by large individuals (62.5%) while third position was occupied equally often by medium and large fish (both 37.5%). Since large fish never occupied first position in natural groups, they were never replaced by the dummy fish in the corresponding mixed groups. Under mixed-group conditions, large fish occupied second and third position equally often (both 50%). In the remaining trials, second position was most often occupied by medium

Table 1. Numbers of observed events when fish swam alone, in pairs or as a group of three into the testing area during the 120 minute observation time when tested in natural conditions

Group	One fish	Pair of fish	Group of three fish
1	11	11	1
2	35	8	3
3	16	8	10
4	16	8	44
5	40	7	3
6	15	8	3
7	11	11	7
8	28	6	24
Total	172	67	95

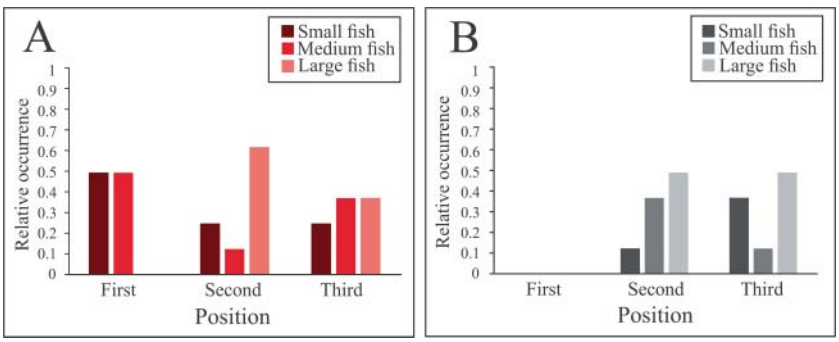

Fig. 2. The relative occurrence of the differently sized fish swimming at the three group positions in the natural (A) and mixed (B) conditions. The graph is divided into the three positions (x-axis: first, second and third) and for each the relative occurrence (y-axis) of the differently sized fish (natural: small = dark red, medium = medium red, and large = pale red; mixed: small = dark grey, medium = medium grey, large = pale grey) that were swimming at the respective positions. $N = 8$ groups.

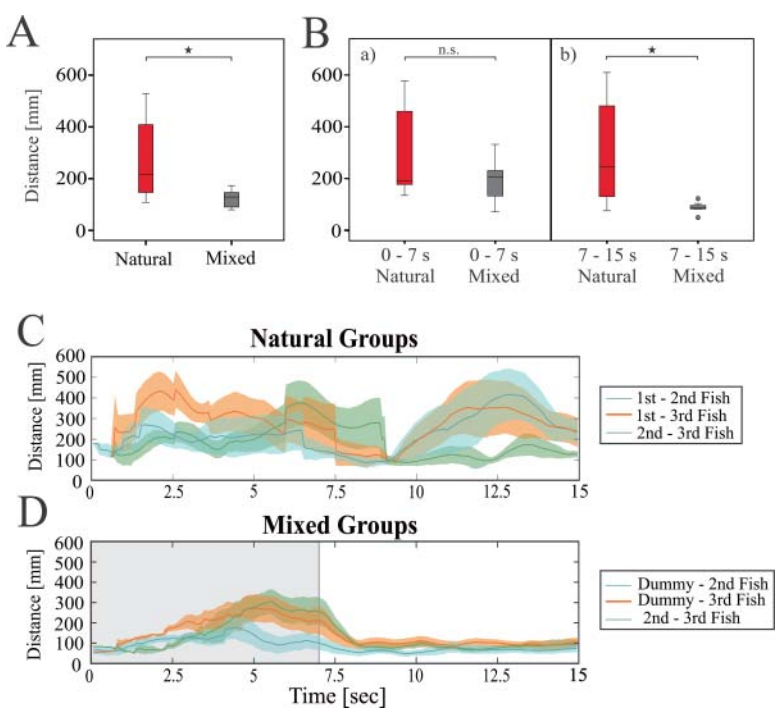

Fig. 3. The median of averaged centre distances between all *M. rume* in natural versus mixed groups (A). In (B), median centre distances are displayed for all fish of both natural and mixed groups for the first half (a) and second half (b) of a trial. The circles represent outliers. Mean centre distances (mm) between group members in natural (C) and mixed groups (D) are shown. The lines display the mean centre distance between group members per frame over the eight groups tested. The transparent coloured area above and below the lines displays the standard errors. Owing to the different numbers of fish entering the testing area, standard errors vary to some extent. In mixed groups (D), the grey shaded area marks the 7 seconds during which the dummy fish was moving through the testing area. $N = 8$ groups.

fish (37.5%) followed by small fish (12.5%). Small fish swam in third position in 37.5% of trials and medium fish in 12.5% (Fig. 2B).

For the comparison of distances between fish in natural and mixed groups, we averaged the distances over the duration of a trial (15 seconds) and for all group members together. A paired-samples *t*-test revealed that the members of natural groups swam significantly further apart from each other than the live fish and the dummy did in mixed groups ($t_7 = 2.81$, $P = 0.026$; Fig. 3A). However, when dividing mixed-group trials into a first part with the dummy moving (seconds 1–7) and a second part with the dummy stationary (seconds 8–15), there was no significant difference in mean centre distances between natural and mixed groups during the first 7 seconds of a trial (Wilcoxon signed-rank test: $z = -1.120$, $P = 0.263$; Fig. 3Ba). In the second part of a trial, again members of mixed groups swam significantly closer together than members of natural groups ($t_7 = 3.015$, $P = 0.020$; Fig. 3Bb). When nearing the end of the testing area (end of the tank), fish in natural groups remained in one corner together resulting in small distances between fish around second 8 (Fig. 3C). Then, in most instances the first fish left the group and swam back towards the living area and the distances between fish increased accordingly. In mixed groups, on the other hand, the dummy reached its end position after 7 seconds and remained there while continuing to emit playback EODs. The two live fish remained close to the dummy, often swimming around, beneath or over it (Fig. 3D).

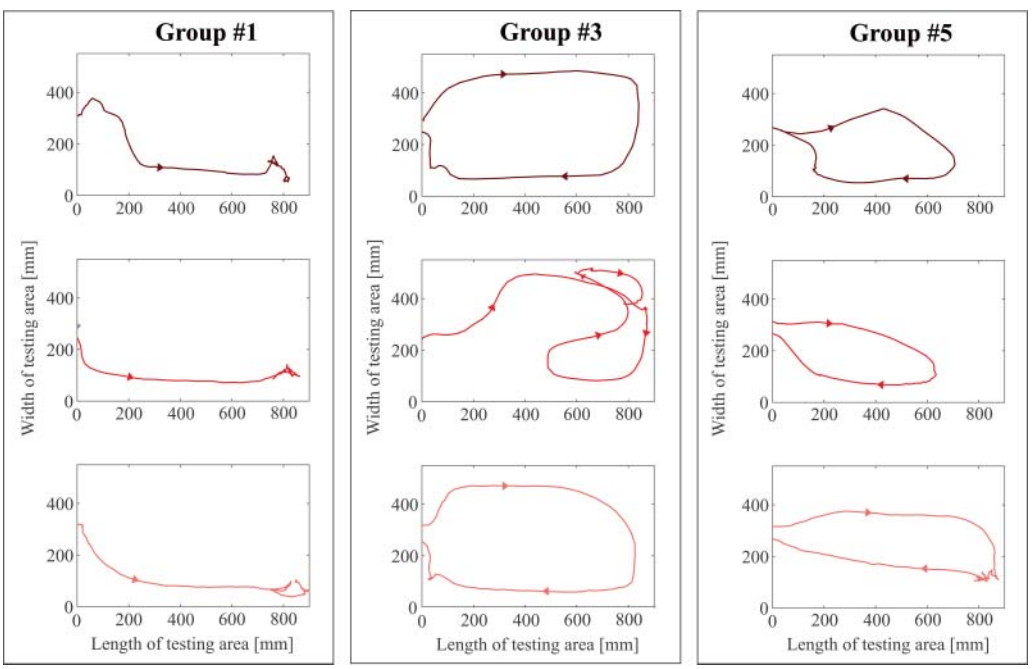

Fig. 4. Individual swimming trajectories of natural groups #1, #3, and #5. In group #1, all fish remained in the testing area throughout the trial. In group #3, the second fish showed a different trajectory and the third fish a similar trajectory to the fish swimming at the front. In group #5, all fish left the testing area before the end of the trial. The trajectories of the first fish are displayed in the first row (dark red), those of the second fish in the middle row (medium red), and those of the third fish in the bottom row (pale red).

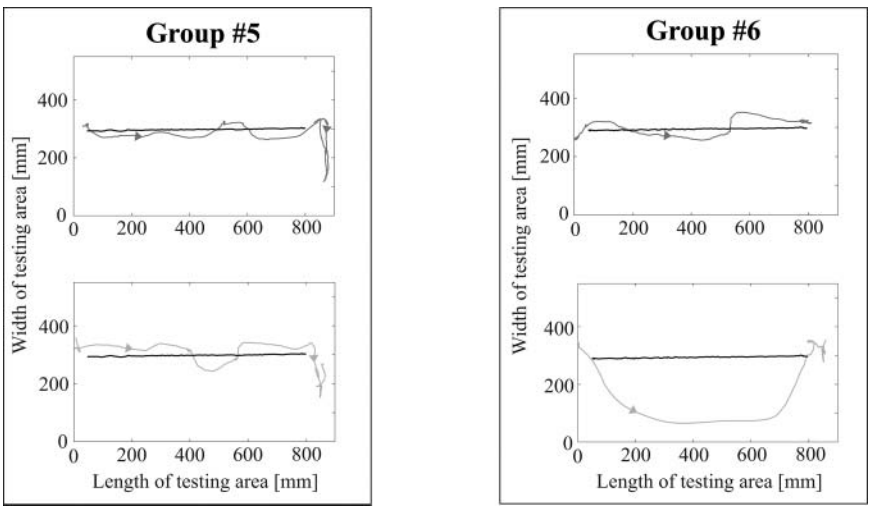

Fig. 5. Swimming trajectories of the fish in position two (top row, medium grey), the fish in position three (bottom row, pale grey), and the dummy (black) in mixed groups. Examples include: both fish following the path of the dummy in a similar manner (group #5); the fish in position two following a similar trajectory to the dummy but the fish in position three swimming along the aquarium wall (group #6).

Generally, when entering the testing area, the first fish of natural groups followed a route close to the right or left side-wall and swam towards the other end of the testing area, where it remained until the 15 seconds of a recording was up (Fig. 4, group #1) or swam back into the living area before that time (Fig. 4, groups #3 and #5). In both cases, most of the fish did not swim straight through the centre of the tank.

In successful trials, at least one of the fish occupying positions two and three was following the fish at the front – either one fish followed a different trajectory and the other fish a similar trajectory to the first fish (Fig. 4, group #3), or both fish followed the same trajectory as the first fish (Fig. 4, groups #1 and #5). In six groups (#1, #4, #5, #6, #7, and #8), fish occupying positions two and three followed the same trajectory as the first fish, while in the other two groups (#2 and #3) one fish followed a different trajectory to the fish in front.

In mixed groups, two distinct patterns of motor behaviours were observed: either both live fish followed a similar trajectory to the dummy (Fig. 5, group #5), or the fish in position two followed the dummy through the centre of the testing area while the fish in position 3 swam along the right or left wall of the tank (Fig. 5, group #6). In three groups (#1, #4, and #5), both fish followed the dummy precisely, whereas in five groups (#2, #3, #6, #7, and #8), only the fish in position two followed the dummy's trajectory and the fish in position three swam close to one of the walls of the tank. When reaching the end of the testing area, in all mixed groups both live fish remained close to the dummy while it was at a standstill.

Electric behaviour

To determine whether the positions occupied by fish or their behaviour and actions might be linked to specific electric behaviour (i.e. IDI patterns), we plotted the IDI graphs and indicated the respective behaviour of the fish, giving three examples (Fig. 6, groups #1, #4,

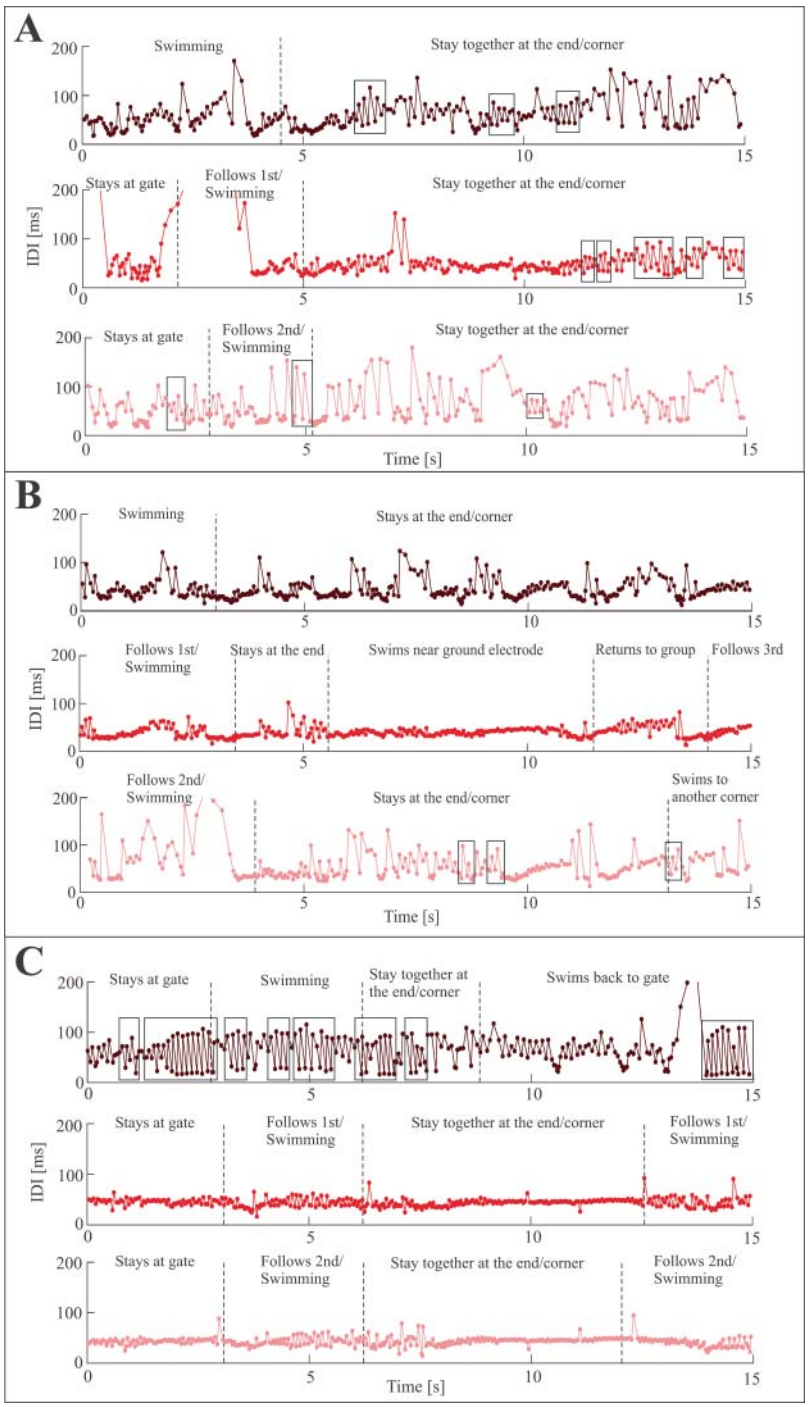

Fig. 6. Electric behaviour of three *natural* groups of three fish each. Shown are IDIs versus time graphs of individuals of natural groups #1 (A), #4 (B), and #8 (C). Vertical lines indicate a change of behaviour described above the respective sequence. The black boxes mark sequences of double-pulses of the first (dark red), the second (medium red), and the third fish (pale red).

and #8). During all trials, a general pattern of motor behaviour with minor variations between groups was observed: at the beginning some fish remained close to the gate through which they entered the testing area, followed by a swimming phase at varying speeds and distances between group members. Upon reaching the end of the testing area, fish swam close to one another usually in one corner of the tank. When comparing the IDI sequences of all individuals in all natural groups and their swimming or following phases from the gate to the other side of the tank, no uniform IDI pattern can be discerned across groups.

For the same three groups (groups #1, #4, and #8), IDI graphs are displayed for the mixed-group conditions with the dummy replacing the first fish of each natural group. Thus, the electrical behaviours of the fish in positions two and three are most important since the dummy emitted the same signals as the first fish in natural groups (Fig. 7). Again, no uniform IDI patterns were observed during the different behaviours of fish or regarding their swimming positions.

Double-pulses occurred in almost all tested fish to differing extents. Fish produced similar median numbers of double-pulses per trial regardless of their position in a group in both natural (Friedman's two-way ANOVA: $\chi^2_2 = 0.600$, $P = 0.741$) and mixed-group conditions ($\chi^2_2 = 1.750$, $P = 0.417$; Fig. 8A). The second and third fish in natural groups produced similar numbers of double-pulses as the two live fish in mixed groups (Wilcoxon signed-rank test: $z = -1.400$, $P = 0.161$).

We also tested whether more double-pulses were produced at specific points in time during a trial (Fig. 8B, C). In both natural and mixed-group conditions, double-pulses were produced repeatedly throughout all trials. In addition, we could not link the production of double-pulses to any specific behaviours, such as following conspecifics, swimming alone or remaining stationary (Figs. 6, 7).

As a measure of *regularization* of electric discharge behaviour, colour-coded autocorrelation functions are shown in Fig. 9. In the given example (group #4), sequences of slightly higher regularizations are evident for the live fish swimming in position two both in natural (Fig. 9A) and mixed groups (Fig. 9B). Also, fish in position three sometimes showed more regularizations, although at different points in time than the fish in position two. In this example, the fish at the head of natural groups (and thus also the dummy in mixed groups) regularized its discharges least of all.

The median values of the maximum autocorrelation coefficients of fish occupying the first, second, and third positions in all eight test groups showed no significant differences between natural and mixed groups (Fig. 9C). Also, a comparison of fish occupying positions two and three only (i.e. excluding the first fish and the dummy) resulted in a non-significant difference between the natural and mixed groups (Wilcoxon signed-rank test: $z = -0.840$, $P = 0.401$). For natural groups, no significant difference in fish at different positions was observed (ANOVA: $F_{2,0.212} = 1.008$, $P = 0.382$), although there was a slight tendency for an increase in regularizations from the first to the third fish. In mixed groups, there was a tendency for the most regularizations to be by fish in position two (Friedman's two-way ANOVA: $\chi^2_2 = 3.250$, $P = 0.197$; Fig. 9C).

In natural groups, most *discharge pauses* were produced by the individuals that swam at position three, while in mixed groups, neither live fish produced any pauses at all (Table 2). This led to a significant difference in the mean number of pauses produced by fish in positions two and three in natural compared with mixed groups (paired-samples *t*-test: $t_7 = 2.546$, $P = 0.038$). Furthermore, the longest IDI per fish was on average significantly longer in natural than in mixed groups (Wilcoxon signed-rank test: $z = -2.100$, $P = 0.036$).

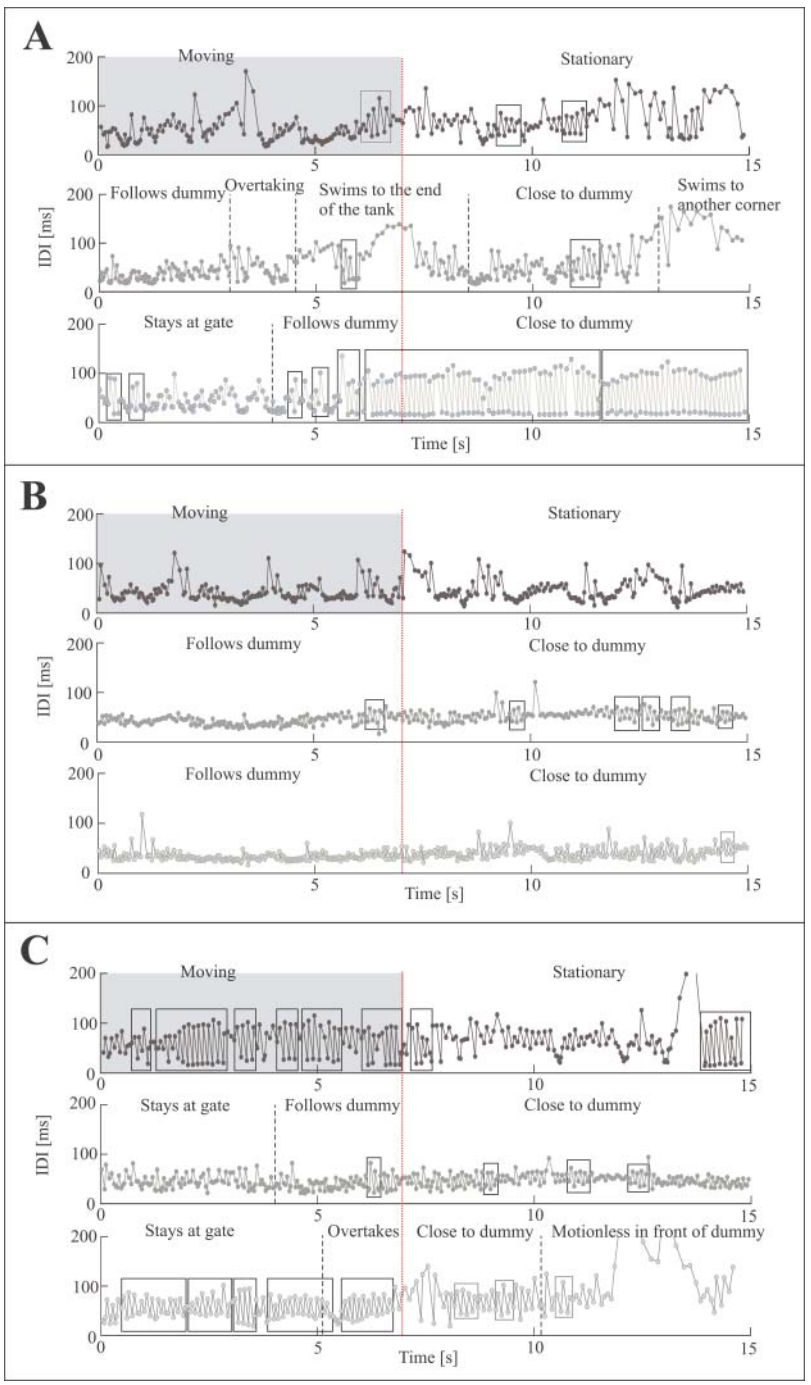

Fig. 7. Electric behaviour of three *mixed* groups of two fish and one dummy each. Shown are IDIs versus time graphs of individuals of mixed groups #1 (A), #4 (B), and #8 (C). Black dashed vertical lines indicate a change of behaviour described above the respective sequence. The black boxes mark sequences of double-pulses. The grey shaded section and red lines indicate the time up until which the dummy fish was moving through the testing area.

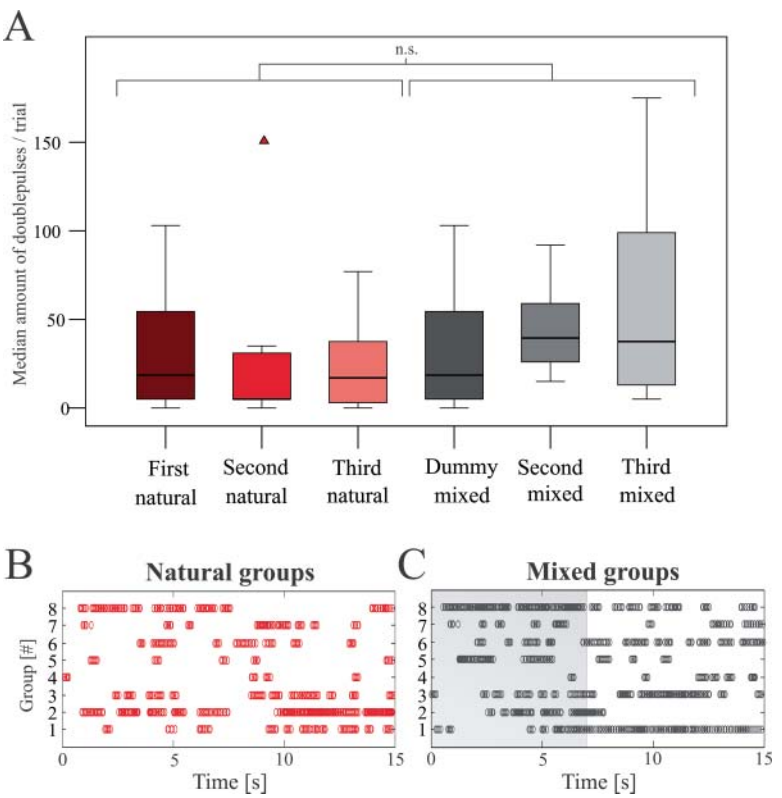

Fig. 8. (A) Median amounts of double-pulses per trial emitted by fish at different positions in natural (shades of red) and mixed groups (shades of grey) ($N = 8$). The triangle represents an extreme outlier. Double-pulses generated by all three fish of each test group are shown in (B) for natural and (C) for mixed groups. Each circle represents one double-pulse that was produced at the respective time. The grey shaded area marks the period during which the dummy was moving through the testing area in mixed groups (C).

Table 2. The longest IDIs (ms) are given for each group member of all eight natural and mixed groups

	Natural			Mixed		
Group	First	Second	Third	Dummy	Second	Third
1	170	**1005 (2)**	179	170	174	135
2	114	146	130	114	88	89
3	126	272	**1042 (4)**	126	257	138
4	123	101	210	123	120	117
5	**694 (3)**	**467 (1)**	**495 (1)**	**694 (3)**	94	204
6	204	273	**2724 (1)**	204	114	182
7	**756 (1)**	**449 (1)**	247	**756 (1)**	125	139
8	230	92	94	230	94	271

Note: Bold font indicates 'pauses', i.e. IDI with a duration of over 450 ms. The numbers in parentheses represent the total number of pauses throughout a trial. The dummy played back the same electrical signal sequences as the first fish in natural groups (grey background).

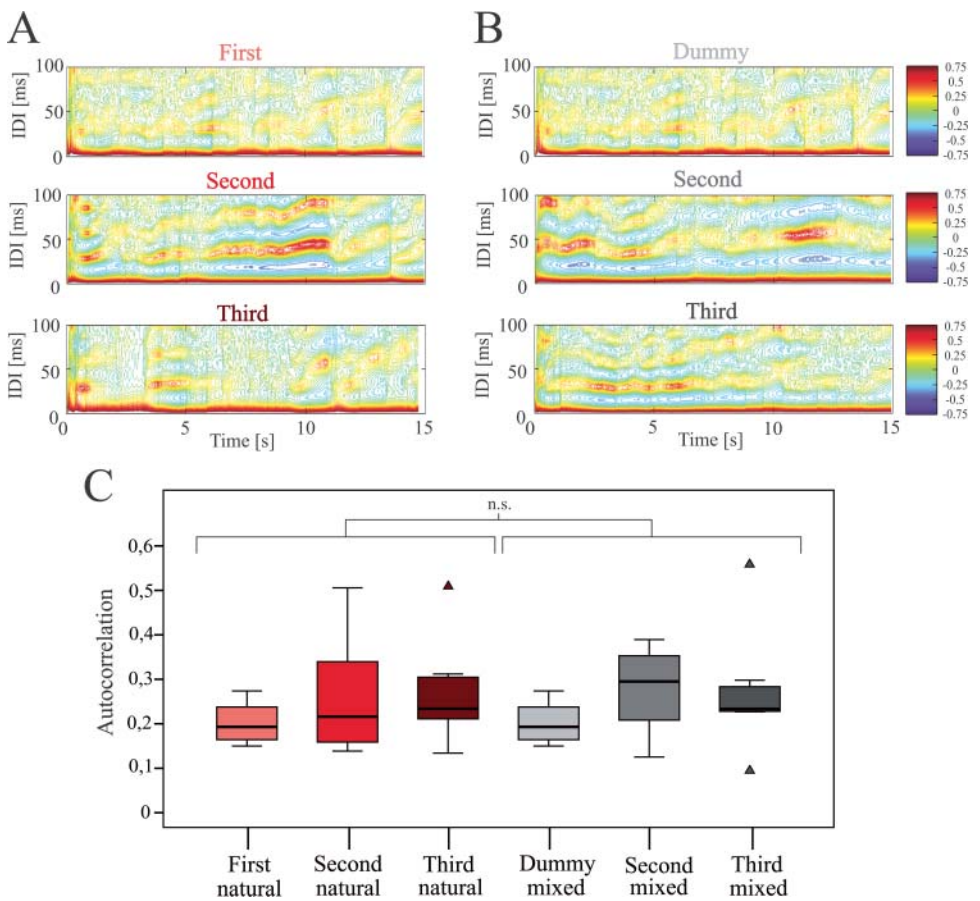

Fig. 9. Example autocorrelation diagram of group #4 in the natural (A) and mixed (B) group conditions. The autocorrelation coefficients are colour-coded, with warmer colours displaying higher numbers of regularizations. (C) Boxplots display the median of the autocorrelation coefficients that were averaged over the whole trial duration for each fish position for all natural (shades of red) and all mixed groups (shades of grey). $N = 8$ groups. The triangles represent extreme outliers.

Even though the dummy emitted the same IDI sequence as the fish swimming at the front in natural groups, its presence resulted in significantly different pausing behaviour of the fish following it.

Electrical interactions

Echo-response histograms of all pair combinations pooled over all eight groups are shown in Fig. 10. The green lines mark the expected distribution of relative IDI frequencies under the assumption that IDI sequences of both fish were generated independently. Bars above that line indicate echo-responses, as the frequency of echoes at the respective latency to the other fish's EOD is higher than expected for an independently discharging fish. When comparing the corresponding echo-quotients, which indicate the ratio of observed over

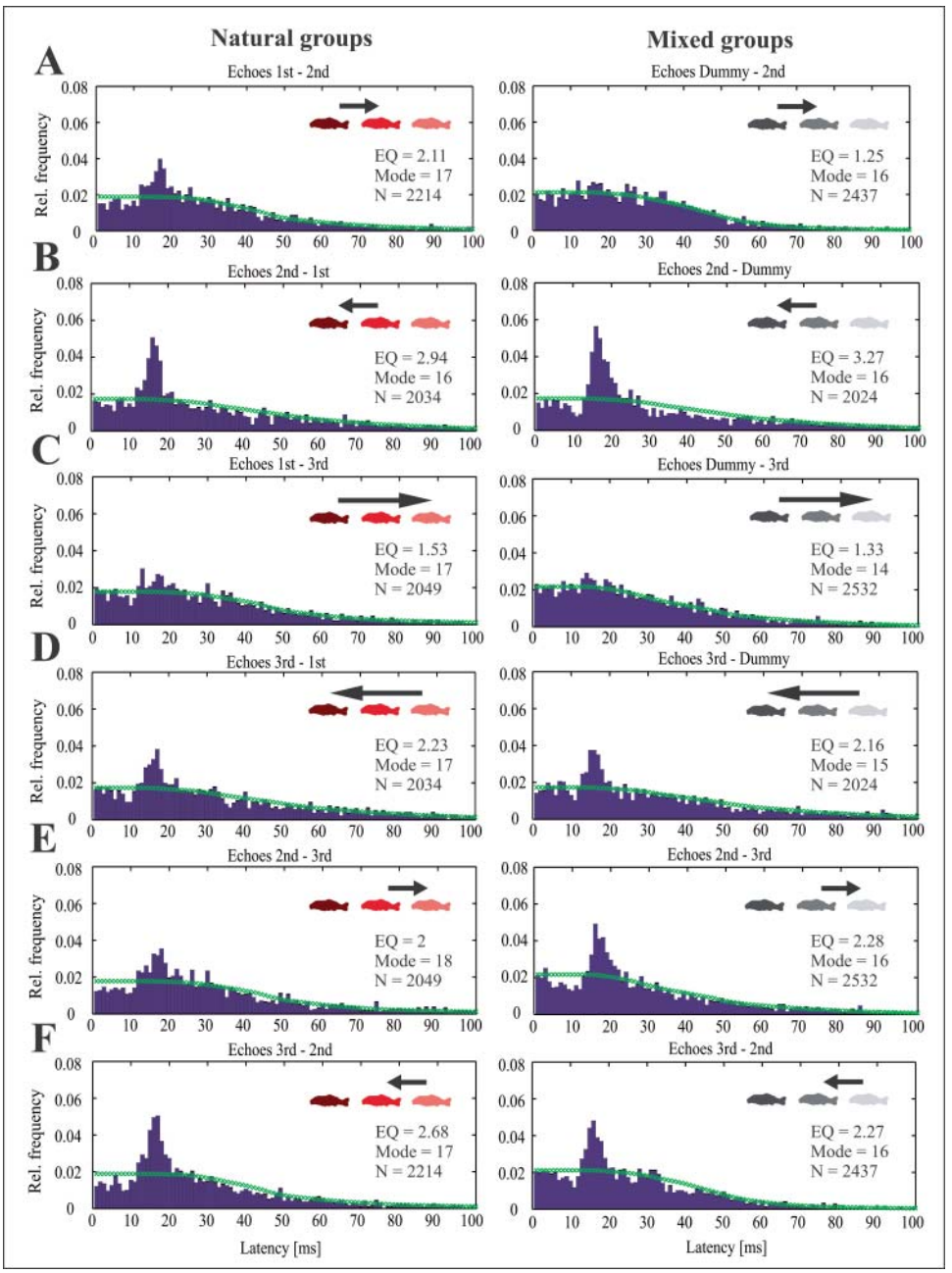

Fig. 10. Echo-response histograms displaying the observed response latencies of EODs (ms) and their relative frequencies for each group member to all possible partners and pooled for all eight groups (blue bars). The left histograms show latencies of individual responses to respective partners in natural groups and the right histograms those for mixed groups: Echoes of the first to the second (A), second to first (B), first to third (C), third to first (D), second to third (E), and third to second (F) positions. The expected distributions of latencies if both fish generated EODs independently are displayed by the green line. Echo quotients (EQ), the mode between 14 and 20 ms, and the number of EODs that were produced by the reference fish (N) are given. Bin size: 1 ms.

expected echoes at modes between 14 and 20 ms, we found that in natural groups fish in position two produced more echoes to the fish swimming ahead of it than to the fish swimming behind. The same was true for mixed groups, with the live fish in position two producing more echoes to the dummy than to the live fish behind it. Furthermore, in natural groups echo-responses were more often produced to immediate neighbours than to next-but-one neighbours, which was also the case for echoes produced by the live fish in position three in mixed groups.

The echo-responses and echo-quotients were also calculated for the playback of the dummy as a kind of control for how well it replaced the real substituted fish and if the echo-responses coincidently produced by the dummy resembled our calculated expected IDI distribution. In fact, the echo-responses of the first fish to the third fish in natural groups were as frequent as the coincidental echoes produced by the dummy to the live fish in position three in mixed groups. Echoes by the dummy to the live fish in position two were less frequent than the echoes produced by the first fish to the second fish in natural groups. Interestingly and maybe as a consequence, the live fish in position two in mixed groups produced considerably more echoes to the dummy than to the real first fish in natural groups, while the echo-quotient of the fish in position three to the first fish and the dummy was similar.

Discharge *synchronizations* represent electrical interactions of two fish mutually generating echo-responses to one another's EODs over a longer period. They occur when the fish emit consecutive EODs in a phase-locked manner over a longer period of time (Gebhardt *et al.*, 2012a). To test for synchronizations, the time-series of EODs of two fish were correlated and the resulting cross-correlation coefficients are shown in colour-coded contour plots (Fig. 11). Strong temporal correlations of the discharges of two fish resulted in high cross-correlations (shown in red), no correlation is represented by a value of 0 (green), and highly negative values indicate the avoidance of the respective response times (blue).

In all trials, both in natural and in mixed-group conditions, several episodes of synchronizations between different pairs of fish were observed (Figs. 11, 12). As an example, Fig. 11 illustrates IDIs versus time (above) and the corresponding synchronization graphs (below) over the trial duration and of all communication partners of natural group #4 (A, B, C). Synchronizations are visible in both plots: a high degree of synchronization in the IDI plots can be seen in the overlap of the two individual traces, while in the contour plots it becomes evident by longer horizontal red bands.

To look at the timing of synchronizations, single episodes of high synchronization are labelled with lower-case letters (a–g) in Fig. 11. In episode 'a', for example, there are synchronizations between fish 1 and fish 2, and between fish 2 and fish 3. The most important general information that can be drawn from this example shown in Fig. 8 is that the synchronization events alternate between the different communication partners. That means that a fish usually synchronizes only with one partner at a time and switches partners in the following episode. For example, during 'a', the first fish synchronized its discharges exclusively with the second fish, followed by an episode of synchronization mainly between the second and third fish (episode 'b'). Sometimes, overlapping synchronizations can occur, as in episode 'b'. Here, fish 3 synchronized its discharge with fish 2, and fish 2 synchronized simultaneously with fish 1. As a consequence, fish 3 also displayed some synchronization with fish 1 (Fig. 11). In most cases, an interaction between two individuals was bilateral for the most part.

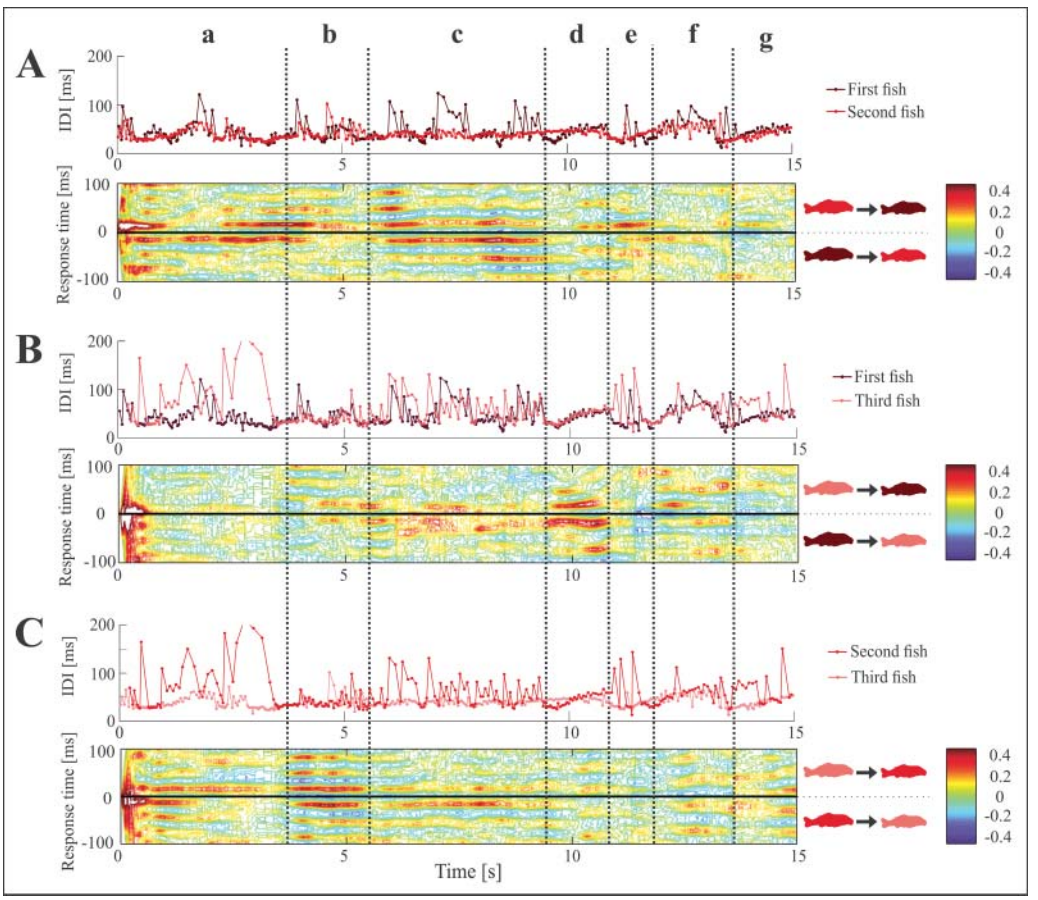

Fig. 11. IDIs (ms) and cross-correlation diagrams for natural group #4. The first fish is displayed in dark red, the second in medium red, and the third in pale red. Electric discharge behaviour (top) and synchronization (bottom) between the first and second fish (A), the first and third fish (B), and the second and third fish (C). In the cross-correlation diagrams, correlation coefficients are colour-coded with high correlation coefficients in warm and low coefficients in cooler shades. Positive response times (0 to 100 ms) represent synchronization of the signalling behaviour of the first to the second fish, whereas negative response times (0 to –100 ms) represent synchronization of the second to the first fish. This is indicated by the fish symbols shown at the right. The dotted lines divide episodes of high correlation between group members and indicate that one group member synchronizes its discharges with only one other group member at a time. The letters denote the single episodes (a–g).

When comparing the overall median amount of synchronizations in natural and mixed groups, synchronization levels were found to be similar (paired-samples t-test, $t_7 = -0.428$, $P = 0.682$; Fig. 12). In addition, the mean amount of synchronization of all live fish in both natural and mixed groups was above 'dummy level', which was defined as the median cross-correlation coefficient calculated for the dummy as it was not capable of synchronizing its discharge. In natural groups, the amount of synchronization of all individuals was independent of their position in the group (Friedman's two-way ANOVA: $\chi^2_2 = 5.071$, $P = 0.407$). Similar to the echo-response, however, there was a non-significant tendency

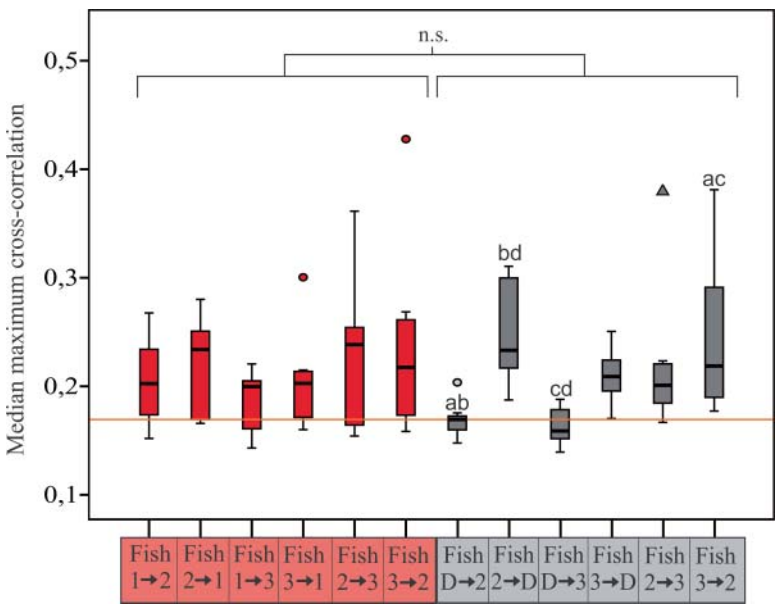

Fig. 12. Median of mean maximum cross-correlation of group members in 15 seconds towards all possible partners in natural (red) and mixed groups (grey) for all eight groups tested. The orange line displays the 'dummy level' of the highest median synchronization randomly produced by the dummy towards real fish. Same letters indicate significant differences of median synchronization between communication pairs in mixed groups. Circles indicate outliers and triangles extreme outliers. $N = 8$ groups.

for the fish in position two to synchronize its discharge more with the fish ahead of it than with the fish swimming behind (paired-samples t-test: $t_7 = -1.954$, $P = 0.092$). In mixed groups, an overall significant difference was observed (Friedman's two-way ANOVA: $\chi^2_2 = 25.500$, $P \leq 0.001$). The fish in position two synchronized its discharges to a significantly higher degree with the dummy ahead of it than to the fish swimming behind (Wilcoxon signed-rank test: $z = -2.521$, $P = 0.012$).

DISCUSSION

In this study, the weakly electric fish *Mormyrus rume* engaged in group behaviour when three unfamiliar individuals swam together in a large tank containing areas with and without places to hide. Group behaviour becomes apparent when the fish leave their hiding place and swim into an open area that does not provide any cover. In about 50% of their excursions into this potentially dangerous open area, fish swam either as a pair (19.82%) or as a group of three fish (28.11%; Table 1). While little is known about the ecology and social behaviour of tapir fish in their natural habitats, group formation has been observed in previous laboratory studies, such as during foraging in open spaces (Gebhardt *et al.*, 2012b), and during reproduction in the laboratory (Schugardt and Kirschbaum, 2004). In contrast, when resting or hiding, no overt group behaviour was observed (Gebhardt *et al.*, 2012b). This suggests that *M. rume* may be a 'facultative schooler' (following the definition of Breder, 1967) or a 'shoaler',

gathering together but not living in groups permanently, which is what we have observed previously in our laboratory as well as in the present experiment. We found that tapir fish swim in groups when faced with an unknown, uncomfortable (bright, without hiding places), and therefore potentially dangerous environment. Even though the tested individuals seemed to prefer to swim alone most of the time (52.07% of all excursions), some test groups showed a high number of occasions when fish swam together as a group of three (28.11% of all excursions; Table 1). It might be that either an optimal group size exists (Davies *et al.*, 2012), which remains unknown for *M. rume* and was not investigated here, or that groups were 'inharmonic' or unstable. Because they were artificially assembled, fish did not have a choice with whom to form a group. When in mixed groups, the live fish followed the dummy more reliably into the open area than they followed a live fish (see Materials and Methods – Experiments). In previous studies, it was shown that fish were recruited by the mobile dummy mainly in response to electrical signals (Donati *et al.*, 2016) and followed it largely independently of the presented playback patterns (Worm *et al.*, 2017). In addition, the body shape or size of the fish in front did not play any role (Worm *et al.*, 2018a).

We found that in both natural and mixed groups, the front fish or dummy had a marked influence on the swimming trajectories of the fish following it, and that it took on some kind of 'leader' function (Figs. 4, 5) (Krause *et al.*, 2000). Interestingly, position one was never occupied by the largest fish in a group; instead, it was equally often occupied by the small or medium-sized individual (Fig. 2). It has also been reported for other species that the individuals at the front of a group or shoal of fish are often of lower nutritional status, as they are the first to encounter a new food source (Krause, 1993; Krause *et al.*, 2000). Thus, the smaller fish at the front of a group might have been driven by hunger to leave shelter in the hope of a higher food uptake when encountering a new food source first (Harcourt *et al.*, 2009). Fish in positions two and three might have the advantage of observing and reacting to the first fish (Reader *et al.*, 2003).

When swimming into the open area in natural groups, the fish followed each other one by one, a behaviour called 'single file swimming' by Moller (1976) for the mormyrid *Marcusenius cyprinoides*. The median distance between *M. rume* individuals was about 20 cm here, which corresponds to about two fish lengths (Fig. 3). During these excursions, the fish did not show any aggressive physical contacts, such as head butts or lateral displays (Bell *et al.*, 1974; Kramer, 1974). In the mixed groups, similar single file swimming was observed with the fish following the moving dummy at a similar distance as the real fish in natural groups during the first part of the trials (Fig. 3).

The second part of the trials depicted a rather different situation in both natural and mixed groups. The fish in natural groups showed several different kinds of group behaviours: either they gathered together in one corner of the tank (Fig. 4, group #1), one fish separated from the group (Fig. 4, group #3), or they all swam back into the living area keeping their distance from one another (Fig. 4, group #5). These behaviours suggest some kind of 'leader' function on the part of the fish in front, even if it was performed at rather large inter-fish distances. In most trials, when the first fish remained in the testing area, the two followers did the same. And when the first fish swam back to the living area, the other two fish followed (Fig. 4).

Comparing these behaviours with those observed during the second part of the mixed-group trials reveals some differences. When the dummy stopped moving after 7 seconds but continued to emit EODs, the live fish remained significantly closer to it than they did to the first fish in natural groups (Fig. 3). Fish appeared to be inspecting the dummy and did not

leave until the end of the playback. The lack of physical and electric interactions on the part of the dummy towards the live fish might be one reason for the observed short distances between the live fish and the dummy in the mixed groups. The fact that the fish orientated most towards the dummy (Figs. 4, 5) shows that the first fish in natural groups was successfully replaced by the dummy and suggests that group cohesion was similar in natural and mixed groups.

When swimming into the open area, fish constantly emitted electric signals presumably for the purpose of both active electrolocation and electro-communication (von der Emde, 2006; Hopkins, 2009; Worm *et al.*, 2017, 2018a). Fish swimming at the front in natural groups did not show any consistent features of electric behaviour across groups, which might have elicited the following behaviour in their conspecifics. When testing for characteristic electric behaviour of fish swimming in positions two or three, we also did not find any clear differences in the occurrence of certain electric emission patterns, such as double-pulses, pauses or regularizations. Furthermore, fish in natural and mixed groups behaved similarly in most respects. In contrast, when considering *interactive* electrical behaviours (echo-responses, synchronizations), clear differences between fish at different positions within the groups and between natural and mixed groups became apparent.

In all trials, very little aggression was observed, which was reflected in the relatively low occurrence of aggressive or threatening electrical displays (e.g. double-pulses). While both in natural and mixed groups and at all swimming positions a median of less than 20 double-pulses per trial (15 seconds duration) was recorded in our experiments (Fig. 8), about 70 double-pulses per 15 seconds (median) were emitted by two *M. rume* engaging in an aggressive competition for a single shelter (Kersten, 2017), and several hundred double-pulses were occasionally produced by fish responding to 14 seconds of electric double-pulse playbacks (Worm *et al.*, 2017).

Regularizations occurred both in natural and mixed groups, and there was a tendency for fish swimming at position two or three of the group to regularize more strongly than the fish at the front (Fig. 9). Regularization linked to an increase in frequency leads to a better spatio-temporal resolution during active electrolocation because of the constant adaptation state of the electroreceptors (von der Emde, 1992; Carlson, 2002; Hofmann *et al.*, 2013), and fish following another individual might have had a greater need to track the movement of the fish in front. Other studies have shown that regularizations also have a communicative function, but what that function is remains a matter of debate (Moller, 1970; Baier and Kramer, 2007; Machnik and Kramer, 2011; Worm *et al.*, 2017, 2018a). Since in the present study regularizations were not very pronounced and were not associated with any particular behavioural situations, we suggest that it served a mainly electrolocation purpose during single file swimming.

The production of EOD pauses was the only IDI pattern that differed between natural and mixed groups, since it was not observed in the latter (Table 2). Fish paused during different behaviours, such as when leaving the shelter, during swimming/following or when close to other group members. Pauses longer than 450 ms were mainly produced by large fish, followed by medium-sized and small fish. In our experiments, we suggest that pauses might have been used by the fish to 'listen' to the signals of other individuals without producing signals themselves, i.e. a type of eavesdropping (Moller, 1995). However, during a pause, a fish does not receive any sensory input through its active electrolocation system, which among other things provides information about the position of neighbouring fish. In a natural group, where live fish behave in a predictable way, this would not matter as much as in a mixed group, in which the dummy behaved in a rather unnatural and unpredictable

way. This might have led to the finding that in mixed groups no pauses were produced at all (Table 2).

In contrast to the above-mentioned signalling patterns, interactive signalling between two individuals differed for individuals swimming at different positions in a group and also to some degree between natural and mixed groups. One type of electric interaction is the so-called echo-response, which was observed both in natural and in mixed groups and also included echoing towards the dummy (Fig. 10). This had already been reported by Worm *et al.* (2017, 2018b), where single *M. rume* produced echo-responses as a reaction to electrical playback by a dummy fish. The echo-response has been proposed to be a jamming avoidance response during active electrolocation (Heiligenberg, 1976; Schuster, 2001). However, in a study by Schumacher *et al.* (2016), *Gnathonemus petersii* was perfectly able to perform an object-discrimination task in the presence of electrical jamming signals. Echoing leads to less temporal overlap between the signals of two individuals, and thus also could serve a jamming avoidance function during electro-communication. By avoiding overlap of their signals, fish might be better able to perceive the waveform of the EODs of their neighbour and thus could better recognize the individual swimming close by, since EOD waveforms are used for species, sex, and individual recognition purposes in several mormyrid species (Bell *et al.*, 1974; Graff and Kramer, 1992; Hopkins, 1999; Carlson, 2002).

In a recent study, Worm *et al.* (2018b) proposed another possible function of the echo-response: echoing might provide a simple mechanism by which mormyrids can specifically address nearby individuals during electro-communication to attract their attention. Thus, echoing in our study might have enabled a fish to mutually allocate social attention, and to communicate with specific individuals at any one time. Interestingly, echoing in mixed groups was often stronger than in natural groups (Fig. 10). This might have been due to the fact that the dummy never engaged in echoing of its own and therefore was more often addressed by the live fish to make it aware of their presence. We observed that live fish produced more echo-responses to fish in front than to fish swimming behind it (Fig. 10). Since a fish cannot see the individual swimming behind, the latter might more often address the fish in front to ensure it of its presence and social intentions.

When echo-responses are continuously produced over a long time, they result in synchronizations of the two fishes' discharges (Gebhardt *et al.*, 2012b). The synchronization of EOD sequences was observed in all groups tested, between all group members, and in all behavioural contexts (Fig. 11). Most often, a fish synchronized its discharge with only one other group member at a time. Bilateral synchronization therefore describes the interactive signalling of two fish over an extended period of time. Fish periodically changed their synchronization partners and thus might have addressed all individuals of the group, including the dummy. We assume that this electric behaviour leads to strong social cohesion and ensures coordinated action of the group. Since the dummy was not participating in echoing and synchronizations, the live group members might have increased their efforts to address the dummy (Figs. 11, 12) as well as reducing swimming distances (Fig. 3) to be better able to predict the movements of the non-responding dummy swimming at the front of the group.

In summary, the smaller individuals of a group mostly occupy the front position during single file swimming. Furthermore, neither the amount of double-pulses nor the regularization of discharges were dependent on the position of the fish in the group and did not differ between natural and mixed groups. Pauses, on the other hand, occurred in natural groups but were completely absent in mixed groups. Echo-responses and the synchronization of

EODs with the remaining group members were addressed more often to the individual swimming ahead than to the one swimming behind. These interactive behaviours tended to occur even more often in mixed than in natural groups. Differences in a fish's interactions with the dummy could have been due to the 'abnormal' motor behaviour and the lack of interactive electric signalling of the dummy.

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