Animal–Robot Interactions: Electrocommunication, Sensory Ecology, and Group Dynamics in a Mormyrid Weakly Electric Fish

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Erklärung

Hiermit erkläre ich, die vorliegende Arbeit persönlich, selbstständig und nur unter Zuhilfenahme der angegebenen Mittel angefertigt zu haben. Inhaltliche und wörtliche Übernahmen anderer Werke wurden durch Zitate als solche gekennzeichnet.

Bonn, den 25. Mai 2018

Martin Worm

Teile dieser Arbeit sind veröffentlicht oder zur Veröffentlichung eingereicht:

1. Worm, M., Landgraf, T., Prume, J., Nguyen, H., Kirschbaum, F., and von der Emde, G. (2018). Evidence for mutual allocation of social attention through interactive signaling in a mormyrid weakly electric fish. *Proceedings of the National Academy of Sciences* (Accepted).

2. Worm, M., Kirschbaum, F. and von der Emde, G. (2018). Disembodying the invisible: Electrocommunication by passive reception of a moving playback signal. *Journal of Experimental Biology* 221: jeb-172890.

3. Worm, M., Kirschbaum, F. and von der Emde, G. (2017). Social interactions between live and artificial weakly electric fish: Electrocommunication and locomotor behavior of *Mormyrus rume proboscirostris* towards a mobile dummy fish. *PLOS ONE* 12 (9): e0184622.

4. Donati, E., Worm, M., Mintchev, S., van der Wiel, M., Benelli, G., von der Emde, G. and Stefanini, C. (2016). Investigation of collective behaviour and electrocommunication in the weakly electric fish, *Mormyrus rume*, through a biomimetic robotic dummy fish. *Bioinspiration & Biomimetics* 11: 066009.

Teile dieser Dissertation basieren auf veröffentlichten (Kapitel 4, 8 und 9), bzw. zur Veröffentlichung angenommen Manuskripten (Kapitel 5). Die Literaturquellen aller Kapitel sind am Ende der Arbeit zusammengefasst.

Diese Arbeit enthält außerdem Daten und Ergebnisse, die von Studentinnen im Rahmen ihrer Abschlussarbeiten am Zoologischen Institut in der Abteilung Neuroethologie/Sensorische Ökologie erhoben wurden. Diese Arbeiten wurden im Rahmen meines Dissertationsprojektes angefertigt und wurden während aller Phasen ihrer Entstehung durch mich mitbetreut:

1. Die Daten der Versuche mit konstanten Entladungsfrequenzen (Kapitel 4) wurden von Rowena Toma für ihre Bachelorarbeit "Auswirkungen verschiedener Dummy-generierter Playbacks auf die motorische und elektrische Antwort von *Mormyrus rume* (Mormyridae, Teleostei)" (Toma, 2014b) erhoben.

2. Die Kontestversuche zur Untersuchung der Kommunikationsfunktion elektrischer Doppelpulse (Kapitel 4) wurden von Anna Kersten im Rahmen ihrer Projektarbeit "Das motorische und elektrische Kommunikationsverhalten von *Mormyrus rume*: Antagonistische Begegnungen in Zweiergruppen" (Kersten, 2017a) durchgeführt und ausgewertet.

3. Das Programm zur Imitation der Echoantwort (Kapitel 5 und 11) wurde von Julia Prume mitentwickelt und kam bei der Anfertigung iher Projektarbeit "Elektrische und motorische Reaktionen des freischwimmenden, schwach elektrischen Fisches *Gnathonemus petersii* auf aggressive Signale und interaktive "Echo Antworten" einer elektrokommunizierenden Attrappe" (Prume, 2015b) und ihrer Bachelorarbeit "Elektrische Reaktionen des freischwimmenden schwach elektrischen Fisches *Gnathonemus petersii* auf interaktive 'Echo Responses' und statische Playbacks einer elektrokommunizierenden Attrappe" (Prume, 2015a) erstmals zur Anwendung.

Alle Übernahmen sind an entsprechender Stelle als solche kenntlich gemacht.

Table of Contents

I. General Introduction

Weakly electric fish offer a unique opportunity to investigate a sensory system specialized for active environmental sensing and study the implications of this sensory modality for social communication. By generating electric organ discharges (EOD), members of the African *Mormyridae* create their carrier signal for environmental perception (Nelson and MacIver, 2006), which makes them mostly independent of visible light in their activity. This active electrolocation ability (von der Emde *et al.*, 2008) allowed mormyrids to develop a particular ecological niche that made them one of the most species-rich and abundant families of African freshwater fish. At the same time, electrical signaling opened a relatively private channel for elaborate communication displays of extraordinary temporal precision. With specialized receptor organs and neural pathways that are dedicated to communication, mormyrids can detect even smallest differences in EOD-waveforms and analyze signaling patterns generated through the variation of inter-discharge intervals (IDI) by other individuals (Baker *et al.*, 2013a). While the EOD itself provides identity information about the sender, temporal patterns of successive signals enable mormyrids to communicate behavioral states. Hence, IDI-variation provides essential information during aggressive encounters, courtship displays, or in group settings (Carlson, 2002a; Hopkins, 1986). Because active electrolocation and electrocommunication rely on the very same signals to serve their respective purposes, the two functions are closely linked and provide a fruitful but challenging research area to understand how animal communication arises from a sensory system designed for environmental monitoring and object detection.

This thesis is structured along three main research topics that are connected on several levels. After exploring the mechanisms of electroreception and electrogenesis, and their implications for the sensory ecology of weakly electric fishes, in Part One, the general principles of electrocommunication will be discussed and systematically investigated in playback experiments with the mormyrid weakly electric fish *Mormyrus rume proboscirostris*. In Part Two, the development of classical ethological studies towards the modern field of ethorobotics will be reviewed and experimentally explored by testing the potential of a mobile fish robot to replace a conspecific individual based on its biomimetic properties. The sensory cues that are provided by this dummy are subsequently reduced experimentally to isolate the stimuli that are necessary to induce social behaviors in *M. rume*, and to identify behavioral and sensory mechanisms by which these behaviors are guided. Part Three of this thesis deals with the mechanisms responsible for collective behavior and group dynamics in shoals of fish. Here, the insights from previous sections will be employed to investigate the influence of a playback-emitting, mobile dummy fish on small groups of weakly electric *M. rume* in an interactive study on electrocommunication, thus exploring the feasibility of mixed live–artificial experiments with mormyrid weakly electric fish.

1. Weakly Electric Fish as Model Organisms

1.1 Electroreception in aquatic vertebrates

Electroreception is a widespread sensory modality among aquatic vertebrates (Figure 1.1). It is found in several phylogenetically old fish taxa including sharks and rays (Elasmobranchii) (Kalmijn, 1971), ratfish (Holocephali) (Fields and Lange, 1980), coelacanths (Actinistia) (Northcutt, 1980), lungfish (Dipnoi) (Watt *et al.*, 1999) bichirs (Cladistia) (Jørgensen, 1982), sturgeons (Teeter *et al.*, 1980) and paddlefish (Chondrostei) (Wilkens *et al.*, 2002), as well as in some amphibians (Apoda and Urodela) (Himstedt *et al.*, 1982). Electroreception is considered to be an ancient sensory modality that was already present in the basal lineage of lampreys (Cyclostomata) (Bodznick and Northcutt, 1981) and has been lost in teleost fishes and higher vertebrates (Bullock *et al.*, 1983). It has re-evolved several times independently in at least two lineages of teleost fishes (Baker *et al.*, 2013b) and even in some mammalian cetaceans (Czech-Damal *et al.*, 2012) and the monotremes (Scheich *et al.*, 1986). Among teleosts, electroreception occurs in catfish (Siluriformes) (Dijkgraaf, 1968) and the South American weakly electric Gymnotiformes, as well as in the not directly related African weakly electric Mormyriformes (Szabo, 1965)

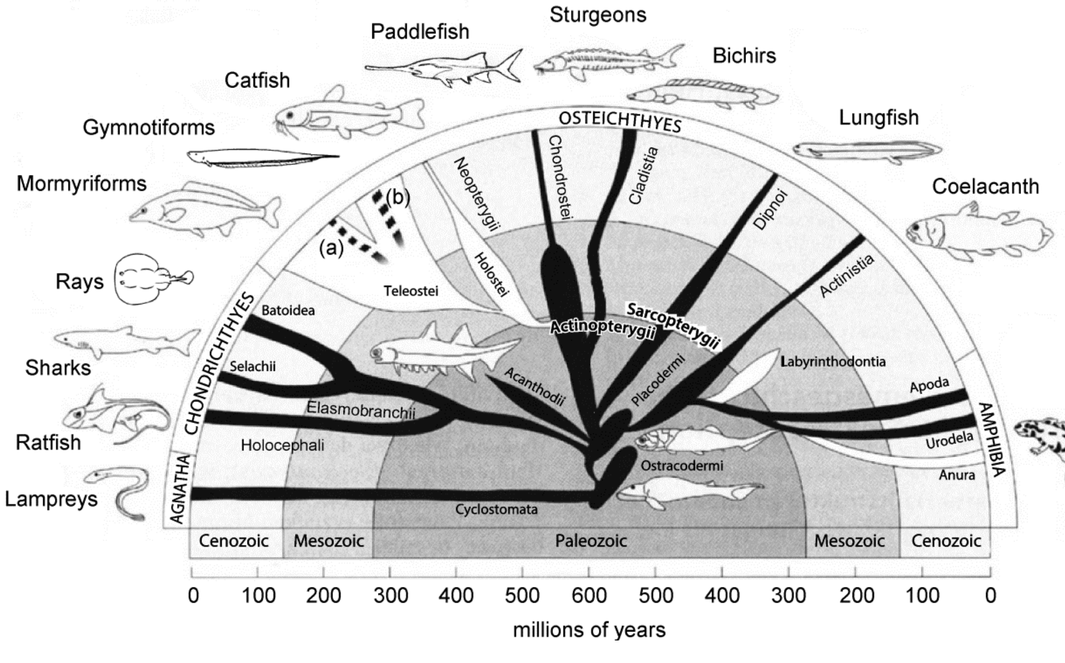

Figure 1.1: Phylogenetic distribution of electroreception in fishes. Electroreception was presumably already present in the most basal fish lineages that gave rise to all extant fish and higher vertebrates (black branches). It was lost in the neopterygian fishes comprising holosteans and modern teleosts (white branches). Among teleosts, it re-evolved at least twice. Electroreceptive species are found within the basal group of bony-tongued Osteoglossomopha (a), including all Mormyriformes and the Xenomystinae as well as within the Ostariophysi (b), of which the Gymnotiformes and their sister taxon, the Siluriformes, are electroreceptive. Modified after von der Emde (2013).

and some African knife fish (Xenomystinae) in their sister group, the Notopteridae (Bullock and Northcutt, 1982).

Elasmobranch electroreceptor organs have long been known as Ampullae of Lorenzini, named after the Italian physician who described their occurrence in the *Torpedo* ray (Lorenzini, 1678). Their function as electrosensory organs was, however, not revealed before the middle of the 20th Century through electrophysiological (Murray, 1960) and behavioral (Dijkgraaf and Kalmijn, 1962; Kalmijn, 1971) experiments. Evidence for the existence of electroreceptors was by then also accumulated for mormyriform and gymnotiform weakly electric fish (Bullock *et al.*, 1961; Fessard and Szabo, 1961). Although the ampullary receptor organs in weakly electric fish and other teleosts are not homologous to those found in non-teleost fishes, the basic morphological principles are very similar (Jørgensen, 2005). Ampullae are composed of a dermal cavity that is connected to the outside water by a canal pore (Figure 1.2A). These canals are filled with a highly conductive jelly, whereas tight junctions between flattened epithelial cells cause a high electrical resistance of the canal walls (Bennett, 1971b; Jørgensen, 2005). In mormyrids, the sensory epithelium at the base of the pore contains three to eight secondary receptor cells that form synapses with a single afferent neuron (Szamier and Bennett, 1974). Electrical potential gradients across the sensory epithelium activate voltage-gated calcium channels and lead to a modulation of transmitter release from the sensory neurons, which in turn modulates the tonic activity of the afferent neuron depending on stimulus polarity (Bodznick and Montgomery, 2005). In either case, the resulting sensory systems are very sensitive (Peters *et al.*, 2007), reacting to electrical potential differences as low as 5 nV cm^{-1} in marine elasmobranchs (Kalmijn, 1982), and 40 µV cm^{-1} in the weakly electric fish *Gnathonemus petersii* (Engelmann *et al.*, 2010). Electrosensory systems based on ampullary receptor organs can thus detect variations in electric DC fields and are tuned to low-frequency electrical stimuli (Bodznick and Montgomery, 2005; Engelmann *et al.*, 2010; Peters *et al.*, 2007). Such weak electric fields are generated by sources external to an electroreceptive animal in the environment. They emanate from aquatic organisms and are modulated for example by their respiratory, osmoregulatory or neuromuscular activity, and also result from differences in physical or geochemical properties of the environment (Kalmijn, 1974; Peters and Bretschneider, 1972). In the ampullary canal systems of marine elasmobranchs, movements relative to the earth's magnetic field can generate induction voltages, which may enable these animals to use geomagnetic cues for orientation (Kalmijn, 1982; Meyer *et al.*, 2005; Peters *et al.*, 2007). Passive electroreception thus aids in prey detection, predator avoidance, finding mates, orientation, and navigation (Wilkens and Hofmann, 2005).

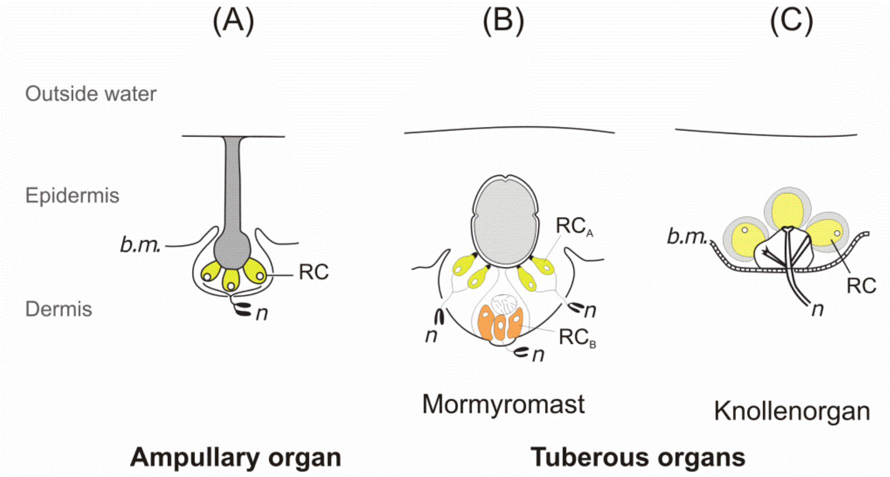

Figure 1.2: Morphology of mormyrid electroreceptor organs. (A) Ampullary receptor organs are sensitive to low-frequency electric stimuli from the environment. Receptor cells within the dermal cavity are connected to the outside water via a jelly-filled canal of a high conductance (grey) and are innervated by a single afferent neuron. (B) Mormyromasts are tuberous receptor organs and detect the self-generated EOD during active electrolocation. They consist of an outer chamber that connects to the apical end of amplitude-sensitive type A receptor cells (yellow), and an inner chamber that contains type B receptor cells, which are sensitive to both amplitude and waveform changes of the local EOD. (C) Knollenorgans are tuberous receptor organs that respond to the high frequencies contained in pulse-type EODs and are time-coders dedicated to electrocommunication. Their large receptor cells are very sensitive and respond to stimuli with a single spike that is transmitted with a high temporal precision that allows detecting characteristics in EOD-waveforms and IDI-patterns of other fish. RC = receptor cell; b.m. = basement membrane; n = afferent neuron. Modified after Hopkins (2009).

1.2 Electrogenic fishes

Electroreception via ampullary receptor organs is a passive sensory system and relies on electric signals generated by external sources. Several lineages of fish taxa have also evolved the capability to actively generate electricity by using specialized electric organs (Bennett, 1971a). Electrogenic fishes can be divided into strongly and weakly electric fish, based on the strength of the generated signals and the purposes for which they are employed. The effect of the electric organ discharges of strongly electric fish species has been known to humans long before they had established any concept of electricity (Finger and Piccolino, 2011b; Moller, 1995). Indeed, early observations made on electric eels and rays contributed significantly to our scientific understanding of electricity and physiology (Catania, 2015b; Piccolino and Bresadola, 2002). By constructing an 'artificial electric organ,' which was inspired by the strongly electric *Torpedo* ray, Alessandro Volta (1800) famously invented the electric battery in an attempt to refute Luigi Galvani's concepts of animal electricity (Finger and Piccolino, 2011a). Electric eels (*Electrophorus electricus*) produce discharges of several hundred volts to stun prey (Catania, 2014; Catania, 2015a) and in self-defense (Catania, 2016). Prey capture aided by strong electrical discharges has also been studied in the electric ray *Torpedo marmorata* (Belbenoit and Bauer, 1972) and the electric catfish *Malapterurus electricus* (Bauer, 1968). The function of the electric organ discharges generated by the perciform marine stargazers (Uranoscopidae) remains speculative (Baron, 2009; Pickens and McFarland, 1964).

The anatomical resemblance of the electric organs of weakly electric fish species to those of strongly electric fishes had long been noticed. However, the apparent uselessness of the weak impulses produced by weakly electric *Mormyrus*, or by the electric ray *Raja clavata*, has puzzled scientists, including Charles Darwin, for a long time. To Darwin, the existence of such organs constituted a mystery he was not yet able to explain with his theory of natural selection (Moller, 1995). The continuous presence of such weak electric discharges was, however, first discovered in *Gymnarchus niloticus* by Lissmann (1951). It was later established that these animals, as well several other species of weakly electric fish, can use distortions of their self-generated electric field for object detection (Lissmann and Machin, 1958) during the process of active electrolocation (Bastian, 1986; Heiligenberg, 1977; von der Emde, 1999). It was soon suggested that electric signals also have a social significance (Lissmann, 1958; Möhres, 1957) and electrocommunication has since been recognized as an important function of electric signaling (Kramer, 1990; Moller, 1995).

Electric organs evolved multiple times independently in several lineages of teleost fishes (Alves-Gomes, 2001), and the capabilities of active electrolocation and electrocommunication developed within two separate orders, the South American Gymnotiformes and the African Mormyriformes. With a few exceptions (Kirschbaum, 1983), all electric organs are derived from muscle precursors that lost their contractibility over evolutionary times but retained their ability to generate electrical potentials (Bass, 1986). Electric organs that are capable of producing weak discharges also developed in synodontid catfish (Baron *et al.*, 1994; Hagedorn *et al.*, 1990) and marine skates (Rajidae), where they are employed during intraspecific communication (Bratton and Ayers, 1987).

The signals that are emitted by electric organs can be classified into pulse-type and wave-type EODs based on their discharge mode (Figure 1.3). Wave-type EODs are generated by several families of South American knife fish and by the African weakly electric *Gymnarchus niloticus* (Hopkins, 1988). Their discharges are produced continuously at rates as low as 24 Hz in *Sternopygus,* and up to 2200 Hz in *Apteronotus* (Albert and Crampton, 2005). Wave-type EODs and are mainly composed of single component frequencies and their higher harmonics, often resulting in almost sinusoidal waveforms

(Heiligenberg, 1977). Mormyrids exclusively produce pulse-type EODs, which are emitted at variable rates and are separated by inter-discharge intervals (IDI) that are considerably longer than the signal itself (Hopkins, 1988). Pulse-type EODs are short, often biphasic, and are composed of a higher bandwidth of component frequencies (Heiligenberg, 1977).

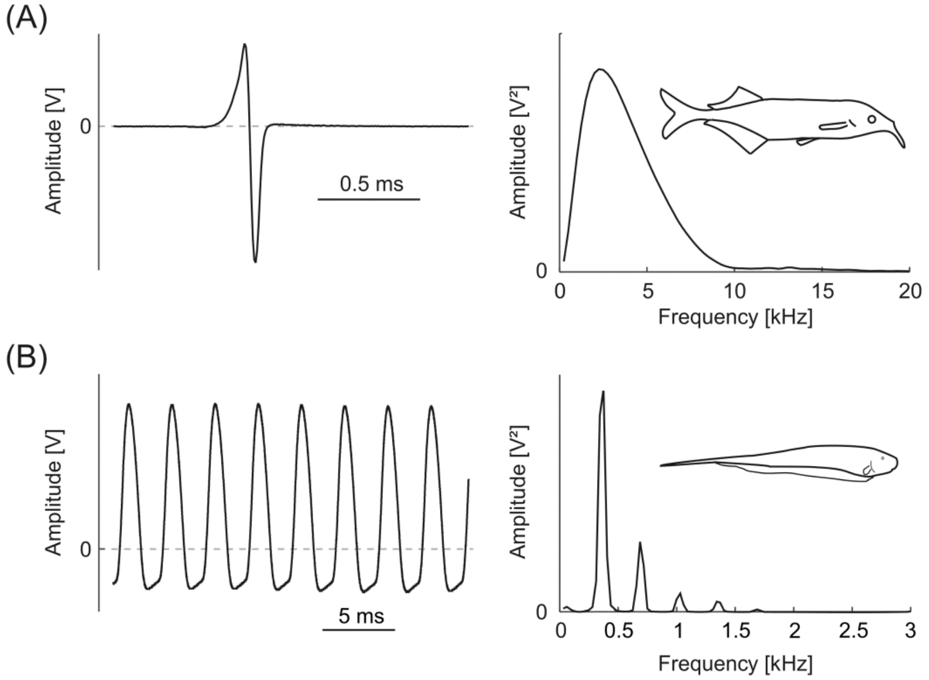

Figure 1.3: Types of electric organ discharges. (A) Pulse-type EOD of *Gnathonemus petersii* (left). The signal is short and biphasic and emitted by the fish at variable intervals that are several times longer than the duration of the EOD. The component frequencies contained in pulse-type EODs are relatively high and have a broad power spectrum (right). (B) The wave-type EOD of *Eigenmannia sp.* (left) is emitted continuously at a single and relatively constant frequency. This discharge frequency and its harmonics constitute the main components of the power spectrum due to the almost sinusoidal nature of the wave-type EOD (right). Modified after von der Emde (1999).

1.3 The electric sense of mormyrid weakly electric fish

Electric organs in mormyrids are located within the caudal peduncle and are composed of four columns of stacked electrocytes (Harder *et al.*, 1964) (Figure 1.4A). Electrocytes are flattened, disc-like cells that are unilaterally and individually innervated, each by a single electromotor neuron through a stalk, which depolarizes their caudal membrane (Bennett, 1971a; Westby, 1984) (Figure 1.4B). Electric organ discharges are initiated by a command nucleus (CN) in the medulla, which generates a command signal that is sent via a relay nucleus to the electromotor neurons that innervate the electric organ (Bell *et al.*, 1983; Grant *et al.*, 1999). Upon activation, electrocytes depolarize unilaterally and generate a small potential difference that can be measured between the anterior and the posterior face of the cell. These voltages add up to the final strength of the EOD due to synchronous activation of electrocytes representing a series connection within the electric organ (Bennett, 1971a). The final amplitude of the signal amounts to just a few volts in weakly electric fish (Bell *et al.*, 1976). Biphasic signals result from subsequent depolarization of the opposite faces of the electrocytes (Westby, 1984). The final waveform (Figure 1.4C) can be more complex, and its characteristics depend on the morphology of electrocytes, their repertoire of ion-channels and their kinetics, as well as their innervation patterns and the complexity of stalks (Caputi *et al.*, 2005).

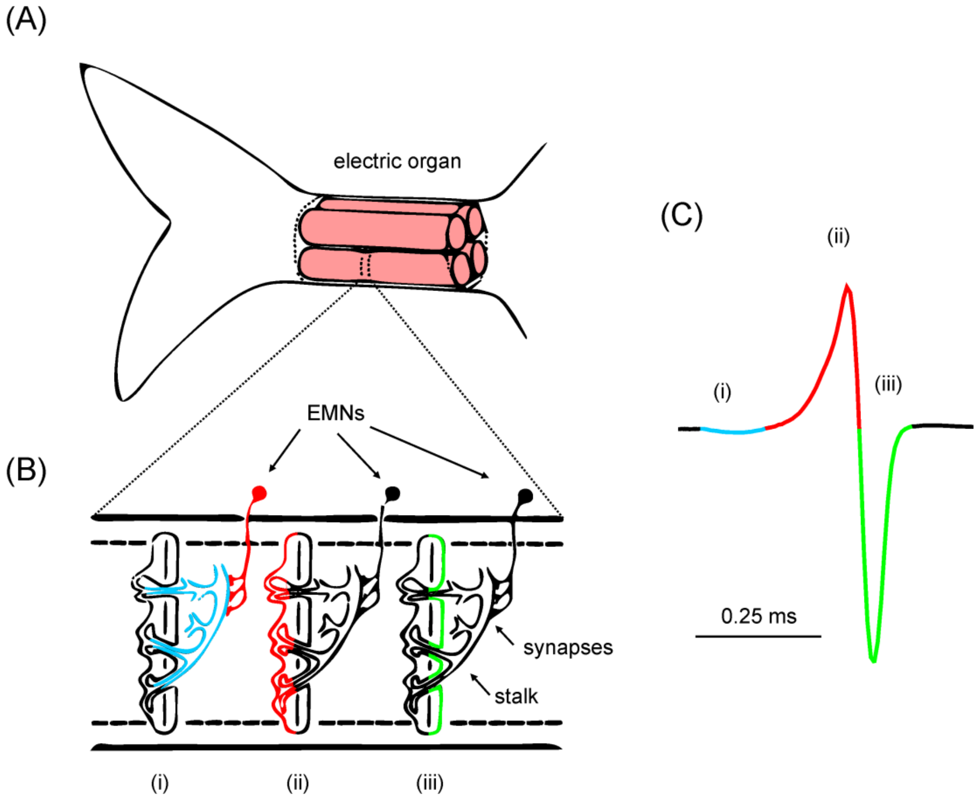

Figure 1.4: Electric organ discharge generation in mormyrids. (A) Location of the electric organ in the caudal peduncle of a mormyrid fish. The organ is composed of four columns of electrocytes, and each column represents a series connection of biogenic voltage sources. (B) Series connection of three electrocytes magnified from a single column in (A). Each electrocyte is innervated by a single electromotor neuron (EMN) via a perforating stalk, which passes through the electrocyte and connects to its caudal face. Central activation of EMNs leads to a depolarization of the stalks (i) and causes a small negative pre-phase of the EOD (C, blue). Depolarization of the stalks activates the caudal membranes of the electrocytes (ii), which generate the primary positive phase of the EOD (red), and eventually the rostral faces (iii), which generates the main negative phase (green). All electrocytes are activated simultaneously, and their voltages add up to the final strength of the EOD. (C) Waveform of the EOD with colors corresponding to the description of sequential activation in (B). Modified after Westby (1984), based on Caputi (2011).

Each discharge of the electric organ instantaneously generates a non-propagating, approximately dipole-shaped electric field around the fish (Hopkins, 1999) (Figure 1.5), and the resulting signal is mainly composed of frequencies way higher than those detected by ampullary receptor organs during passive electroreception. To detect EODs during active electrolocation and electrocommunication, weakly electric fish have additional receptor organs that are tuned to the high frequencies contained in their self-generated EOD (Hopkins, 1981a). These tuberous receptor organs (Figure 1.2B, C) are covered by an epithelial plug, which serves as a capacitor between the outside water and the receptor cells, and therefore renders the receptor insensitive to low-frequency and DC electric fields (Bennett, 1971c). While both electrocommunication and active electrolocation are mediated by the same types of receptors in Gymnotiformes (Caputi *et al.*, 2002; Caputi and Nogueira, 2012), each of the two functions is mediated by a specialized type of tuberous electroreceptor organ in mormyrids.

Mormyromast electroreceptor organs (Figure 1.2B) are specialized to detect the self-generated electric field during active electrolocation in mormyrids (Bell *et al.*, 1989). They are distributed across large areas of the fish's skin (Amey-Özel *et al.*, 2012; Harder, 1968; Hollmann *et al.*, 2008) and contain two different types of sensory cells (Szabo and Wersäll, 1970). Type A cells respond to local changes in EOD-amplitude, whereas type B cells are additionally sensitive to time-shifts, which represent waveform changes of the EOD (von der Emde and Bleckmann, 1992). Nearby objects with an impedance different from the surrounding water are detected and discriminated by the fish during active electrolocation. This is because these objects modulate the self-generated electrical field due to their resistive and capacitive properties (Figure 1.5A), thus generating a two-dimensional electric image on the fish's skin (Caputi *et al.*, 1998; von der Emde and Schwarz, 2002). Parameters extracted from the electric image enable the fish to distinguish objects based on distance (von der Emde *et al.*, 1998), size and shape (von der Emde and Fetz, 2007), as well as material composition (von der Emde and Ringer, 1992). The discrimination of material composition relies on the detection of amplitude and waveform modulations of the local EOD caused by the complex impedances of objects that can be assigned an 'electric color' depending on their resistive and capacitive properties. This electric color is retained independently of distance or size of an object (Budelli and Caputi, 2000; Gottwald *et al.*, 2017a). Because capacitive properties are a commonality of living organisms, this provides mormyrids with a general capability to distinguish animate from inanimate objects and facilitates prey detection in complex environments (von der Emde, 1990; von der Emde, 1994; von der Emde and Bleckmann, 1998). Although electric images cannot be focused on the fish's electroreceptive skin by mechanisms analogous to those of visual systems, active electrolocation provides weakly electric fish with a three-dimensional representation of their immediate surrounding (von der Emde *et al.*, 2010). It also allows them to detect and discriminate between behaviorally relevant features of the environment (Fechler *et al.*, 2012; Fechler and von der Emde, 2013). Consequently, active electrolocation is also used for orientation and navigation (Cain *et al.*, 1994; Walton and Moller, 2010). The active nature of electrolocation in mormyrids is further emphasized by

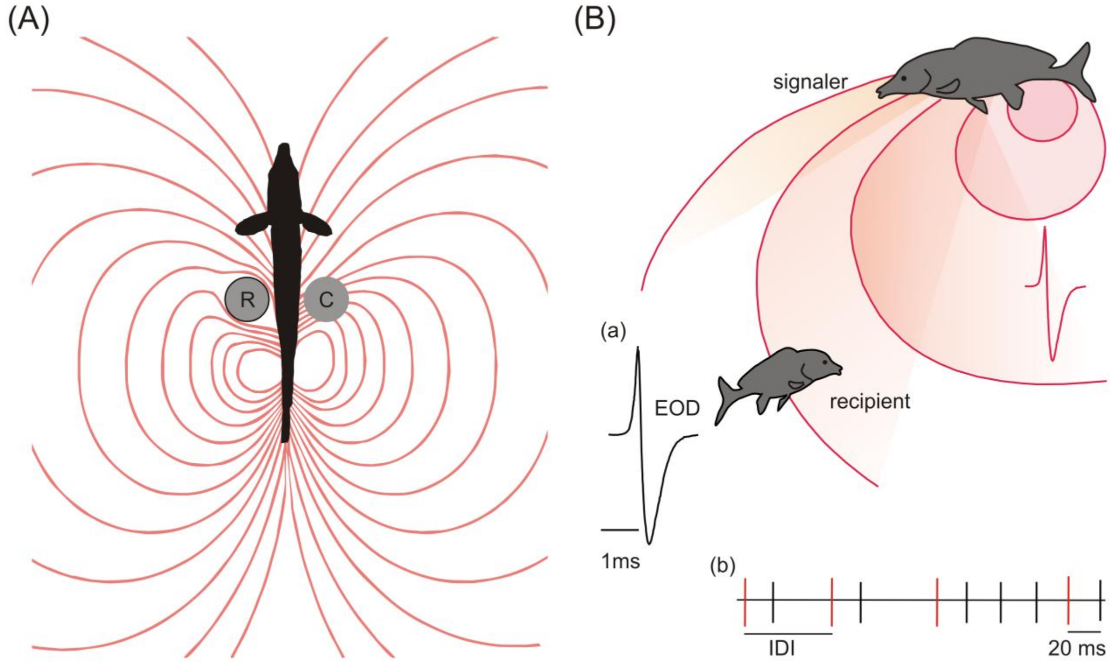

Figure 1.5: General principles of active electrolocation and electrocommunication. (A) During active electrolocation, discharges of the electric organ generate an electric field around the fish. This field is detected by electroreceptor organs, the mormyromasts, which cover large areas of the fish's skin. Objects with electrical properties different from the surrounding water distort the electric field and thereby modulate the amplitude of the local EOD that is detected by individual mormyromasts. Objects with a conductivity higher than that of the surrounding water (C) will focus the field vectors of the electric field onto the electroreceptive skin, thereby increasing the amplitude of the EOD. Objects that are resistive compared with the surrounding water (R) will spread the field lines, thus leading to a local decrease in EOD amplitude. Objects with capacitive properties will additionally distort the waveform of the EOD. Modified from Stoddard (2002a). (B) Electrocommunication is based on the perception of a signaler's EOD by a recipient via the knollenorgan pathway (Figure 1.7). Information about a signaler's identity is contained in the waveform of his EOD (a), whereas current behavioral states and motivations can be communicated through variations of inter-discharge intervals (IDI). The IDI represents the duration between successive EODs of an individual (b) and can lead to distinct discharge patterns. Communication can also arise from interactive signaling, which is characterized by fixed latencies between the EODs of signaler (red) and recipient (black). Inspired by Hopkins (2005).

the display of stereotypical motor patterns that accompany explorative behaviors (Hofmann *et al.*, 2014; Toerring and Belbenoit, 1979). Morphological pre-receptor adaptations facilitate active sensing by focusing the electric field onto electroreceptive foveae with higher receptor densities in the head region of the fish (Bacelo *et al.*, 2008; Pusch *et al.*, 2008). Additionally, discharge rates are dynamically regulated to adapt electrosensory sampling rates according to the current requirements of environmental sensing (Post and von der Emde, 1999; Toerring and Moller, 1984; von der Emde, 1992). Nevertheless, the detection range of active electrolocation is restricted to approximately one standard length of the fish, and the capability to discriminate between objects is usually confined to a distance below 5 cm in *G. petersii* (von der Emde *et al.*, 2010).

One reason for the limited range of active electrolocation is that the sensory threshold of mormyromasts is relatively high (Bennett, 1971c). Together with the axons of the ampullary receptor organs, mormyromast afferents project exclusively to the electrosensory lateral line lobe (ELL) of the hindbrain. Here, somatotopic representations are formed in three separate layers for inputs from passive electroreception, as well as those of type A cells and type B cells of the mormyromasts (Bell and Maler, 2005). A somatotopic map of the active electrosensory system is also retained in the nucleus lateralis of the torus semicircularis in the midbrain, which receives input from the ELL and where phase and amplitude information are likely to be processed (Hollmann *et al.*, 2016). The electrosensory input to the central nervous system is refined by an intricate corollary discharge system, which enhances the sensitivity to reafferent input from the self-generated EOD by mormyromast afferents in the ELL (Bell, 1989). This corollary discharge system also regulates IDI-duration by inhibiting the mesencephalic precommand nucleus (PCN) and the thalamic dorsal posterior nucleus (DP) of the electromotor system, both of which provide excitatory input to the command nucleus, which initiates the EOD (Carlson, 2002b; 2003; von der Emde *et al.*, 2000). Corollary discharges are initiated by activity in the command nucleus and eventually activate the dorsal region of the ventroposterior nucleus (VPd) of the torus semicircularis, which provides inhibitory input to DP and PCN (Carlson, 2003; Carlson and Hopkins, 2004a). This prevents excitatory input of these nuclei to the command nucleus, thus forming a central pattern generator in the mormyrid electromotor system that regulates discharge activity via recurrent inhibition provided by the corollary discharge (Carlson, 2003; von der Emde *et al.*, 2000) (Figure 1.6). In *Brienomyrus brachyistius*, selective stimulation of neurons in DP and PCN induced distinct signaling patterns that typically occur during social interactions, while excitatory stimulation of neurons in VPd caused elongated IDIs indicative of resting behavior (Carlson and Hopkins, 2004a; b). These findings demonstrate the significance of central pattern generation in the electromotor system for active sensing and electrocommunication (Carlson, 2002a).

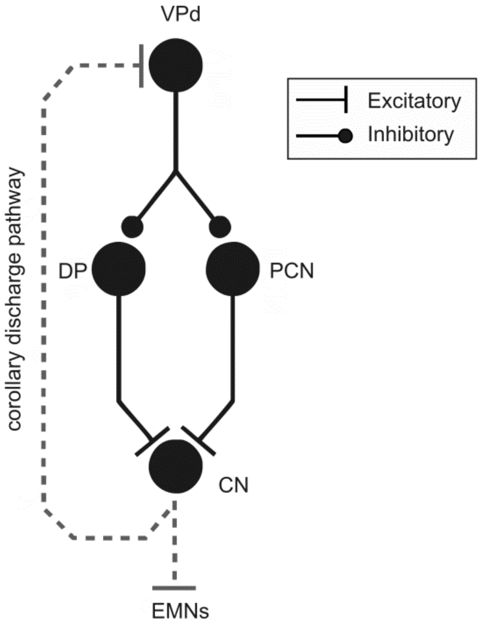

Figure 1.6: Central pattern generator of the mormyrid electromotor system. Activation of the medullary command nucleus (CN) initiates the EOD as well as a corollary discharge that provides excitatory input to the dorsal ventroposterior nucleus (VPd) in the midbrain. Activity in VPd inhibits the dorsal posterior nucleus (DP) and the precommand nucleus (PCN), thus preventing them from activating the command nucleus. Excitatory stimulation of DP and PCN initiates EOD patterns used during electrocommunication, whereas inhibition of DP and PCN via activation of VPd leads to IDI-sequences that are typical of resting behavior. Direct connections are represented by solid lines, indirect connections by dashed lines. Modified from Carlson (2003).

Electrocommunication in mormyrids is based on their second type of tuberous electroreceptor, the so-called knollenorgan (Figure 1.2C). Knollenorgans are composed of 1–9 relatively large secondary sensory cells, each mounted on a subsensory platform within a sensory chamber, and innervated by branches of a single afferent neuron (Derbin and Szabo, 1968; Szabo, 1965). Several lines of evidence unequivocally link knollenorgans to the perception of signals generated by other individuals, thus proving the function of these receptor organs during communication. The overall sensitivity of knollenorgans (0.2-0.5 mV) exceeds that of mormyromasts by more than one order of magnitude (Bennett, 1971c). This makes them ideally suited for the detection of EODs emitted by other weakly electric fish, whose signals can consequently be detected from distances far beyond the range of active electrolocation (Moller *et al.*, 1989).

Knollenorgan receptor cells respond to stimulation with a single spike, which is generated with a short latency of 0.2 ms at the inner face of a sensory cell and transmits electrically to the afferent nerve fiber innervating the receptor organ. At the same time, the outer face of a receptor cell serves as a capacitor that blocks low-frequency signal components (Bennett, 1965). The preservation of timing information during signal transmission is very accurate but comes at the expense of intensity and spatial information (Bell, 1989; Bell and Grant, 1989). Knollenorgan afferents project to the nucleus of the electrosensory lateral line lobe (nELL), where their input is inhibited through a corollary discharge during the animal's EOD. Thus, mormyrids effectively prevent their own signals from being processed beyond the hindbrain (Bell and Grant, 1989). Temporal information about the signals of other fish is passed on to the nucleus exterolateralis of the mesencephalic torus semicircularis (Szabo *et al.*, 1979), where lesions abolished signaling responses to conspecific individuals in *G. petersii* (Moller and Szabo, 1981). Mormyrids belonging to a subgroup termed clade A, show a functional division of the nucleus exterolateralis into nucleus exterolateralis pars anterior (ELa), and nucleus exterolateralis pars posterior (ELp). This neuroanatomical differentiation enables these species to distinguish EOD-waveforms (Baker *et al.*, 2013a; Xu-Friedman and Hopkins, 1999). Because knollenorgans on opposite sides of the fish's body respond to opposing slopes of an external EOD due to their AC-coupling (Bennett, 1965), responses from knollenorgans on different body regions provide the information necessary to differentiate between EOD-waveforms (Hopkins and Bass, 1981). On a neural level, such waveform discrimination is implemented by a delay-line anticoincidence detection mechanism that processes signals transmitted from knollenorgans on different sides of the body in ELa (Baker *et al.*, 2013a; Lyons-Warren *et al.*, 2013; Xu-Friedman and Hopkins, 1999). At low stimulus intensities, waveform differences are discriminated based on a population code of knollenorgans with different sensory properties (Lyons-Warren *et al.*, 2012).

Discharge patterns emitted by other fish are detected through successive activation of the same knollenorgans and are analyzed by sensory neurons in ELp, which receives the output of ELa (Baker *et al.*, 2013a). ELp contains neurons that have low-pass, high-pass, or band-pass filter properties and selectively respond to short, long, or intermediate stimulus IDIs, respectively (Baker and Carlson, 2014; Carlson, 2009). This enables the fish to detect even smallest variations within specific communication patterns (Baker *et al.*, 2016). Knollenorgans and the corresponding neural pathways can thus simultaneously decode the temporal aspects of EOD-waveforms and IDI-patterns, both of which contain behaviorally relevant information (Baker *et al.*, 2013a). The central neuroanatomy of electrocommunication in the mormyrid brain is summarized in Figure 1.7. The functional relationship of the knollenorgan pathway to the central pattern generator of the electromotor system described above is currently unresolved.

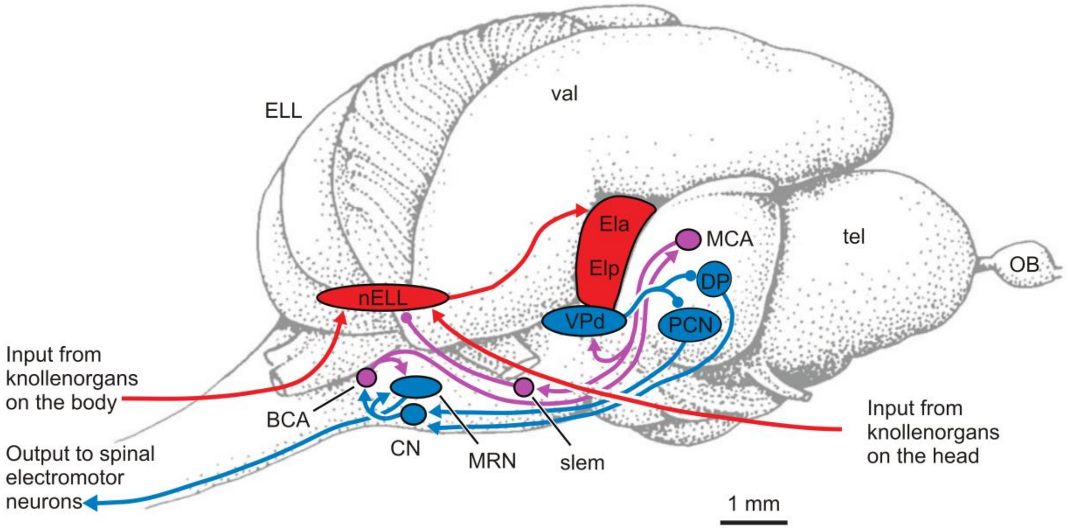

Figure 1.7: Central neuroanatomy of electrocommunication in the mormyrid brain. The mormyrid electromotor system (blue) consists of a medullary command nucleus (CN) that initiates the generation of an EOD via a medullary relay nucleus (MRN), which activates the spinal electromotor neurons that innervate the electric organ (Figure 1.4). Activation of CN also induces a corollary discharge that shapes both electromotor output and sensory perception by recurrent inhibition through dedicated neural pathways (purple). Corollary discharges are relayed via the bulbar command-associated nucleus (BCA) to the mesencephalic command-associated nucleus (MCA), which provides excitatory input to the dorsal ventroposterior nucleus (VPd). VPd belongs to the central pattern generator of the electromotor system (Figure 1.6) and blocks activity in the dorsal posterior nucleus (DP) and the precommand nucleus (PCN). Both DP and PCN provide excitatory input to CN and play an essential role in regulating IDI-duration. Via the sublemniscal nucleus (slem), the corollary discharge pathway provides recurrent inhibition to the nucleus of the electrosensory lateral line lobe (nELL). The nELL is the first central relay that receives sensory input from the peripheral knollenorgans (red). Reafferent signals from the fish's EOD are thus blocked at the level of the nELL. Signals of other fish are relayed to the nucleus exterolateralis, of which the anterior part (ELa) processes EOD-waveform information, while IDI-patterns are decoded in the posterior region (ELp) of this nucleus. ELL: electrosensory lateral line lobe; val: valvula cerebelli; tel: telencephalon; OB: olfactory bulb. Arrows represent excitatory connections; inhibitory connections are marked with circles. Modified from Baker *et al.* (2013a).

1.4 Habitat and sensory ecology of weakly electric fishes

Weakly electric fishes inhabit African, as well as South and Central American tropical freshwater ecosystems. Gymnotiform species are widely distributed throughout the major hydrogeographic regions of the humid Neotropics from northern Argentina to southern Mexico (Albert and Crampton, 2005) (Figure 1.8A). Mormyriformes are mainly riverine species that inhabit the majority of African river systems and lakes and are distributed throughout the main ichthyofaunal regions from the Nile basin and south of the Sahara to north of the Cape (Hopkins, 1986; Hopkins *et al.*, 2008) (Figure 1.8B).

On both continents, the habitats of weakly electric fishes are frequently associated with turbid water conditions (Lissmann, 1958; 1961). Several species tend to display nocturnal activity patterns with hiding or shoaling behavior during the day, and foraging migrations into open waters at night when there is no risk from visual predators (Lissmann, 1961; Moller *et al.*, 1979). A nocturnal lifestyle is also implied by circadian variation of electric discharge activity (Moller, 1995; Stoddard *et al.*, 2007) and retinal adaptations that trade off spatial resolution for improved detection of low-contrast stimuli, such as large, fast-moving predators, under dim light conditions (Francke *et al.*, 2014).

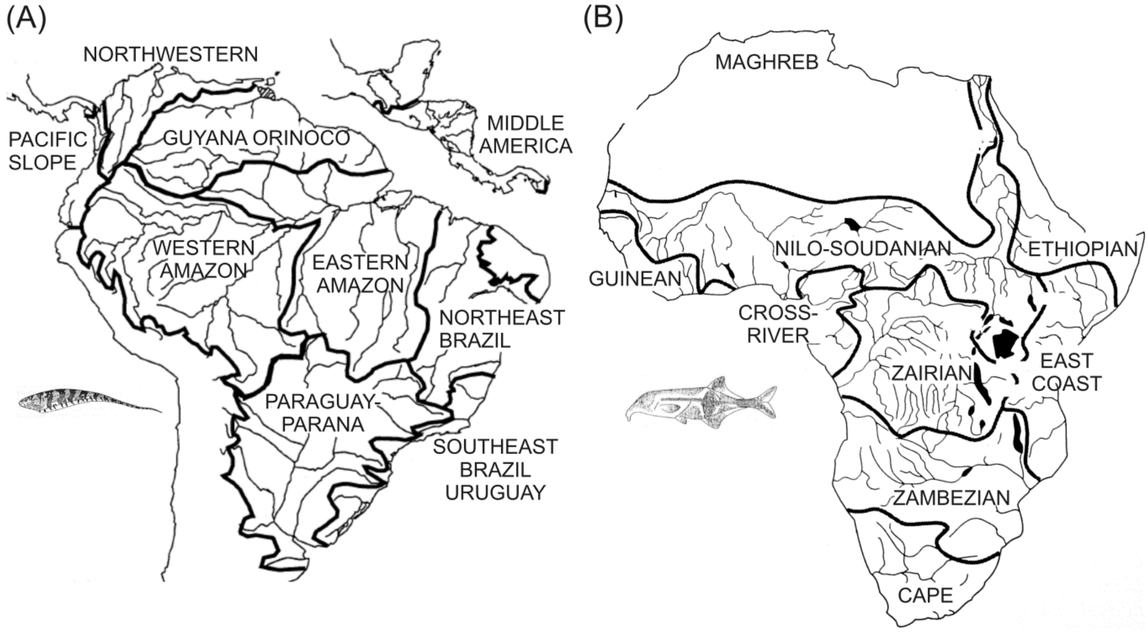

Figure 1.8: Geographical distribution of weakly electric fishes. (A) Neotropical habitats of South and Central American Gymnotiformes based on hydrogeographic regions. Adapted from Albert and Crampton (2005). (B) Geographical distribution of mormyrids based on the main African ichthyofaunal regions. Mormyrids are absent from the southern Cape and the northern Maghreb regions. Modified after Moller (1995).

Electric organ discharges of both gymnotiform and mormyriform weakly electric fishes are highly diverse in discharge type, waveform, and frequency (Hopkins, 1988). A possible explanation for such specifications are environmental constraints related to the specific habitat of a species. In a comparative study on the habitats of several Gymnotiformes, Lissmann (1961) noticed that species with a high-frequency wave-type EOD (> 800 Hz) were only encountered in fast-flowing waters, mostly among rocks and sandy grounds without dense vegetation, whereas no such preference was observed in species with lower frequency discharge modes. Exempt from this rule were those fish with the lowest-frequency wave-type EOD (< 100 Hz), which preferred calm and stagnant waters. Pulse-type species generally exhibited rather sedentary and sluggish behaviors and were associated with calm waters featuring dense vegetation and complex root systems (Lissmann, 1961). This relationship was contested by Hopkins and Heiligenberg (1978), who suggested that EOD-types represent a behavioral rather than an ecological adaptation, with wave-type fish being more active and agile in their behavior, thus requiring a higher resolution during active electrolocation of fast moving objects compared with the more sedentary pulse-type fish. The correlation between habitat and discharge mode was later confirmed by Crampton (1998), who also found that the EOD-repetition rates of pulse-type species in habitats with water flow is higher than in standing water bodies. He suggested that in particular wave-type species with high discharge rates need a high temporal resolution to detect objects, such as prey that move fast in relation to the fish in simple environments, whereas pulse-type species specialized in detecting capacitances in complex environments with dense vegetation and relatively static prey (Crampton, 1998). High-frequency wave-type *Apteronotus* can efficiently capture small Daphnia in open water in the dark using active electrolocation (MacIver *et al.*, 2001). Crampton (1998) demonstrated that high-frequency species tend to feed on such planktonic prey, whereas lower frequency and pulse-type fish rely on stationary prey, such as *chironomid* larvae. However, wave-type fish are in principle also able to discriminate resistive from capacitive objects (von der Emde, 1998).

Except for piscivorous *Mormyrops* (Arnegard and Carlson, 2005), mormyrids mainly feed on invertebrates (Lauzanne, 1988). *Chironomid* larvae constitute a major food source for many mormyrid species, which can, therefore, be classified as benthic invertivores (Kouamélan *et al.*, 2006; Kouamélan *et al.*, 1999). All mormyrids emit pulse-type EODs, and their capability to discriminate capacitive objects is inversely correlated with EOD-duration (von der Emde and Ringer, 1992). Shorter pulse-durations were observed mainly in more gregarious species and were hypothesized to be an adaptation that reduces the probability of EOD overlaps in groups (Hopkins, 1980), thereby aiding to prevent jamming during active electrolocation (Heiligenberg, 1976; Schuster, 2001).

Mormyrids are frequently preyed upon by electroreceptive catfish (Hanika and Kramer, 2000; Merron, 1993). The evolution of short, biphasic electric signals in both gymnotiform and mormyriform species may have served in cloaking weakly electric fish from such predators because of a reduction of the lower component frequencies of the signal, to which passive electrosensory systems are most sensitive (Stoddard, 1999; 2002b). EOD-waveforms of many mormyrids are sexually dimorphic, especially during spawning, and males tend to emit EODs with longer duration (Carlson *et al.*, 2000; Hopkins, 1986), which is considered to be a dominance marker that effectively makes them more vulnerable to predation (Hanika and Kramer, 2000; 2005). Evolutionary adaptation of EOD-waveforms to predation pressure may thus have initiated sexual selection based on signal recognition and thereby triggered the extensive speciation in both groups of weakly electric fishes (Stoddard, 2002b).

Indeed, the Mormyridae are the most abundant group within the otherwise species-poor Osteoglossomorpha, comprising more than 200 species in 21 genera (Miller and Sullivan, 2017). Sexual selection based on EOD-diversification is considered to be the primary driving mechanism behind this relatively recent and ongoing speciation of mormyrids (Arnegard *et al.*, 2010a; Feulner *et al.*, 2008; Sullivan *et al.*, 2002). For instance, differential expression of genes that code for voltage-gated ion channels may play a role in shaping EOD-waveforms and consequently contribute to speciation by mate choice based on EOD characteristics in mormyrids (Nagel *et al.*, 2017). Hence, new species and even genera of mormyrids have recently been described based on EOD-waveform (Kramer and van der Bank, 2000) and molecular data (Maake *et al.*, 2014; Sullivan *et al.*, 2016).

Lissmann's discovery sparked considerable interest in the investigation of active electrolocation, and a great deal of advance has been made unveiling the morphological, physiological, and behavioral parameters and principles that underlie this ability. Weakly electric fish even inspired attempts to translate their sensory capabilities into technical applications (Bleckmann *et al.*, 2004; Caputi, 2017; von der Emde *et al.*, 2009) (see also Part Two). Several strategies for electrocommunication have been described for both mormyriform and gymnotiform species and will be dealt with in more detail in Part One. However, little is known about the behavior of weakly electric fishes in their natural habitats, especially concerning how they interact and communicate with other individuals or in social groups of different sizes. Several authors have addressed geographical distribution, species diversity, food habits, and the influence of anthropogenic impact on populations of weakly electric fishes in ecological field studies (Blake, 1977; Kouamélan *et al.*, 2006; Sullivan *et al.*, 2002). Such studies have demonstrated that some mormyrids are gregarious and form schools (Hopkins, 1981b), while others, such as *Brienomyrus*, are territorial and occupy individual shelters (Friedman and Hopkins, 1996; Hopkins and Bass, 1981). Breeding in mormyrids is induced by the environmental changes that occur during the rainy season (Kirschbaum, 1975). During this time, many mormyrid species were observed to migrate from their river habitats into smaller streams and flooded areas to spawn, and from there the juveniles migrate back to the rivers in large schools when the dry season begins (Hopkins, 1986; Kirschbaum, 1995). Social behavior of mormyrids is thus versatile. Whether individuals are gregarious and aggregate in shoals or display territorial and agonistic behavior, may depend not only on species, sex, and age, but may also vary seasonally or depending on the time of day (Carlson, 2016; Hopkins, 1986; Moller *et al.*, 1979). Detailed behavioral observations of mormyrids during their nocturnal activity in the wild were only reported for *Mormyrops anguilloides*, who gather in small groups to hunt for cichlids in Lake Malawi (Arnegard and Carlson, 2005). Observations of riverine mormyrids under semi-natural conditions revealed complex interaction patterns and electrocommunication even among different species (Scheffel and Kramer, 2006). Recordings of electrocommunication behavior with high spatial and temporal resolution from a natural habitat have only recently been accomplished for gymnotiform *Apteronotus rostratus*. These observations suggested that data obtained from the laboratory need not necessarily represent what happens under natural conditions (Henninger *et al.*, 2017).

Laboratory studies have documented a multitude of signaling strategies that mormyrids engage in during active electrolocation and social interactions. Social signaling occurs for instance during aggressive encounters (Bell *et al.*, 1974; Kramer and Bauer, 1976; Terleph, 2004; Werneyer and Kramer, 2002), courtship and spawning (Baier and Kramer, 2007; Bratton and Kramer, 1989; Werneyer and Kramer, 2005; Wong and Hopkins, 2007), as well as during group activities like resting, swimming, and foraging (Gebhardt *et al.*, 2012a; Gebhardt *et al.*, 2012b; Scheffel and Kramer, 1997). Apart from IDI-variations that result in characteristic modulations of discharge frequencies and behavior-specific signaling patterns, mormyrids can also generate so-called echo responses with a fixed latency of only a few milliseconds to EODs of nearby individuals (Kramer, 1974; Russell *et al.*, 1974). Prolonged episodes of mutual echoing lead to interactive electric signaling patterns that are characterized by synchronized discharge sequences between two individuals. This may serve in jamming avoidance (Heiligenberg, 1976), but it is also assumed to have a communicative function, possibly by facilitating group cohesion (Arnegard and Carlson, 2005; Gebhardt *et al.*, 2012a).

2. Study Aims and Objectives: Mobile Dummy Fish for the Investigation of Electrocommunication

A major challenge when studying electrocommunication is the correct assignment of EODs to individual fish in groups of two or more unrestrained animals, which can be time-consuming and difficult. Such difficulties may in part be overcome by playback experiments, which can evoke stereotypical communication behavior in mormyrids (Kramer, 1979). Such tests have been used to demonstrate the capability of mormyrids to differentiate EOD-waveforms (Graff and Kramer, 1992; Hanika and Kramer, 2005; Machnik and Kramer, 2008a) and IDI-patterns of other fish (Kramer and Kuhn, 1994). However, playback electrodes usually lack the locomotor behavioral component that is important during interactive communication displays involving mutual feedback between signaler and recipient (Crockett, 1986). In other words, a stationary playback source does often not behave according to the signals it emits. Besides, responses with communicative intent by the receiving fish do not affect the agent that initiated communication. This may induce a perceptual mismatch between what is communicated by a playback, and what is acted upon by the receiving animal. This mismatch may jeopardize the validity of an observed behavioral reaction as an appropriate response to the original communication signal.

In the present thesis, the potential of using mobile fish dummies to overcome such limitations during the investigation of electrocommunication will be explored in playback experiments with the pulse-type weakly electric mormyrid *Mormyrus rume*, mainly represented by the subspecies *proboscirostris*. *Mormyrus rume proboscirostris* originates from the middle Congo River (Kirschbaum, 1995) and can be reliably bred in captivity (Schugardt and Kirschbaum, 2004). This thesis is based on the work of Gebhardt (2012), who provided detailed descriptions of signaling patterns and strategies occurring during various behavioral situations in groups of up to five individuals of *M. rume*, including their reactions to electrical playback of such signaling patterns. In the present work, electrical playbacks of IDI-patterns were systematically extended, and their presentation was refined by mounting playback electrodes on mobile fish dummies. This allowed behavioral control and repeatability of stimuli across experimental trials, as well as including spatial aspects of social interactions into the analysis of behavioral responses.

Part One of this thesis focuses on strategies employed by *M. rume* during electrocommunication. From an electromotor perspective, such strategies can rely on variations in overall discharge frequency, the emission of distinct temporal discharge patterns, or on interactive signaling behavior based on fixed temporal relationships between the discharge activity of two fish. Locomotor strategies include the display of stereotyped motor patterns and spatial relationships between the playback source and the receiving fish. In the first series of experiments, a behavioral setting based on following-behavior was created that allowed to systematically investigate the influence of electrical playback of naturally occurring discharge patterns that were prerecorded from freely behaving fish during various behavioral contexts. Natural discharge sequences were characterized either by a certain average discharge frequency or by a particular IDI-pattern. Responses of *M. rume* to natural patterns were contrasted with those to artificial sequences of constant frequency discharge patterns. The behavioral implications of differential expression of response patterns are discussed and interpreted with respect to the behavioral contexts implied by the playback signal and the hierarchical relationships of the fish. In a second setup, the role of interactive signaling was tested by contrasting responses to static playbacks of naturally occurring but randomly arranged IDIs with responses to an interactive playback that dynamically responded to signals emitted by the fish by mimicking the mormyrid echo-response. Implications of echoing for social communication in mormyrids are discussed.

Part Two explores the contributions of different sensory modalities of *M. rume* in a second set of experiments to identify the sensory basis of the following-behavior observed in Part One. In a combination of classical ethological experiments and a state-of-the-art ethorobotic design, single individuals and small groups of *M. rume* were confronted with a biomimetic robotic dummy fish mimicking live fish in morphology, size, and motility cues, additionally to electric playback generation. In a subsequent test series, sensory cues from vision, the lateral line system, and active electrolocation were experimentally excluded, narrowing down the perception of the signal source to passive electric sensing, probably mediated by the knollenorgan pathway. Actively generated electrical signals were thus identified as the critical stimuli that are both necessary and sufficient to initiate and mediate the social interactions displayed by *M. rume*.

Based on the conclusions from previous sections, Part Three inquires the significance of electrocommunication during social interactions in small groups of different sizes with the long-term goal of establishing mixed societies of live fish and interactive dummies based on electric signal generation. By closing the feedback loop between the behavior of live fish and the mobile dummy on the two levels of locomotor behavior and electrical signaling, the general feasibility of such an approach was evaluated for a mormyrid weakly electric fish. Through detailed analysis of behavioral observations and simultaneously recorded signaling interactions, a possible function of interactive signaling for addressing individuals within a group via electrocommunication could be identified.

II. Part One: Communication

3. Introduction to Animal Communication

3.1 Definitions and the evolution of communication systems

Communication is a universal feature of living organisms, ranging in its complexity and dimensions from molecular interactions on a subcellular level to the use of recursive language in human societies and the transmission of digital information around the globe. This omnipresence of communication processes substantiates the importance to delineate and define animal communication systems in a way that allows formulating hypotheses that can be tested in experimental studies. Watzlawick *et al.* (2007) may be right in noting that all behavior is essentially communication, and hence not to communicate is as impossible as not to behave. This understanding, however, does not provide an operational definition for hypothesis-driven research on animal communication.

Generally speaking, communication can be defined as a transfer of information (Hurd and Enquist, 2005). By defining communication in a comprehensive sense that includes all procedures by which two mechanisms affect each other, Shannon and Weaver (1998) point out a sequence of three levels of problems that need to be solved during communication. These include the accuracy of the transmission of communication symbols, the precision with which these symbols convey the desired meaning, and the effectiveness with which the perception of that meaning affects a receiver's behavior (Shannon and Weaver, 1998). From a technical point of view, communication signals generated by a signaler need to be coupled to a medium and then propagate to the location of the receiver, who in turn needs to detect, decode, and classify the signal (Bradbury and Vehrencamp, 2011). Detailed knowledge about the effectors that generate communication signals, the receptors that perceive such signals, and the influence of environmental conditions that affect their transmission, is thus essential for the study of animal communication systems.

Animal communication systems, however, require a shared repertoire of signs, as well as semantic and pragmatic rules to transport context-specific information, and such features cannot emerge from one-way interactions during evolution (Witzany, 2013). Wilson (1975) defines communication as actions of an organism that influence the probability of occurrence of another organism's behavior patterns adaptively. From an evolutionary point of view, it then becomes clear that communication systems can only evolve to the net-benefit of both sender and receiver (Bradbury and Vehrencamp, 2000). Empirically, the existence of animal communication systems can be identified where a sender's signal modifies the response of a receiver, and where different signals lead to varying responses by that receiver. Therefore, a simple 'action-response game,' where the sender selects a signal, and the receiver chooses an appropriate response (Figure 3.1), is the minimum requirement for an operational definition of animal communication (Hurd and Enquist, 2005).

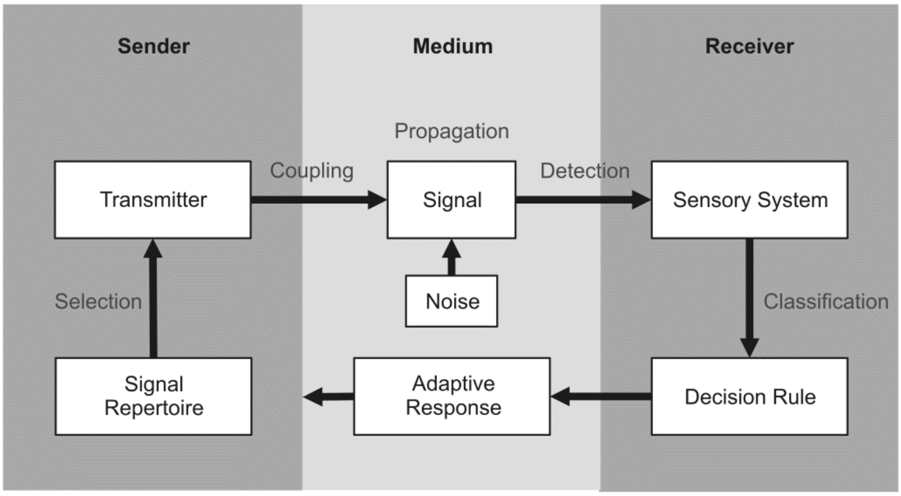

Figure 3.1: Schematic illustration of animal communication. Communication involves a sender, who generates a signal, and a receiver, who reacts to that signal. The sender selects an appropriate signal from his repertoire based on a behavioral context or an internal motivation to communicate. The signal is coupled to a medium via the transmitting effector organ chosen by the sender. It is then propagated through the medium to the receiver and may be affected by environmental noise in the process. The receiver will detect the signal with a dedicated sensory system that is responsive to stimuli in the sensory modality the sender chose for communication. The signal is then decoded and classified by the receiver and evokes an adaptive response based on the receiver's internal decision rules. Ultimately, this response also affects the sender. Inspired by Shannon and Weaver (1998) and Bradbury and Vehrencamp (2011).

Communication is often directly associated with human language. In a narrow sense, however, the faculty of language requires recursion, i.e., the capability to use a finite amount of expressions to generate an infinite amount of meanings (Hauser *et al.*, 2002). The hierarchical syntactic structure necessary to accomplish this task has not been identified in any animal communication system other than human language so far (Bolhuis *et al.*, 2014). Thus, while language can represent, and, if necessary, communicate complex and arbitrary concepts in past, present or future independently of the modality used for its externalization (Fitch, 2000), communication needs to be interpreted in the context of the respective environment and does not necessarily depend on a conscious intent to communicate (Watzlawick *et al.*, 2007).

The study of animal communication is concerned with the nonverbal, or rather prelingual aspects of communication, which humans share with other animals. For instance, visual communication can convey basic emotions such as happiness, anger, sadness, fear, surprise, and disgust via facial expressions that are understood by humans across different cultures (Ekman, 1970). Similarly, dominance relationships can be inferred from the display of gestures and postures humans adopt during interactions (Bente *et al.*, 2010). Independently of the verbal content of a conversation, a speaker's emotional state can be inferred from variations in speaking rate, intensity, fundamental frequency and other spectral parameters of the voice (Scherer, 2003). Especially olfactory communication can be entirely subliminal. The evidence is accumulating that mate choice also in humans is influenced by the unconscious olfactory perception of a potential partner's Major Histocompatibility Complex (MHC I). This leads to increased physical attraction to individuals with an MHC I that differs from one's phenotype and thus ensures protective variability in the immune system of prospective offspring (Jaworska *et al.*, 2017).

Comparative studies on animal communication systems can, therefore, shed light not only on nonverbal human communication but also on the evolution of human language, because they can identify the ecological constraints that led to the evolution of prerequisites for language in other animals (Hauser *et al.*, 2002). These include the capability for vocal imitation as a precondition for speech, which is absent in nonhuman primates (Fitch, 2000) but common for instance in cetaceans (Janik, 2000). Similarly, ontogenetic parallels between language acquisition in humans and vocal learning in birds offer the possibility to study neuroanatomical and developmental principles in an analogous vertebrate communication system (Wilbrecht and Nottebohm, 2003). Finally, the discovery of 'mirror neurons' has interesting implications for communication. These neurons were identified in the premotor cortex of monkeys, where they are active both when the animal acts, as well as when it observes a similar action in another individual (Rizzolatti and Arbib, 1998). This finding, led to the hypothesis that the communication of intentions by use of spoken language might have evolved from gestural communication via the mirror neuron system. In this context, mirror neurons could establish a link between actor and observer, who then become sender and receiver of communication signals (Rizzolatti and Arbib, 1998). Such considerations also stress the potentially multimodal or even crossmodal nature of communication.

The fact that almost all sensory modalities play a role during communication gives reason to ask the question if there are universal principles that underlie the evolution of communication signals in different sensory systems. Through careful observations of animal behavior, ethologists like Konrad Lorenz and Nico Tinbergen inferred that signaling displays evolved from displacement and intention movements via ritualization (Tinbergen, 1952b). Complex and innate behavioral sequences displayed for instance during courtship behavior of three-spined sticklebacks (*Gasterosteus aculeatus*) (Tinbergen, 1952a) and contests between male fighting fish (*Betta splendens*) (Lissmann, 1932) have been identified and described in many animals.

Communication systems can emerge from behavioral or sensory pre-adaptations. All animals use their sensory systems to monitor their environment for cues that provide information relevant to survival and reproduction and evolve decision rules that enable them to react to such information in adaptive ways. If a particular behavior offers useful cues to a receiver and is thus favored by the receiver's decision rules, this behavior may become ritualized into a signal if both parties benefit from communication (Bradbury and Vehrencamp, 2011). On the other hand, senders may evolve signals that exploit pre-existing sensory preferences and decision rules in receivers. There is, therefore, a minimum of reliability that a potential signal must have before either sender or receiver benefit by engaging in communication (Bradbury and Vehrencamp, 2000).

Communication is of particular importance in reproductive and competitive behavioral contexts. Sexual selection by female choice lies at the heart of many communication systems and can, for instance, evolve if males generate signals that appeal to female perceptual biases, which initially evolved to serve other purposes such as food detection or predator avoidance (Ryan and Cummings, 2013). Intrasexual competition, especially male-male competition for resources, is another important context where communication systems are expected to occur. Males of many species establish and defend territories that grant them access to food and females and thus increase the fitness payoffs for a territory holder, but energy intensive and potentially harmful territorial fights will diminish such payoffs. The capability to assess one's resource holding power (RHP) in relation to a contestant is thus essential to avoid fitness costs associated with unnecessary fights (Parker, 1974).

Based on game-theoretical considerations, Maynard Smith and Price (1973) derived the concept of the evolutionary stable strategy (ESS) to explain why so many male-male contests are resolved without serious injury, even though the contestants are often equipped with weaponry suitable for escalated fighting and would undoubtedly maximize their fitness in case of winning. According to this concept, behavioral strategies are evolutionary stable if a population that behaves accordingly cannot be 'invaded' by individuals adopting a 'mutant' strategy with higher fitness payoffs for these individuals (Maynard Smith and Parker, 1976). Such strategies are adaptive because, in situations where there is a conflict of interest between individuals, the best strategy to maximize one's fitness depends on what strategies other individuals adapt (Maynard Smith, 1976). An ESS for fighting behavior would thus predict that competitions over resources between contestants with asymmetric RHP are settled 'conventionally' and that escalated fights occur only where such differences are not obvious, for instance, when opponents are similar in size (Maynard Smith and Parker, 1976).

Based on these considerations, Enquist and Leimar (1983) predicted the existence of sequential assessment strategies for conflict resolution. Sequential assessment involves multiple stages of repetitive, ritualized behavioral displays, which allow each contestant to sample information about the opponents fighting abilities in successive rounds of increasingly costly displays. Based on this information, each contestant can evaluate his probability to win an escalated fight and will decide to either leave the contest and give up the resource, or to stay in the competition, proceed to the next level, and risk an escalated fight (Enquist and Leimar, 1983). The existence of sequential assessment strategies during aggressive encounters between males was confirmed in behavioral experiments with the cichlid fish *Nannacara anomala* (Enquist *et al.*, 1990).

3.2 Classification of communication signals

Several approaches can be used to classify communication signals (Bradbury and Vehrencamp, 2011). One way is to categorize signals based on the information they provide about the sender, who conveys information either about his identity or concerning his current motivation. Identity information may reveal a signaler's species, but also his membership of a social group, for instance through vocal dialects in humans, cetaceans (Strager, 1995; Weilgart and Whitehead, 1997), and birds (Rothstein and Fleischer, 1987). Individual identity can be determined for instance through facial or voice recognition, and individual identification based on auditory cues has been demonstrated for the calls of ravens (*Corvus corax*) (Boeckle and Bugnyar, 2012) and the signature whistles of bottlenose dolphins (*Tursiops truncatus*) (Tyack, 1997). In addition, senders convey information about their sex, which is most apparent in sexually dimorphic species (Owens and Hartley, 1998). Furthermore, identity information may concern an individual's reproductive state (Semple and McComb, 2000) and social status (Maynard Smith and Harper, 1988), the latter of which is of particular importance in group-living animals that establish social hierarchies.

Motivational signals communicate emotional or intentional information that can relate to different contexts. Examples are courtship and mating signals in the context of reproduction, as well as aggressive threats and dominance signals during competition for mates, territories, and food. Social integration signals maintain bonds between partners (Young *et al.*, 2011), parents and their offspring (Iacovides and Evans, 1998), as well as group cohesion in herds, flocks, and schools of animals (Parrish and Hamner, 1997). Environmental signals are concerned with food sources and predator avoidance. Examples are the waggle dance of honey bees (*Apis mellifera*), which provides other workers with information about distance and direction to a nectar source (von Frisch, 1965), and the alarm calls of vervet monkeys (*Cercopithecus aethiops*), who have different codes for different types of predators (Seyfarth *et al.*, 1980).

Communication signals can also be classified based on the mechanisms that guarantee signal reliability and thereby assure mutual benefit for sender and receiver (Hurd and Enquist, 2005) (see Figure 3.2). For a communication system to be evolutionary stable, receivers require some form of assurance that reacting to a signal increases their fitness payoffs and not merely tricks them into behaviors that only serve the interest of the signaler. Such reliability guarantees are essential if there is a conflict of interest between the two parties (Maynard Smith, 1991). This means that in cases where there is no direct causal relationship between a signal and the quality a sender intends to communicate about himself, either the signaler needs to prove he is not cheating, or receivers need strategies to call a bluff. Hurd and Enquist (2005) subdivided adaptive signals into 'performance signals,' which cannot be faked and can only be produced by senders with a certain quality directly related to the signal, and 'strategic signals,' which can in principle be produced by anyone and are therefore vulnerable to cheating. Performance signals do not need to provide a reliability guarantee because there is a direct causal relationship between the signal and the signaled quality that reserves the signal for individuals who have that quality. Performance signals are therefore 'honest' by default. Such signals are 'index signals' if the physical condition of the sender constrains their expression. Classic examples are the relationship between dominant croak frequency and size of male toads (*Bufo bufo*), which cannot be faked by smaller individuals (Davies and Halliday, 1978), and the roaring activity of red deer (*Cervus elaphus*), which indicates the body condition of a harem holder (Clutton-Brock and Albon, 1979). Performance signals may also be constrained by information that the signaler holds (Hurd and Enquist, 2005). They can, for instance, be used by prey to deter a predator by signaling that its approach has been detected, or by demonstrating the ability to escape a potential attack (Leal, 1999).

Signals that are not directly constrained by some inherent property of a sender are strategic. For strategic signals to be evolutionary stable, senders must be willing to bear costs as a reliability guarantee (Hurd and Enquist, 2005). 'Handicap signals' are costly to senders and thereby demonstrate that the signaler can afford to generate the signal (Grafen, 1990; Zahavi, 1975). Signaling costs thereby either incur during signal production, because senders must be willing to expend resources for signal generation, or they arise as a consequence of signal display, for instance, due to increased predation risk (Hurd and Enquist, 2005). 'Conventional' signals do not underlie such constraints. Their meaning is not necessarily associated with any property of the signal itself and may thus easily be produced by any individual (Guilford and Dawkins, 1995; Hurd and Enquist, 2005). Conventional signals are therefore vulnerable to cheating (Dawkins and Guilford, 1991). However, costs are imposed on senders of conventional signals by the reaction of receivers, who may challenge the sender and retaliate upon detection of dishonest signaling (Dawkins and Guilford, 1991).

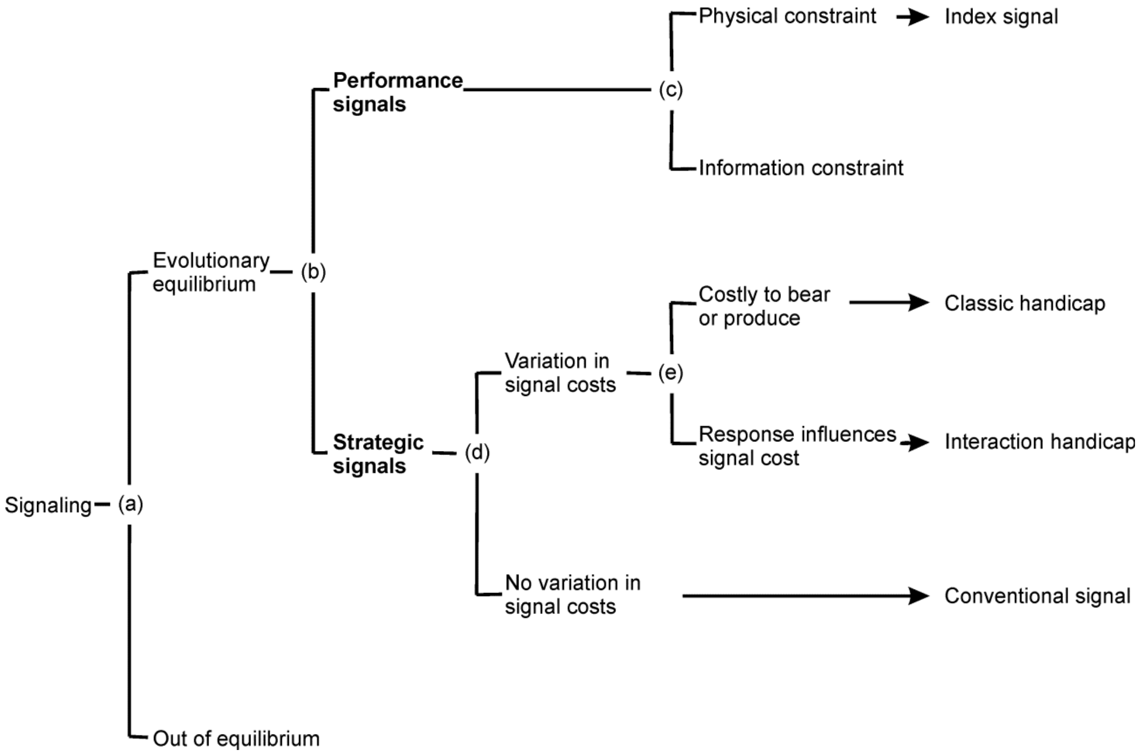

Figure 3.2: Taxonomy of signal types. A signaling system that is beneficial to both sender and receiver is expected to attain a state of evolutionary equilibrium (a). Signals from evolutionary stable communication systems (b) can be subdivided into performance signals and strategic signals. Performance signals (c) are reliable because they are constrained by a sender's physical properties or by information held by the sender. Any individual may use strategic signals (d). Their reliability is only guaranteed by the sender's ability to bear costs that arise as a consequence of signaling. Signaling costs can be inflicted through handicaps and vary depending on the senders quality because only high-quality individuals can afford strong handicaps (e). The costs of conventional signaling are not dependant on either the message or the signaler's condition. The reliability of conventional signals is guaranteed by receiver probing and retaliation in case of dishonest signaling. Modified from Hurd and Enquist (2005).

3.3 Communication in fishes

Fishes have a multitude of very sensitive sensory systems that enable them to acquire information through several sensory modalities. In many species, some sensory systems are highly specialized and constitute adaptations to very particular habitats and ecological niches. Sensory information is used for long and short range orientation and navigation, food detection, and predator avoidance. Most senses are, however, also used for communication. While visual communication among fishes is most apparent to the human observer, responses to chemical and acoustic communication signals are very common as well.

Both mormyriform and gymnotiform weakly electric fishes make use of all the standard sensory systems during their natural behavior (Moller, 2002; Schuster, 2006). Additionally, their active electrosensory systems not only allow them to acquire sensory information about the environment (see section 1.3) but also open communication channels with significant implications for their lifestyle and social behavior.

3.3.1 Visual communication

Vision is the dominant sensory system for prey detection, orientation, and navigation, as well as social communication for a large number of fish species, many of which possess big eyes and devote large areas of their brains to the processing of visual information (Kotrschal *et al.*, 1998). The importance of visual signals for social communication is apparent from the vast amount of colorful displays that are characteristic for many species, particularly among teleosts. Fish visual systems have adapted to photic environments with visual conditions as different as the high lighting intensities of tropical coral reefs and the virtual absence of sunlight in deep-sea habitats (Douglas, 2001). Many fishes have excellent color vision, a trait that dates back at least 540 million years to the agnathan vertebrate lineages represented only by lampreys and hagfish among extant species (Collin and Trezise, 2006). Several teleosts, such as the goldfish (*Carassius auratus*), possess four types of photoreceptor cones (Bowmaker *et al.*, 1991) and have tetrachromatic color vision (Neumeyer, 1992). The spectral sensitivity of various fishes exceeds the range of light that is visible to humans. Some species can use the relatively private channel of short-wavelength ultraviolet radiation for social communication (Siebeck, 2013). Others rely on red fluorescent light in marine habitats below 10 meters, where the long-wavelength components of ambient sunlight become attenuated (Anthes *et al.*, 2016).

Visual communication displays may consist of behavioral elements, such as ritualized postures and movement patterns, as well as morphological features, like elongated fins, or of conspicuous color patterns. All these attributes allow specific and goal-directed signaling. Coloration can result from structural elements that cause refraction of light and selective reflection of particular wavelengths, and from pigment cells that selectively absorb wavelengths based on the chemical properties of pigment molecules. Especially pigment-based coloration allows for active regulation of color displays (Shawkey and D'Alba, 2017). Consequently, coloration can vary according to dominance relationships (Howard, 1974; O'Connor *et al.*, 1999), stress levels (Backström *et al.*, 2015), and visual background conditions of the environment (Kelley *et al.*, 2016). Additionally, various social contexts cause specific color changes (Rodrigues *et al.*, 2009), and especially males of many species adopt intense nuptial colorations for courtship displays (Allender *et al.*, 2003; Bakker and Milinski, 1993; Gumm *et al.*, 2011). Color patterns can be designed to camouflage individuals from a distance, while simultaneously serving in social signaling at closer ranges (Marshall *et al.*, 2003). Visual signals are essential for recognizing species (Siebeck *et al.*, 2010) and even individuals (Kohda *et al.*, 2015; Satoh *et al.*, 2016). They are important during mate choice and play a crucial role in the species radiation of African cichlids, which is based on female selection for male nuptial coloration (Seehausen *et al.*, 2008). During agonistic and territorial interactions, aggressive behavior can be induced by coloration (Tinbergen, 1948), ornaments like black stripes (Bachmann *et al.*, 2016; Morris *et al.*, 1995) or eye-spots (Beeching, 1993), as well as by postures like erected fins and gill covers (Simpson, 1968). Visual information is also crucial for the formation of large schools (Partridge and Pitcher, 1980) and can influence the decision to join a shoal based on similarities between individuals (Rosenthal and Ryan, 2005). It has also been hypothesized that visual perception of stripes and banded patterns in mackerel (*Scomber scombrus*) mediates the optimal spatial relationship between individuals in large schools of fish (Denton and Rowe, 1998).

Visual signals may manipulate behavior by way of similarity with behaviorally relevant objects such as eggs in mouthbrooding African cichlids (Amcoff *et al.*, 2013) or eyes in the cichlid oscar (*Astronotus ocellatus*) (Beeching, 1993). They can emphasize or exaggerate a signaler's body size, e.g., through extended fin appendages in male swordtails (*Xiphophorus hellerii*) (Rosenthal and Evans, 1998). Red coloration often results from carotenoid pigments that animals can only acquire through ingestion. Thus the intensity of red signals provides reliable information concerning the nutritional state and thereby the quality of a sender (Sefc *et al.*, 2014). Negative correlations between male red coloration and parasite infestation have been observed in the Lake Victoria cichlid (*Pundamilia nyrerei*) (Maan *et al.*, 2006) and the three-spined stickleback (*Gasterosteus aculeatus*) (Bakker and Milinski, 1993).

A particular case of visual communication exists among mesopelagic lanternfishes (Myctophidae). These animals generate bioluminescent light through ventral photophores for counter-illumination that provides camouflage against downwelling light in their otherwise featureless habitat. However, they also produce bioluminescent signals with lateral photophores that may serve in species recognition and could give an explanation for speciation via sexual selection in a deep-sea habitat devoid of obvious reproductive barriers (Davis *et al.*, 2014).

Mormyrids are rather inconspicuous regarding coloration, and their visual system is assumed to be reduced in favor of the electrosensory system (Wullimann and Northcutt, 1990). Consistent with their nocturnal lifestyle, mormyrids are adapted to dim light conditions and posses a grouped retina that provides a relatively poor spatial resolution (Landsberger *et al.*, 2008). However, the ability of *Gnathonemus petersii* to discriminate visual patterns under low light conditions has been demonstrated (Schuster and Amtsfeld, 2002) and vision was shown to play a role during various behaviors including social interactions among conspecifics (Moller *et al.*, 1982). It has been suggested that the characteristic vertical white stripes of *G. petersii* serve a function in group cohesion (Moller, 2002), but other species, such as *M. rume,* lack such characteristics altogether. Whether the few visually detectable sexual dimorphic traits, such as anal fin expansion in mature males of *M. rume proboscirostris,* have any bearing on communication, or merely functional implications during spawning, has never been addressed experimentally (Brown *et al.*, 1996; Moller *et al.*, 2004). Consequently, electrosensory discrimination of EOD-waveforms has taken over as the sensory basis for species recognition and mate choice in mormyrids (Carlson and Gallant, 2013). However, the characteristic subdivision of the nucleus exterolateralis that allows such waveform discriminations (see section 1.3) is absent in most members of the mormyrid subfamily Petrocephalinae (Carlson *et al.*, 2011). In these species, eye size and the optic tectum are enlarged, and it has been suggested that vision may play an important role in their social behavior due to their inability to discriminate EODs based on signal waveform (Stevens *et al.*, 2013).

3.3.2 Acoustic communication

Unlike terrestrial vertebrates, who rely on periodic pressure differences in the air for auditory perception, fish hearing relies on the linear acceleration that is caused by relative movements between a relatively inert otolith organ with high density, and a sensory membrane containing directionally sensitive hair cells. This system makes fishes highly sensitive to low-frequency acoustic signals and infrasound (Sand and Karlsen, 2000). Fishes that are considered to be 'hearing specialists' have additionally developed accessory structures, such as Weberian ossicles, which enhance their hearing capabilities and extend the sensitivity of the auditory system to higher frequencies by connecting the inner ear to the gas-filled and therefore compressible swim bladder (Popper and Lu, 2000). The primary function of hearing in fishes may be to monitor the 'acoustic landscape' for objects like predators or prey, as well as for cues for orientation and navigation (Popper and Fay, 1993; Sand and Karlsen, 2000). Still, many species have developed mechanisms to produce sound for social communication, mainly in the context of either agonistic interactions, courtship, or distress (Ladich, 2004).

Prominent examples of vocalizing fishes are the croaking gouramis of the genus *Trichopsis*, who produce double pulses of 'croaks' during male agonistic encounters and courtship by using modified pectoral fins (Ladich *et al.*, 1992). Male toadfishes, like the midshipman (*Porichthys notatus*), use sonic swim bladder muscles to produce continuous 'hum' sounds at night to advertise for females (Feng and Bass, 2016). In Nile tilapia (*Oreochromis niloticus*), both males and females vocalize while guarding their territory (Longrie *et al.*, 2013). In some cichlids, vocalizations, in addition to visual signaling, may provide species information during multimodal courtship displays (Escobar-Camacho and Carleton, 2015). In general, associations between signal parameters, such as dominant frequency and body mass or size of a sender, may provide fertile females or rivaling males with reliable information about the quality of a potential mate or opponent (Bertucci *et al.*, 2012; Ladich *et al.*, 1992). A particular case of sound production occurs in herring (*Clupea pallasii* and *C. harengus*), who generate fast, repetitive tick sounds through gas-bubble expulsion from the anal duct. This has been hypothesized to play a role as contact signals for the mediation of social cohesion during shoaling at night (Wilson *et al.*, 2004).

Mormyrids are hearing specialists that are unique in having swim bladder-derived, gas-filled sacs associated with their inner ear (Stipetić, 1939). This adaptation makes *Gnathonemus petersii* sensitive to frequencies of up to at least 2.5 kHz (McCormick and Popper, 1984; Werns and Howland, 1976). *Gnathonemus* also vocalizes by generating acoustic 'click' sounds during agonistic interactions with conspecifics (Rigley and Marshall, 1973). Characteristic tonal 'hoot' vocalizations, emitted during agonistic territorial interactions, were reported for mormyrids of the genera *Marcusenius* (Lamml and Kramer, 2007) and *Petrocephalus* (Crawford, 1997; Lamml and Kramer, 2008).

An especially prominent role play the vocalizations of 'strongly acoustic' mormyrid species of the genus *Pollimyrus* during social communication (Crawford, 1997). While 'click' sounds ('pops') and 'hoots' were observed during agonistic encounters of both sexes in *Pollimyrus*, their vocal repertoire also includes sounds that are generated by males during courtship behavior (Crawford *et al.*, 1986). Males produce alternations of characteristic 'grunts' and 'moans' while courting females that enter their territory, as well as 'growls' in response leaving females and while patrolling the territory (Crawford, 1997; Crawford *et al.*, 1986). Male courtship signals are generated by a drumming muscle that connects to the swim bladder (Crawford and Huang, 1999) and are elicited by female inter-discharge interval patterns (Crawford, 1991). Qualitative variations in vocalization between individuals may provide information that influences a female's decision to spawn (Crawford, 1997), and differences between the vocalizations of closely related species suggest that vocalizations may enable species recognition (Lamml and Kramer, 2006), a task that is usually attributed to EOD-waveform discrimination in mormyrids. The communication of identity information may be assumed by vocalizations in *Pollimyrus* because the duration of their EOD might be too short to mediate this task (Crawford and Huang, 1999).

3.3.3 Chemical communication

Chemical compounds are behaviorally highly relevant for fishes during food detection and predator avoidance. But chemicals are also involved in social behaviors such as individual, sex and species recognition, territorial interactions, courtship displays and mating, parent-offspring interactions, schooling, and migration (Liley, 1982). The behavioral significance of chemical cues was highlighted by von Frisch's (1941) discovery that chemicals released from the skin of injured minnows (*Phoxinus phoxinus*) serve as an alarm substance for conspecifics and evoke predator avoidance responses. Behaviors mediated by the olfactory perception of this 'Schreckstoff' include erratic swimming movements, increased shoaling tendencies, bottom-dwelling, hiding, and fleeing (von Frisch, 1941). As with many chemosensory guided behaviors, the question whether this reaction represents a true signaling system, evolved to the net-benefit of senders and receivers, or constitutes merely an adaptation to environmental cues by receivers, remains debated (Bradbury and Vehrencamp, 2011; Liley, 1982).

Olfaction relies on specific binding of odor molecules to the odorant receptors of primary sensory neurons in the olfactory epithelium (Buck and Axel, 1991). The olfactory system of fish is morphologically and functionally subdivided to detect information relevant to all vital aspects of fish behavior. In the crucian carp (*Carassius carassius*), microvillous cells detect food odorants, ciliated cells mediate the alarm response, and sensory crypt cells respond to sex pheromones, while the primary sensory afferents of all of these morphologically different cell types project to defined regions of the olfactory bulb (Hamdani and Døving, 2007). This functional organization is maintained by fibers of the secondary mitral cells, which form distinct bundles of the olfactory tract that connects the olfactory bulb to the telencephalon. Stimulation of individual bundles of the olfactory tract in Atlantic cod (*Gadus morhua*) induced behavioral responses typical of feeding, alarm reaction, or courtship, thus demonstrating the close connection between olfactory sensory perception and adaptive behavioral reactions in response to odorants (Døving and Selset, 1980).

Pheromones play an essential role in social behavior and reproduction of fishes. Numerous studies with various species have demonstrated the capability of male fish to identify receptive females based on olfactory perception (Liley, 1982). The abundance of sensory crypt cells, which detect sex pheromones in crucian carp, varies seasonally and peaks during the spawning season (Hamdani *et al.*, 2008). Female goldfish (*Carassius auratus*) release steroid-derived maturation hormones as pre-ovulatory sex pheromones. These hormones induce sperm maturation in males and synchronize spawning, which is ultimately stimulated by the receptive female's release of prostaglandin derived pheromones (Sorensen, 1992). Male goldfish respond with different behavior patterns to the distinct components of female sex pheromones (Poling *et al.*, 2001). In the olfactory bulb of males, but not females, of the closely related crucian carp, single neurons were shown to respond selectively to individual pheromones. This demonstrates how precisely the reproductive behavior of these fish is tuned to olfactory signals (Lastein *et al.*, 2006).

Pheromones not only provide cues regarding reproductive state, but they can also signal an individual's social status and thereby provide information about dominance relationships or the quality of a potential mate. For instance, male Mozambique tilapia (*Oreochromis mossambicus*) excrete urine pulses with odorant signals during aggressive interactions. This allows them to signal dominance to other males in social hierarchies (Barata *et al.*, 2007) and to advertise to females, who prefer dominant males for spawning (Barata *et al.*, 2008). Odorants allow fish to identify members of their species (Plenderleith *et al.*, 2005), discriminate kin from unrelated conspecifics (Thünken *et al.*, 2014), and even recognize individuals (Keller-Costa *et al.*, 2015). Such abilities are of particular importance for parent-offspring relationships, for instance in cichlids that engage in parental care (Keller-Costa *et al.*, 2015).

Outside the reproductive context, the behavioral preference that many fishes exhibit towards water that contained conspecifics suggests that chemical cues play a role in social cohesion, schooling, and migration (Liley, 1982). Anadromous salmonids imprint on the odor composition of their natal river habitat (Scholz *et al.*, 1976) and use olfactory guidance for homing in river systems (Wisby and Hasler, 1954) and potentially even at sea (Døving and Stabell, 2003). According to the pheromone hypothesis of migration (Nordeng, 1977), homing salmons could use odorant cues emitted by descending smolts to guide them back to their spawning grounds. Possible odorant cues to mediate such behavior are bile acids, which are released by fish into the water. These chemicals are structurally highly diverse and evoke responses of the salmonid olfactory system at extremely low concentrations (Døving *et al.*, 1980). This mechanism has been shown to underlie the anadromous migrations of sea lamprey (*Petromyzon marinus*) (Sorensen *et al.*, 2005).

In addition to the olfactory sense, the gustatory system, which is responsible for taste perception, also processes chemical information. In contrast to olfaction, the sense of taste is a close-range sensory system that mainly evaluates food items, which have been detected or tracked using far-range sensory systems like vision or olfaction (Valentinčič, 2004). It is mediated by taste buds, which are sensory organs containing several secondary chemosensitive receptor cells (Hansen and Reutter, 2004). Communication is not a primary function of the gustatory sense. However, in many fishes, taste buds are not exclusively developed within the oral cavity. They are also distributed externally on the body surface (Gomahr *et al.*, 1992), as well as on appendages like the barbels of catfish (Finger and Böttger, 1990) or the elongated pelvic fins of anabantid *Trichogaster* (Scharrer *et al.*, 1947; Weber, 1963). Such appendages may be used for food detection in combination with tactile cues (Bisazza *et al.*, 2001; Kasumyan, 2011; Weber, 1963), but especially for the elongated pelvic fins of the Anabantidae, social functions through taste perception have been proposed (Picciolo, 1964; Vierke, 1978). However, a negative effect of the removal of these fins on mating success could not be confirmed (Pollak *et al.*, 1978). Gustatory cues are conceivably also important for parent-offspring relationships in mouthbrooding species (Liley, 1982).

The chemoreceptive sensory systems of mormyrids have hardly been investigated (Moller, 2002; Schuster, 2006). Chemosensory information has been shown to play a role in food detection in *Gnathonemus petersii* (von der Emde and Bleckmann, 1998), and odorants derived from food items like tubifex worms and chironomid larvae caused an increase in EOD-frequency (Jäger, 1974). However, the specialized cells that contain the alarm substance in many fishes are absent from the skin of mormyrids (Pfeiffer, 1977), and social responses to odorants have not been investigated.

3.3.4 Communication through tactile stimuli and the lateral line

At close distances, fishes can obtain mechanosensory information about their environment through touch perception and the mechanosensory lateral line system. The lateral line consists of superficial neuromasts for the detection of weak water movements, and of canal neuromasts that react to small pressure differences between adjacent pores of the lateral line canals (Bleckmann and Zelick, 2009). The lateral line enables behaviors such as rheotactic orientation to water currents (Montgomery *et al.*, 1997), prey detection by following hydrodynamic trails (Pohlmann *et al.*, 2001), and, in the case of blind Mexican cavefish (*Astayanax fasciatus*), the detection of objects in an active sensing process (von Campenhausen *et al.*, 1981). The lateral line also mediates communicative functions, for instance for the synchronization of spawning, during aggressive behavior, and for the coordination of schooling fish (Montgomery *et al.*, 2014). In cichlids, male quivering displays during courtship may stimulate the superficial neuromasts of the female's lateral line (Escobar-Camacho and Carleton, 2015) and vibrational signals exchanged between the sexes were shown to induce spawning in red salmon (*Oncorhynchus nerka*) (Satou *et al.*, 1994). During agonistic lateral display behavior of the cichlid *Nannacara anomala*, contesting fish generate water movements directed at each other. The mechanosensory lateral line senses the strength of the resulting water displacements and thereby samples information to assess the opponent's fighting ability. This information enables contestants to decide whether or not to escalate the fight to a more aggressive level (Butler and Maruska, 2016; Enquist *et al.*, 1990).

Tactile perception in fishes is mediated by free nerve endings and Merkel cells, but tactile communication appears to be relatively uncommon (Kasumyan, 2011). A haptic function similar to that of the fingers of primates or mammalian whiskers has been proposed for the Schnauzenorgan of *G. petersii* based on the trigeminal innervation of this characteristic chin appendage (Amey-Özel *et al.*, 2015).

Even though all types of electroreceptors have their origin in the lateral line system, both the electrosensory and the mechanosensory lateral line are present in weakly electric fishes (Szabo, 1965). However, in the mormyrid *G. petersii*, the peripheral sensory structures and receptors of the mechanosensory lateral line are reduced in comparison to other teleosts (Schumacher, 2017). Nevertheless, parallel and antiparallel displays are frequently observed in contesting mormyrids (Bell *et al.*, 1974; Crockett, 1986; Terleph, 2004) and are likely to generate mechanosensory information that helps to assess opponents. In juvenile *Mormyrus rume proboscirostris*, mechanosensory information obtained through touch or the lateral line appears to be important for group cohesion (Khait *et al.*, 2009).

3.4 Electrocommunication

Electrocommunication occurs in both mormyriform and gymnotiform weakly electric fish, as well as in electrogenic skates (Rajidae) (Bratton and Ayers, 1987) and catfish (*Synodontis*) (Baron *et al.*, 1994).

3.4.1 Communication via pulse-type electric signals in Gymnotiformes

In Gymnotiformes, both pulse-type and wave-type species have signaling strategies to communicate electrically in addition to active electrolocation. Black-Cleworth (1970) analyzed social interactions in pulse-type *Gymnotus carapo* and established that unmodified discharge production serves as an identification signal that indicates species, location, and size of the sender. She also described discharge modifications such as SIDs (sharp increases decreases in EOD rate) that she interpreted as aggressive threat signals. Discharge cessations were associated with submissive behavior and were interpreted as appeasement signals of subdominant individuals (Black-Cleworth, 1970). The capability of *G. carapo* to discriminate individuals based on the waveform of their EOD could be demonstrated in electric playback experiments (McGregor and Westby, 1992) and the function of SIDs as aggressive threat signals was confirmed for *Gymnotus omarorum* (Batista *et al.*, 2012). Additionally, *Gymnotus* produces 'chirps,' which are noisy, high-frequency electric field modulations with an amplitude much smaller than that of the regular EOD. These chirps indicated subsequent submission in competitive contests in *G. carapo* (Guariento *et al.*, 2016). In *G. omarorum*, chirps were emitted by the subordinate individual after a dyadic contest was resolved. They were proposed to be a more unambiguous signal of submission than complete discharge cessations because the latter might also be interpreted as electric hiding attempts (Batista *et al.*, 2012). Once the dominance relationship between two individuals was established, the subordinate individual adopted a higher average discharge rate, while that of the dominant individual remained unchanged (Guariento *et al.*, 2016). In addition, dominant individuals engage in a jamming avoidance strategy that benefits their electrolocation ability while tending to jam that of the subordinate individual (Westby, 1979).

3.4.2 Communication via wave-type electric signals in Gymnotiformes

In contrast to pulse-type fish, wave-type species cannot dynamically modify their discharge behavior by context-dependent variation of IDI-duration. Consequently, wave-type weakly electric fish can be characterized by their discharge frequency, which is remarkably constant in individuals and varies between individuals within the frequency range characteristic of a given species. Additionally, there is a large variety of species-specific EOD-waveforms (Crampton and Albert, 2006), which fish could use for species discrimination during electrocommunication (Fugère and Krahe, 2010). *Eigenmannia lineata* can discriminate male and female electric signals based on the waveform of the EOD (Kramer and Otto, 1988). However, Fugère and Krahe (2010) found the production of communication signals in brown ghost knifefish (*Apteronotus leptorhynchus*) to be affected only by signals within the species-specific frequency range, but independent of the EOD-waveform used to generate that frequency.

The performance of active electrolocation in wave-type weakly electric fish is impaired by amplitude modulations that are caused by the interference of EODs with similar discharge frequency. Wave-type fish like *Eigenmannia*, therefore, show a characteristic jamming avoidance response (JAR) that maximizes the frequency difference between their own and a nearby conspecific's discharge frequency (Heiligenberg, 1980; Watanabe and Takeda, 1963). Characteristic asymmetries in JAR behavior of male and female, as well as adult and juvenile *Eigenmannia*, led Kramer (1987) to propose that the JAR may have implications for social communication. Jamming may, however, also occur intentionally, like in *Apteronotus leptorhynchus*, where individuals with lower discharge rates may rise their EOD frequency to actively jam an opponent during aggressive encounters (Tallarovic and Zakon, 2005).

In several species, dominance relationships correlate with discharge frequency, and males and females may utilize different frequency ranges. In *Apteronotus leptorhynchus*, males discharge at higher rates than females, and social dominance among males is positively correlated with discharge frequency (Zakon *et al.*, 2002). On the contrary in *Eigenmannia virescence*, dominant males use the lowest frequencies, while dominant females have the highest discharge rates (Hagedorn and Heiligenberg, 1985). The most prominent social signals generated by wave-type fish are 'chirps,' which are transient increases in EOD-frequency that can be classified according to their duration and to the extent of their frequency excursion. Short duration chirps are aggressive signals that only last about 20 ms, whereas long duration chirps can last hundreds of milliseconds and serve as courtship signals (Zakon *et al.*, 2002). Other signaling behaviors include frequency modulations and complete interruptions of the EOD (Hagedorn and Heiligenberg, 1985; Zakon *et al.*, 2002). Based on the observation that the winners of dyadic contests between male *Apteronotus leptorhynchus* emitted more 'chirps,' while losers produced more gradual frequency rises, Triefenbach and Zakon (2008) suggested that these displays are conventional signals in this species, and that they are used during sequential assessment by opponents competing over a resource. Outside an aggressive context, 'chirping' is also engaged in by male and female wave-type weakly electric fish during courtship (Henninger *et al.*, 2017) and is of particular importance to initiate spawning (Hagedorn and Heiligenberg, 1985).

3.5 Electrocommunication in Mormyriformes

Among the Mormyriformes, the sole representative of the Gymnarchidae, *Gymnarchus niloticus,* is the only African wave-type species of weakly electric fish. *Gymnarchus* uses short interruptions of its discharge activity to signal aggression, while long cessations indicate appeasement by submissive individuals (Hopkins, 1974). Characteristic 'singing' displays, which manifest in regular frequency modulations of variable duration, were described by Bullock *et al.* (1975), who were, however, reluctant to conclude on social signaling behavior, because 'singing' occurred in solitary individuals.

3.5.1 Communication via EOD-waveform information in mormyrids

All mormyrids are pulse-type fish and can potentially use waveform information of the EOD, as well as information encoded in the temporal sequence of inter-discharge intervals (IDI) for electrocommunication. The members of most mormyrid lineages can discriminate EOD-waveforms with a submillisecond temporal resolution, and the peripheral and central mechanisms that underlie this capability are relatively well understood (Baker *et al.*, 2013a). On shorter timescales, EOD-waveforms remain constant and provide identity information about a sender on several levels from species identification (Feulner *et al.*, 2009a; Hopkins and Bass, 1981) to individual recognition (Graff and Kramer, 1992; Hanika and Kramer, 2005; Paintner and Kramer, 2003). Mormyrid EODs vary in duration from 85 µs in *Pollimyrus* (Crawford, 1992) to 8 ms in *Paramormyrops gabonensis* and are characteristic for a given species with respect to shape, the number of positive and negative phases, as well as their polarity (Hopkins, 1980). Identity information conveyed via the waveform of the EOD is thus considered to be one of the main factors responsible for the species radiation based on sexual selection among mormyrids (Arnegard *et al.*, 2010a; Feulner *et al.*, 2009b). In many species, EOD-waveforms are also sexually dimorphic, at least during the breeding season, when especially male signals are affected by hormonally induced changes of the electric organ (Bass, 1986; Bass and Hopkins, 1983). In species with sexually dimorphic signals, male EODs tend to be of longer duration than those of females, thus providing information about a sender's sex and reproductive state (Hopkins, 1999). EOD-duration is positively correlated and varies dynamically with social dominance and may thus communicate the relative status of an individual within a social context (Carlson *et al.*, 2000; Terleph and Moller, 2003). Again, such changes appear to be under hormonal control (Carlson *et al.*, 2000). The lower frequency components of longer EODs go hand in hand with higher energetic costs (Hopkins, 1999) and a higher risk of predation by electroreceptive predators such as catfish (Hanika and Kramer, 2000). The costs that increased predation imposes on males with longer EODs make this trait a reliable indicator of a signaler's quality that can be used by females for mate selection (Machnik and Kramer, 2008a).

Apart from waveform information, the amplitude of the EOD may provide reliable information about a sender. Because EOD-amplitude correlates positively with a fish's size, anti-parallel displays, during which contestants mutually position their electric organ close to the opponents head, may be used to determine relative fighting ability based on the strength of the generated signal (Bell *et al.*, 1974; Crockett, 1986; Terleph, 2004; Terleph and Moller, 2003).

3.5.2 Communication via IDI-variation in mormyrids

The second way for mormyrids to engage in electrocommunication is to encode information into the temporal sequence of their discharges by varying IDI-duration. In contrast to the constant nature of the EOD itself, the resulting discharge patterns are highly variable and depend heavily on the current behavior of the fish.

Vast amounts of electrical signaling behaviors have been documented and can be associated with context-specific behavior in mormyrids. Such signaling patterns can be classified according to the behavioral contexts in which they are observed, as well as by the strategies the fish use to encode information into communication sequences. These strategies can be based on general discharge frequency and overall distribution parameters, on distinct and stereotypical discharge patterns, as well as on interactive signaling that depends on the discharge activity of other individuals. The use of such signaling strategies by *Mormyrus rume* will be the subject of chapters 4 and 5.

Resting individuals consistently discharge at lower rates compared to foraging or swimming ones (Bauer, 1974; Gebhardt *et al.*, 2012a; Gebhardt *et al.*, 2012b; Sänger, 1967), potentially allowing eavesdropping individuals to monitor the ongoing behavior of nearby conspecifics. Additionally, regularizations of an individual's IDI-pattern occur frequently in response to electric signals (Moller, 1970). The transition from variable to regular intervals may be a means to uncloak electrical communication signals from the random background noise of lightning in the tropics (Hopkins, 1973). Regularized discharge patterns are reliably observed during aggressive interactions in *Gnathonemus petersii* (Bell *et al.*, 1974; Terleph, 2004) and are part of the courtship displays of female *Pollimyrus* (Baier and Kramer, 2007) and *Marcusenius macrolepidotus* (Werneyer and Kramer, 2005). The significance of discharge regularizations during electrocommunication and active electrolocation in *Mormyrus rume* will be investigated and discussed in chapter 4, and a potential function for mutual assessment during agonistic encounters will be considered in chapter 6.

Distinct discharge patterns occur in a variety of behavioral contexts. Overt aggression during agonistic interactions is often expressed through head butts and chasing. In several species, these agonistic behaviors are accompanied by discharge accelerations, which are often preceded by short discharge cessations (Bell *et al.*, 1974; Carlson and Hopkins, 2004b; Gebhardt *et al.*, 2012a; Kramer and Bauer, 1976; Terleph, 2004). These discharge patterns can, therefore, be classified as aggressive threat signals and seem to be mutually understood by different species of mormyrids (Kramer, 1976b).

Outside an overtly aggressive context, *Brienomyrus brachyistius* generates 'scallops,' which are brief accelerations of only 8–12 EODs. They likely function as territorial dominance signals and potentially even provide an individual signature of the sender (Baker *et al.*, 2016; Carlson and Hopkins, 2004b). Similarly, male *Pollimyrus* emit highly regular double pulses, i.e., alternating long and short IDIs, as threat signals during visitations of their nests (Baier and Kramer, 2007). *M. rume* frequently produces such double pulses in response to electrical signals (Gebhardt, 2012), and it will be argued in chapter 4 that double pulses may serve as a conventional signal that expresses aggressive motivation in this species.

Characteristic electrical display patterns are especially prominent in the context of reproduction. Male *Brienomyrus brachyistius* produce 'rasps' during the breeding season to attract females into their territory (Carlson and Hopkins, 2004b; Hopkins and Bass, 1981). After prolonged courtship interactions, partners engage in 'rasp matching' and produce 'creaks' exclusively during spawning (Wong and Hopkins, 2007). Electric signaling displays during courtship and spawning were also described for *Marcusenius macrolepidotus* (Werneyer and Kramer, 2005) and several members of the genus *Pollimyrus*, where they occurred in addition to acoustic courtship displays (Baier and Kramer, 2007; Bratton and Kramer, 1989).

Electric signaling has also been associated with flight in attacked individuals (Kramer, 1976c), and especially animals that turn out to be submissive often stop discharging for extended periods of time (Bell *et al.*, 1974; Wong and Hopkins, 2007). The resulting 'social silence' could constitute an attempt to hide electrically, but may also allow silent individuals to 'listen in' on a conspecific's signaling (Moller *et al.*, 1989).

Interactive discharge sequences can lead to discharge synchronizations between individuals that are mediated by the mormyrid echo response, and to episodes of fixed-order signaling, during which individuals of a group discharge in a specific sequence after each other for some time (Gebhardt *et al.*, 2012a; Gebhardt *et al.*, 2012b). The echo response is characterized by a very brief, fixed latency of only a few milliseconds between the signals of two fish (Kramer, 1974; Russell *et al.*, 1974). Interactive signaling via artificially generated echo responses will be the subject of chapter 5. Although echoing was initially proposed to be a jamming avoidance response (Heiligenberg, 1976), communicative functions of this peculiar electromotor behavior will be considered, and a potential role of the echo response as a strategy that allows addressing specific individuals in a group will be proposed (see also chapter 11).

Compared to the information conveyed by the waveform of the EOD, the adaptive nature of specific IDI-patterns for electrocommunication is less well understood. This lack of understanding is in part due to the dual function of electrical signaling during active electrolocation and electrocommunication, which makes it difficult, if not impossible, to unequivocally assign certain discharge characteristics exclusively to either of the two functions. This problem will be addressed in chapter 4. For investigations aiming beyond a mere description of signaling behavior in animals, two approaches have proven to be valuable for experimental testing of hypotheses on animal communication. Communication strategies for conflict resolution and hierarchy formation can be investigated by staging dyadic contests over an indivisible resource and subsequently interpreting signaling displays with respect to the outcome of the contest (Hardy and Briffa, 2013). However, the game-theoretical considerations that proved to be valuable tools to explain the evolution and adaptive nature of signaling systems in other animals have hardly been applied to the study of IDI-based communication systems in mormyrids (see Terleph (2004) for an exception).

The second strategy is to use playback of communication signals and observe the behavioral responses of focal individuals to artificial signals (McGregor, 2000). Playback has been particularly useful to uncover communication properties of acoustic signal displays such as vocal duets in bird song (Douglas and Mennill, 2010) and the advertisement calls of amphibians (Gerhardt, 1994), but can in principle be generated for any sensory modality. Electrical playbacks have been used in many studies to investigate behavioral responses to the properties of EOD-waveform and IDI-pattern in both mormyrid and gymnotiform weakly electric fish. Playback studies that specifically address potential information content in mormyrid IDI-sequences, and systematically compare behavioral responses to artificial sequences, are relatively rare. Electrical playbacks were used to investigate the capability of mormyrids to recognize species-specific discharge patterns (Hopkins, 1981b; Kramer, 1990; Kramer and Kuhn, 1994; Teyssedre and Serrier, 1986), a sender's sex (Crawford, 1991), and information concerning its behavioral state (Gebhardt, 2012;

Kramer, 1979). Other playback studies addressed female preference for behaviorally relevant discharge patterns (Machnik and Kramer, 2008b; 2011) and the precision with which fish can resolve the temporal structure of specific communication patterns (Baker *et al.*, 2016). Chapter 4 will deal with static playbacks reproducing discharge sequences observed in freely behaving *Mormyrus rume*. The effect of interactivity in a playback sequence, achieved by mimicking the mormyrid echo response, will be the subject of chapter 5.

One advantage of playback experiments is that they allow the systematic manipulation of communication signals, which can then be tested under standardized experimental conditions. A particular difficulty when investigating social communication in weakly electric fish is to distinguish EODs of more than one freely moving individual (Bell *et al.*, 1974; Gebhardt *et al.*, 2012a; Guariento *et al.*, 2016; Jun *et al.*, 2013; Wong and Hopkins, 2007). The use of electrical playback signals can circumvent this problem because the playback sequence is known to the experimenter. However, communication displays that are based on interactivity, or involve senses other than the electrosensory modality, may not be faithfully displayed in response to a stationary electric dipole emitting a static playback sequence. In this thesis, a solution to this problem is approached by using mobile dummy fish as a source of electrical playback sequences of communication signals.

4. Project 1: Social Interactions between Live and Artificial Weakly Electric Fish: Electrocommunication and Locomotor Behavior of *Mormyrus rume* towards a Mobile Dummy Fish

This chapter is based on my contribution to:

Worm, M., Kirschbaum, F. and von der Emde, G. (2017). Social interactions between live and artificial weakly electric fish: Electrocommunication and locomotor behavior of *Mormyrus rume proboscirostris* towards a mobile dummy fish. *PLOS ONE* 12(9): e0184622.

It additionally contains data from

Toma, R. (2014). Auswirkungen verschiedener Dummy-generierter Playbacks auf die motorische und elektrische Antwort von *Mormyrus rume* (Mormyridae, Teleostei). *Bachelor's thesis*. Rheinische Friedrich-Wilhelms-Universität, Bonn,

and

Kersten, A. (2017). Das motorische und elektrische Kommunikationsverhalten von *Mormyrus rume*: Antagonistische Begegnungen in Zweiergruppen. *Unpublished project thesis*. Rheinische Friedrich-Wilhelms-Universität, Bonn.

Both thesis were prepared in the context of the electrocommunication project in the Neuroethology/Sensory Ecology group at the Institute of Zoology under my co-supervision.

4.1 Introduction

Communication is an integral component in coordinating interactions between individuals, spanning a wide range of social contexts from agonistic behavior to the formation of groups and collective decision making. Communication systems have developed within all of the main sensory modalities used by animals including active sensory systems, such as sonar in bats (Altringham and Fenton, 2003) and cetaceans (Tyack, 2000), and the perception of electrostatic fields in weakly electric fishes (Hopkins, 1974).

Mormyrid weakly electric fish have evolved a unique electrosensory capability: by emitting pulse-type electric organ discharges (EOD) they use the same signals both for actively probing their environment, i.e. active electrolocation (von der Emde, 1999; von der Emde and Fetz, 2007), and for communication with conspecifics (Hopkins, 1988). Active electrolocation is based on the perception of these self-generated signals through mormyromast electroreceptor organs (Bell *et al.*, 1989; von der Emde *et al.*, 2008), which are specialized for detecting object evoked amplitude and waveform modulations of the local EODs and are distributed over large areas of the animals' skin (Harder, 1968; Hollmann *et al.*, 2008). Electrocommunication is mediated by a different type of electroreceptor organ, the so-called knollenorgans (Derbin and Szabo, 1968), which are time-coders that respond very sensitively to the EODs of other electric fish. The input of knollenorgans to the brain is inhibited centrally by a corollary discharge signal during the production of the self-generated EOD (Bell and Grant, 1989), demonstrating that the knollenorgan pathway mediates electrocommunication between individuals (Baker *et al.*, 2013a). The EOD itself is an all or nothing signal, whose waveform reveals information about the signaler's identity, such as species and gender (Hopkins, 1981b), its reproductive state (Bass and Hopkins, 1983) and relative rank in a social hierarchy (Carlson *et al.*, 2000). However, the EOD-waveform remains stable on a short to medium duration time scale. In contrast, the inter-discharge intervals (IDI) are highly variable in duration, and their temporal sequence can be related to an animal's current behavioral state (Carlson, 2002a).

Social interactions among mormyrids are accompanied by stereotypical motor patterns (Crockett, 1986), many of which are reminiscent or even identical to those observed during active electrolocation (Toerring and Belbenoit, 1979). Activity-dependent EOD production may vary in overall frequency, with active animals usually discharging at higher rates compared to resting ones (Bauer, 1974; Gebhardt *et al.*, 2012a; Moller, 1970). In addition, regularizations of interval distributions (Moller, 1970) occur in the context of active electrolocation (von der Emde, 1992) and during social encounters (Moller and Bauer, 1973). Apart from general variations in overall discharge rate, distinctive temporal IDI-patterns, occurring in specific behavioral situations, have been described in several mormyrid species. These include accelerations during aggressive encounters (Bell *et al.*, 1974; Carlson and Hopkins, 2004b; Kramer, 1976a), double-pulse patterns during territorial behavior (Baier and Kramer, 2007), as well as 'rasps,' which serve as courtship signals (Hopkins and Bass, 1981). Furthermore, electrocommunication can also result from interactive discharge patterns. In certain situations, mormyrids tend to respond to the signals of a conspecific by discharging at a preferred latency of a few milliseconds (Kramer, 1974; Russell *et al.*, 1974; Schuster, 2001). This so-called 'echo response' has been assigned a function selectively in social contexts (Arnegard and Carlson, 2005; Lücker and Kramer, 1981) and active sensing (Heiligenberg, 1976), and although its occurrence is very stereotypical, its functional implications are still unresolved. Prolonged periods of time-locked discharge activity were shown to lead to sequences of mutual EOD synchronizations that can switch between individuals within a group (Gebhardt *et al.*, 2012b).

Since the emergence of classical ethology as a research discipline, so-called 'dummies' have been widely used in behavioral biology to identify the essential components of various releasing mechanisms that can trigger stereotypical behavior patterns (Tinbergen, 1948). In contrast to using living animals as a stimulus, such an approach guarantees repeatability and allows for a standardized experimental protocol. Analogous to the study of acoustic communication, playbacks of electric signals have, e.g., been used to relate EOD properties to male fighting potential (Hanika and Kramer, 2005), mate recognition (Feulner *et al.*, 2009a), and to decode the communicative value associated with stereotypical IDI-sequences (Kramer, 1979; Kramer and Kuhn, 1994; Moller, 1970; Teyssedre and Serrier, 1986).

Reproducing central features of living conspecifics by constructing biomimetic fish dummies has made it possible to investigate personality traits and individual preferences in a variety of fish species (Abaid *et al.*, 2012; Abaid *et al.*, 2013; Donati *et al.*, 2016; Kopman *et al.*, 2013; Marras and Porfiri, 2012; Phamduy *et al.*, 2014; Ruberto *et al.*, 2017). On a group level, mobile fish dummies have been used to study cohesion and collective decision making in small shoals of three-spined sticklebacks (Faria *et al.*, 2010; Ward *et al.*, 2012) and zebrafish (Butail *et al.*, 2013), as well as dynamic interactions in shoals of guppies (Landgraf *et al.*, 2014). Weakly electric fish may be particularly suited for studying social behavior in such an approach since a central feature of their communication—the emission of electrical signals—is easily manipulated by electrical playback experiments (Donati *et al.*, 2016; Worm *et al.*, 2014).

By presenting a mobile dummy fish, which is capable of producing EOD playbacks with naturally occurring or artificially generated IDI-sequences of different temporal patterns and overall frequencies to single individuals of the weakly electric fish *Mormyrus rume*, this study combines classical dummy experiments with the active production of communication signals in a standardized experimental setup. In a previous study, (see chapter 8) evidence was provided that the presence of electrical playback signals is the primary determinant for the initiation of following-behavior when compared with visual cues and naturalistic motion patterns (Donati *et al.*, 2016). Here, the question remained whether different IDI-sequences influence the likelihood of individual *M. rume* to follow after a mobile dummy fish and whether such sequences can account for different interaction patterns between the mobile dummy and a live fish, both electrically and with respect to locomotor behavior. It was hypothesized that if different IDI-sequences contain varying information, which is registered by the receiving animal, it would also be possible to observe different reactions of the fish to the mobile dummy during electrical signaling and by corresponding motor patterns. It was found that the animals' following-reactions increased when the dummy emitted electrical playbacks, but this was largely independent of the particular playback pattern which was presented. However, certain stereotypical signaling responses occurred in all cases and some electrical response patterns of the animals varied when the dummy produced different IDI-sequences. In particular, the number of double pulses and regularization displays depended on the playback pattern. Interestingly, no adoption of the dummy's overall IDI-distribution was observed in response to playback of naturally occurring discharge patterns, whereas animals reliably time-locked their signaling activity to constant discharge frequencies in the range of the average IDI-distributions that are usually observed during active behavior in *M. rume*. In addition, *M. rume* followed the dummy in a differing spatial relationship when playback was presented compared to an electrically silent control, and specific motor patterns were almost exclusively displayed in response to electric signal presentations. These findings support the idea that electrical IDI-patterns convey information and can play a role in spatial interactions and social cohesion of individuals within groups of weakly electric fish (Khait *et al.*, 2009; Moller, 1976).

4.2 Materials and methods

4.2.1 Animals

Eight individuals of *Mormyrus rume proboscirostris* (standard length: 9.8–17.0 cm) were used in the experiments. Animals were bred in captivity (F. Kirschbaum, Humboldt University of Berlin) and were approximately six years of age at the time of experimentation. Sex (5 males, 3 females) was determined by anal fin morphology (Kirschbaum and Schugardt, 1995), but none of the animals had previously been in a reproductive state. Therefore, a slight possibility remains that some of the females were male, but did not yet express male characteristics. All fish were kept in pairs in tanks under tropical conditions (water temperature ~25°C, light/dark periods 12/12 h), where they were physically isolated by a water permeable barrier, which prevented physical contact but allowed electrocommunication between the individuals. Food was provided in the form of defrosted *chironomid* larvae at least five times a week.

Additional eight *M. rume* (standard length: 14–18 cm) were obtained from Aquarium Glaser GmbH (Rodgau, Germany) and were kept under the same conditions. These animals were used in a subset of experiments performed by Toma (2014b) that involved constant frequencies of electrical playback sequences. Sex and age of these animals were not determined.

All experiments were approved by the Ministry for Environment, Agriculture, Conservation and Consumer Protection of the State North Rhine-Westphalia (MULNV) and were carried out in accordance with the guidelines of German law, with the animal welfare regulations of the University of Bonn, and with the 'Guidelines for the treatment of animals in behavioural research and teaching' (ASAB, 2006).

4.2.2 Experimental setup and electrical playback generation

Animals were individually transferred to an experimental tank with a ground area of 200 cm x 50 cm and a water level of 20 cm at least one day before testing. Water temperature and conductivity were kept constant at 25 ± 2°C and 100 ± 5 µS cm^{-1} during all experiments. The experimental tank (Figure 4.1) was subdivided into a 90 cm long testing area and a 110 cm living area, which were connected through a gate that was 10 cm in width. The living area was subdivided into a sheltered area with hiding places in the rear and an open area in front of the gate.

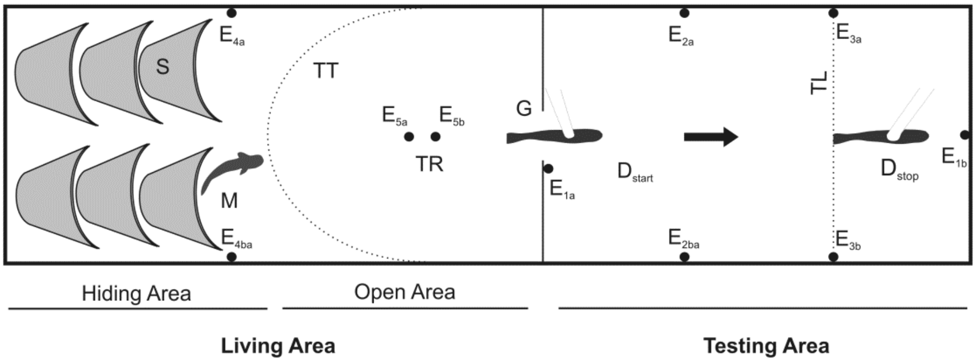

Figure 4.1: Top view of the experimental tank. S) shelter, M) focal fish, TR) trigger electrodes, TT) approximation of the spatial trigger threshold, G) gate, D_{start}) dummy fish at the start position, D_{stop}) dummy fish at the end position, TL) target line defining the following-criterion, E_{xa}–E_{xb}) Electrode pairs. Figure not drawn to scale. Base area: 200 cm x 50 cm.

Playbacks consisted of IDI-sequences that had previously been recorded from freely behaving *M. rume*. They were concatenated from a pre-recorded EOD waveform of a live specimen and presented at a sampling rate of 48 kHz. A total of seven playback sequences were used (compare Figure 4.4A–G and Table A.1 in Appendix A for more detailed descriptions). Playbacks were characterized as either being based on patterns (P) or average frequencies (F), with numbers indicating increasing IDI-duration. They were recorded from fish that were foraging (F_1), hiding (F_4) or displaying aggressive behavior in a group (P_A) (Gebhardt *et al.*, 2012a), following an electrically silent dummy fish (F_2), slowly swimming (F_3), in a subordinate position displaying periods of electrical silence (P_S), as well as emitting a double-pulse pattern containing alternations of long and short IDIs (P_D). A subset of experiments featuring electrical playback of constant frequencies was performed with the second group of animals (Toma, 2014b). Playback EODs were assembled to sequences resulting in discharge rates of 5, 10, 15, 25, 40, and 80 Hz. These frequencies corresponded approximately to the average discharge rates of playbacks F_4 (4.9 Hz), F_3 (11.1 Hz), F_2 (15.8 Hz), and F_1 (31.0 Hz). The playback frequency of 40 Hz represented the upper limit of discharge rates observed in *M. rume*, which is usually not sustained by the fish for extended periods of time. Frequencies as high as 80 Hz were never observed in *M. rume* and constituted an exaggerated stimulus.

A dummy fish was made from a 12 cm black fishing lure (Kopyto-Relax) that was endowed with a pair of carbon electrodes separated by a distance of 9 cm along its longitudinal axis. The dummy was attached to a white plastic rod that was connected to a slide, which could be moved along a track above the testing area of the experimental tank. To establish standardized experimental conditions with a similar relationship of the fish's initial behavior and the activity of the mobile dummy, the onset of every experimental trial was triggered by an EOD of the tested fish. This was accomplished by burying a pair of trigger electrodes within the open area of the living compartment (TR in Figure 4.1). Differential amplification (Brownlee Precision Model 440, Palo Alto, CA) of the signal measured between these electrodes defined an area between the hiding area and the gate (TT in Figure 4.1), where an EOD exceeding a predefined threshold value initiated the start of an experimental trial (see below) via a TTL pulse, which was generated by a digital oscilloscope (Yokogawa DL1620, Yokogawa Electric Corp., Tokyo, Japan). The threshold was adjusted for each fish by adjusting the amplification according to the magnitude of the fish's EOD. This configuration started an experimental trial when the fish produced an EOD after leaving the hiding area. The TTL pulse initiated the execution of an experimental sequence via Spike2 (Version 5.21, Cambridge Electronic Design, Cambridge, UK) starting both movement and electric signal generation of the dummy fish. The respective playback sequences were sent to the dummy via a D/A-converter (CED Power 1401, Cambridge Electronic Design, Cambridge, UK) and an analog stimulus isolator (model 2200, A-M Systems Inc., Carlsborg, WA) capable of reproducing the natural EOD-waveform of *M. rume*. The resulting electric field (see Donati *et al.* (2016) and Figure 8.4 for a characterization in a similar dummy), measured head-to-tail very close to the dummy, had an amplitude of 19.05 $V_{p\text{-}p}$ and thus was slightly stronger than that produced by the largest test fish (13.87 $V_{p\text{-}p}$). A DC motor was used to move the slide with the dummy via a cord linkage, thus moving the dummy fish through the testing area at a speed of 0.11 m s^{-1}. Two control conditions were performed without electrical playback. In one, only the moving dummy was presented (C_1), while in the other (C_2) the dummy remained motionless at the end position D_{stop} (compare Figure 4.1). Only the moving dummy was presented as a silent control (0 Hz) during the test series involving constant playback frequencies.

All experiments were performed in complete darkness with only infrared light illumination (850 nm, IR Illuminator Model SA1-60-C-IR, Itakka, Wattens, Austria), which is invisible for the fish (Ciali *et al.*, 1997). Both the living area and the testing area were monitored with infrared-sensitive video cameras (DBK 21AF04 FireWire Camera with Vari Focal T4Z2813CS-IR CCTV Lens, The Imaging Source, Bremen, Germany) from above.

4.2.3 Experimental protocol

The trigger mechanism for starting an experimental trial was activated only when the test fish sojourned in the hiding area. Once the test fish initiated an experimental sequence, the dummy moved across the testing area for 7.5 s while either emitting one of the playback patterns or remaining silent during controls (C_1). No movement was induced during control condition C_2.

For each animal, two experimental sessions were conducted on non-consecutive days, during which a total of ten successful presentations of each playback sequence were given in random order to each animal. For all but the second control condition (C_2), a presentation was defined to be a successful following-trial, if the test fish followed the dummy across an imaginary target line perpendicular to the rear end of the dummy fish at its end position (TL in Figure 4.1) within 15 seconds after the trial was initiated. Playback conditions were presented in randomized order with inter-trial intervals of at least 10 minutes. Non-successful presentations were repeated. To get the test fish accustomed to the treatment and avoid the possibility of ceiling effects (Martin and Bateson, 2007), each experimental session was preceded by a series of ten trials during which a regular 20 Hz playback sequence was used as a stimulus. This frequency was within the range of natural discharge frequencies displayed by *M. rume*, but differed from all the natural playback patterns used during the actual experimental trials.

Relative following-scores were calculated for the eight fish that were used during the experiments with natural playback sequences, by dividing the number of presentations, during which the following-criterion was met, by the total number of trials of the respective experimental condition.

The experimental protocol for the test series involving constant-frequency electric playbacks was slightly modified. Here, playback presentation was reduced to a duration of ten seconds, and only eight successful presentations were obtained per fish and playback condition.

4.2.4 Data acquisition

Electric signals were recorded via an array of five pairs of silver electrodes mounted in the experimental tank, which were arranged orthogonally to account for all EODs independently of the fish's position in the tank. All signals were amplified, digitized and recorded in Spike2 for subsequent analysis as time series. Simultaneously, all activity in the testing area was recorded to disk at 15 fps. Data were recorded during 30 seconds following the trigger signal for the experiments with natural playback sequences, and during 15 seconds for the experiments with constant playback frequencies.

4.2.5 Hierarchy determination

To determine the relative hierarchy of all individuals, animals were transferred pairwise into an illuminated tank with a white ground area of 60 cm x 30 cm. The single shelter provided was a 20 cm x 5 cm transparent red plastic tube. The animal that acquired ownership over the tube after 20 minutes was considered to rank higher than its opponent.

Ownership was expressed either by occupying the tube or by aggressively preventing the opponent from doing so (compare Terleph (2004)). Each fish was tested against all other fish in successive contests. Individuals were not tested more than once per day to mitigate potential effects of the outcome of previous contests on the following encounter (Chase *et al.*, 1994). Standard length and body weight of all animals were subsequently determined by placing each on laminated scale paper and weighing them wrapped in moist tissue. Hierarchy relationships were not determined for the test fish that participated in the constant-frequency playback experiments.

4.2.6 Locomotor behavior

A total of seven different motor-behavior patterns were quantified from the video recordings, which were randomized to rule out observer bias during the analysis. A 'cut off' occurred when the test fish intercepted the dummy's swimming trajectory and crossed its pathway during the first 7.5 seconds after the onset of the experiment. 'Circling' (Kramer, 1976a) was defined as a full circle by the test fish around the dummy during the first 15 seconds of an experiment. Incomplete circles within the same time frame were counted as 'lateral probing' (Crockett, 1986; Toerring and Belbenoit, 1979). 'Lateral va-et-vient' comprised short forward and backward swimming movements at a constant distance to the dummy, and 'radial va-et-vient' consisted of small tail strokes directed towards the dummy after a turn of 180° (Toerring and Belbenoit, 1979). 'Lateral va-et-vient' was only quantified between seconds 7.5 and 15 when the dummy had already stopped moving. A 'head butt' occurred when the test fish hit the dummy by a strike with its head (Bell *et al.*, 1974; Kramer, 1974) and instances of 'touch' lead to a visible deflection of the dummy fish by physical contact without obvious aggressive intent. A link to a video demonstration of these behaviors can be found in Appendix A. Locomotor behavior was not analyzed during the constant-frequency playback experiments.

4.2.7 EOD data analysis

Recorded EOD data were reduced to time series, and the signals of the fish and the playback were separated for further analysis. Data from the ten replicated trials per experimental condition of the same individual were pooled for histogram representation and averaged for subsequent statistical analysis of distribution parameters. This was done to avoid pseudo-replication due to repeated experimental conditions with the same individuals (Hurlbert, 1984).

Histograms of relative IDI-occurrence for the test series with constant frequencies were obtained from data that were pooled for all eight trials per experimental condition and tested fish for the ten seconds of playback presentation. Similar histograms were produced for the five seconds after the playback had stopped. Modal IDI-duration was determined from the histograms of each fish and translated to instantaneous frequencies before data were pooled again to obtain a single IDI-histogram per playback condition. All histograms were prepared with a bin size of 2 ms.

Adaptive cross-correlations between playback signals and EOD responses were calculated to quantify electric discharge synchronizations of *M. rume* with the mobile dummy fish during the experiments with natural playback sequences. These analyses were performed according to the procedure described in (Gebhardt *et al.*, 2012a). In short, IDI-sequences of fish and playback were transformed to high-resolution time series using exponential filtering. Pearson's correlation coefficients were then determined over the experiment's time for a 'response time' of 100 ms between the two time series. The maximum cross-correlation value within this 100 ms time window was then extracted for the electrical reaction of *M. rume* to the playback sequence from seconds one to 14. Data were averaged over a duration of 1/15 seconds to obtain a single value per video frame. The relative amount of correlation between the fish's signals and the playback signals was then compared for the different playback conditions. In addition, the duration of sequences of video frames with correlation coefficients greater than 0.3 was quantified. The amount of random cross-correlations between playbacks and fish responses was assessed by running the same analysis using IDI-sequences emitted by the fish during the moving control condition C_1 for each playback. A generalized linear mixed model (GLMM) using repeated measures of each playback and individual fish as fixed factors was used to assess the overall statistical difference between random correlations and those resulting from discharge interactions with electrical playback patterns.

The autocorrelation of a fish's discharge sequence was used to quantify the amount of discharge regularization. Autocorrelation was analyzed within a 200 ms response-time frame based on the same high-resolution time series used for the cross-correlation analysis described above. For the responses of *M. rume* to natural discharge sequences, the average amount of autocorrelation was calculated per fish and experimental condition. In addition, the duration of sequences with an autocorrelation coefficient greater than 0.3 was quantified. For the responses of *M. rume* to the constant frequency playbacks, the mean duration per experimental trial during which autocorrelation coefficients exceeded 0.5 was extracted for statistical comparison.

Double-pulse patterns were defined as sequences of alternating long and short IDIs. The minimum definition used for the quantification of a double-pulse pattern in this study was a sequence of at least five consecutive IDIs, where intervals 1, 3, and 5 were ≥ 60 ms and intervals 2 and 4 were ≤ 50 ms. This analysis was performed automatically using a custom-written Matlab script (Version R2013b, The MathWorks Inc. Natick, MA) and was only performed for the experiments with natural playback sequences.

Echo-responses were analyzed by quantifying the relative occurrence of latencies with which each playback EOD was followed by EODs of the fish. These latencies were compared to the distribution that would be expected if the IDI-sequences of playback and fish were two independent time series. Echo responses were quantified according to Kramer (1974) by calculating the ratio of observed to expected latencies at the mode of the observed latency distribution.

Statistical comparisons between experimental conditions were performed in IBM SPSS Statistics for Windows (Version 22.0, IBM Corp., Armonk, NY) using repeated measures ANOVA if data were assumed to be normally distributed as assessed by the Shapiro-Wilk test. In cases where the assumption of sphericity was violated according to Mauchly's test, epsilon (ε) was used to adjust the degrees of freedom according to Greenhouse and Geisser (1959). Data not meeting the criterion of normality were analyzed using the non-parametric Friedman's two-way analysis of variance by ranks. Associations with hierarchy rank were determined based on Spearman rank correlations (ρ). Statistical significance was accepted at the $\alpha = 0.05$ level.

4.2.8 Video tracking

For comparison of swimming-trajectories dependant on the presence or absence of electrical playback signals, all videos recorded for playback condition F_2 and the control C_1 were rectified to correct for radial distortion and subsequently tracked to obtain trajectories and spatial orientations for both the dummy and the focal fish. Tracking was performed using Ctrax (Branson *et al.*, 2009) including the provided Matlab toolboxes for subsequent correction and analysis of tracking data. The distance between test fish and the dummy was determined for each frame as the shortest connection between the snout of the test fish and any point on the ellipse representing the dummy's current position (compare inset of Figure 4.21A). The angular relationship between dummy and fish was determined from the dummy's coordinate system by calculating the absolute angle between the dummy’s orientation and the line connecting the centers of the ellipses representing fish and dummy (compare inset of Figure 4.21B). The average cross-correlation coefficients between electric signal sequences and the temporal occurrence of double pulses were then assigned to each frame. To guarantee synchronicity between EOD- and video recordings, an infrared LED was activated simultaneously with playback presentation and recorded on video.

4.3 Results

4.3.1 Dominance hierarchy

Based on the hierarchy experiments, all animals could be unequivocally assigned to a relative dominance rank within the group of test fish, with fish #1 being the highest and fish #8 the lowest ranking individual. Increase in hierarchy rank was correlated with an increase in the animals' standard length ($\rho_s = -0.93$, $p = 0.001$), weight ($\rho_s = -0.93$, $p = 0.001$) and peak-to-peak EOD amplitude ($\rho_s = -0.71$, $p = 0.047$) (Figure 4.2).

4.3.2 Following-behavior

Analysis of relative following-scores (Figure 4.3A) revealed a statistically significant difference between the treatments ($\chi^2_{(7)} = 30.52$, $p < 0.001$) with all conditions involving natural electrical playback forming a homogenous subgroup ($\chi^2 = 3.44$, $p = 0.75$). Single individuals of *M. rume* were, therefore, less likely to be recruited into the testing area by an electrically silent dummy compared to a dummy emitting EODs (*median* score = 0.48). However, there was no overall effect on following-behavior in response to the different playback sequences (*median* scores: 0.87–1). To test whether animals would enter the testing area and meet the following-criterion independently of the experimental conditions, no stimuli were presented after activation of the trigger during control condition C_2. Statistical analysis (paired-samples t-test, $t_{(7)} = 3.27$, $p = 0.014$) confirmed a significant difference of relative following-scores between the control conditions C_1 (*mean* ± *s.e.m.* = 0.51 ± 0.08) and C_2 (*mean* ± *s.e.m.* = 0.21 ± 0.07) (Figure 4.3B). This indicates that following-behavior did not occur spontaneously, but was instead triggered by the movement of the dummy, even when the dummy was electrically silent.

There was a statistically significant effect of experimental condition on the animals' latency to enter the testing area ($F_{(2.912,\,20.385)} = 11.21$, $p < 0.001$, $\varepsilon = 0.42$) (Figure 4.3C). Without playback, animals took on average 0.79 ± 0.17 (*mean* ± *s.e.m*) seconds longer to enter the testing area as indicated by a Bonferroni adjusted comparison ($p = 0.014$) between the control C_1 (*mean* ± *s.e.m.* = 2.85 s ± 0.16 s) and the average of all conditions featuring natural electrical playback sequences. Latencies for the conditions featuring electrical playback did not differ statistically ($F_{(6,\,42)} = 1.83$, $p = 0.12$).

A positive correlation between hierarchy rank and relative following-scores was observed in all eight individuals (Table 4.1), which was significant for the control condition C_1 ($\rho_s = 0.98$, $p < 0.001$) and the low frequency playback F_4 ($\rho_s = 0.78$, $p < 0.022$). This means that in the latter situations, higher-ranking individuals were more likely to follow the dummy than lower-ranking fish.

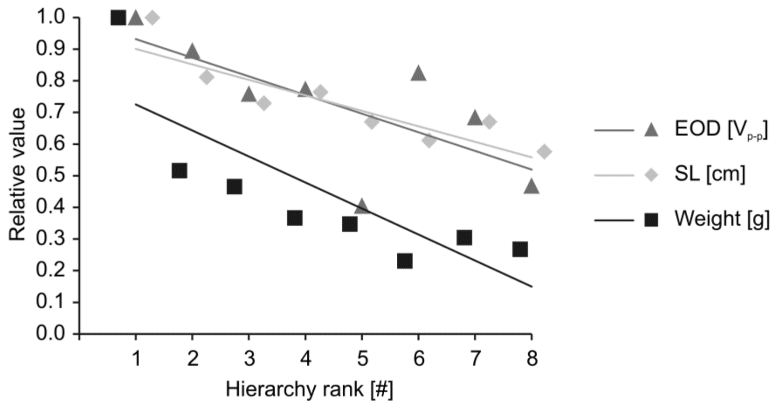

Figure 4.2: Associations between hierarchy rank and fish characteristics. EOD-amplitude (▲), weight (■) and standard length (◆) increased with increasing position within the hierarchy. #1 is the highest- and #8 the lowest-ranking individual.

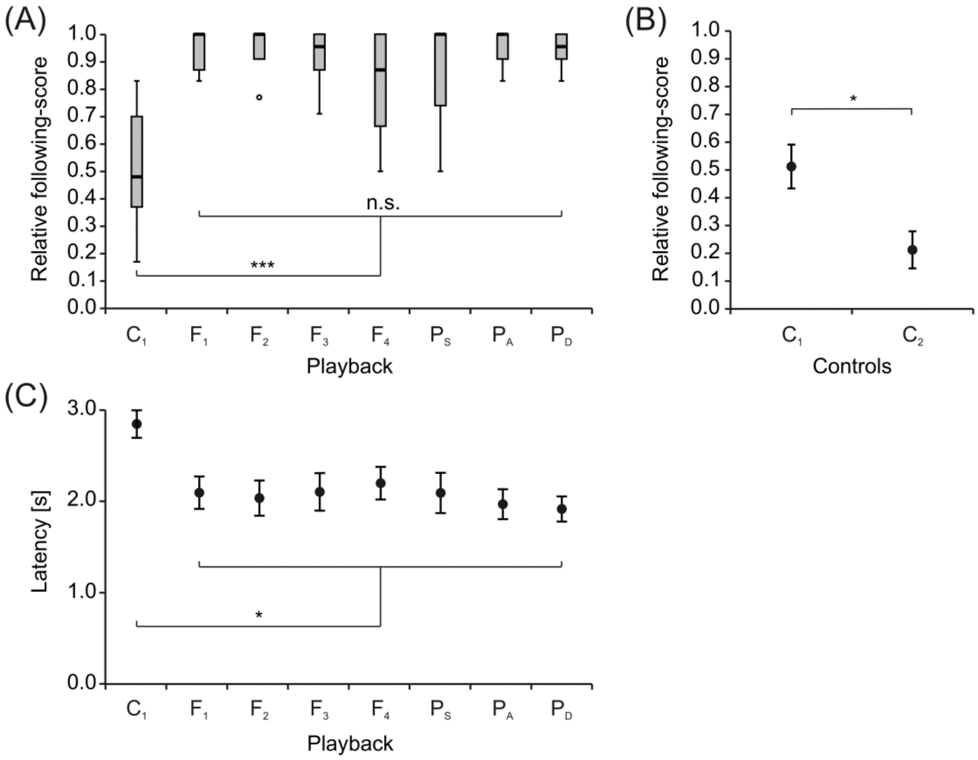

Figure 4.3: Following-behavior based on playback presentation. Different playbacks are given on the abscissa. (A) Box plots of relative following-scores for single *M. rume* following a mobile dummy. Animals followed more often during playback presentation compared to the control condition (C_1). (B) Comparison of relative following-scores (*mean* ± *s.e.*m.) between the control conditions. Animals crossed the target line more often if the electrically silent dummy moved across the testing area (C_1). (C) Latency (*mean* ± *s.e.*m.) of fish to enter the testing area after the onset of the experiment, i.e., after the dummy started moving.

Table 4.1: Associations between dominance rank and following-score. Spearman rank correlations (ρ_s) and corresponding *p*-values are listed for each playback condition.

Playback	**ρ_s**	***p*-value**
C_1	0.98	**< 0.001**
F_1	0.12	0.77
F_2	0.32	0.45
F_3	0.52	0.18
F_4	0.78	**0.022**
P_S	0.44	0.28
P_A	0.36	0.39
P_D	0.48	0.23

4.3.3 Electrical responses

Electrical responses varied considerably between the experiments with natural playback sequences (Figure 4.4) and those with constant-frequency playbacks (Figure 4.6). The electrical responses to the different natural playbacks and control conditions are summarized in Figure 4.4. In the central column, IDI-duration is plotted versus trial duration for all playbacks (red), as well as a typical response of fish #2 (black), to demonstrate the patterning of the respective signal sequences. The relative occurrence of interval lengths and their distributions are depicted on the left-hand side of Figure 4.4 for the presented playbacks (red) and the summed electrical responses of all eight *M. rume* to the respective experimental conditions (black). Statistical comparison of IDI-distribution parameters for 15 s sequences, averaged over the ten trials performed with each individual fish per experimental condition, revealed significant differences between IDI mean ($\chi^2_{(8)} = 36.17$, $p < 0.001$), IDI median ($\chi^2_{(8)} = 29.47$, $p < 0.001$), IDI mode ($\chi^2_{(8)} = 21.38$, $p = 0.006$), and the inter-quartile difference (q75–q25, $\chi^2_{(8)} = 26.93$, $p = 0.001$, Figure 4.5). The same data are plotted for each fish separately as relative cumulative sums (RCS) on the right-hand side of Figure 4.4. These diagrams allow assessing the contributions of individual fish to the overall IDI-distribution in each category. Evidently, animals did not adopt the overall IDI-distribution that was emitted by the dummy. Instead, distribution modes were approximately the same for the electrical responses to all playbacks, including the silent control C_1, and were most reminiscent of the IDI-distribution in playback F_2, with a mode at 64 ms (Figure 4.4B). The motionless control condition C_2 represents an exception (Figure 4.4I; compare Figure 4.5). Here, animals discharged less regularly and with longer intervals, leading to a broader IDI-distribution. From the cumulative histograms in Figure 4.4 it becomes evident that electrical discharge responses were not uniform across individual fish. Particularly for the highest ranking individual fish #1, a second turning point in the histogram indicates a bimodal IDI-distribution (indicative of double pulses, see below) in response to all but the low-frequency playbacks F_4 and P_S (Figure 4.4D, E) and the controls C_1 and C_2 (Figure 4.4H, I).

Electric signaling responses of *M. rume* to playback of constant discharge frequencies are summarized in Figure 4.6 and Table 4.2. On the left-hand side of Figure 4.6, histograms of relative IDI-distributions characterize the responses of fish to different playback conditions. For all but the electrically silent control condition (Figure 4.6A), histograms are displayed separately for IDIs observed during the first 10 seconds when electrical playback was presented (black line), and the five seconds after the playback had stopped (grey area). The IDIs that correspond to the respective playback frequencies are indicated by blue bars. The right-hand side of Figure 4.6 depicts IDI-sequences corresponding to the different playback frequencies (blue) and exemplary responses to each frequency by an individual fish (black). The diagrams show a strong and frequency dependent reaction of the test fish to the signals emitted by the mobile dummy. During the silent control condition (Figure 4.6A), the IDI-distribution of the signaling fish with a mode at 60 ms was comparable to the distributions obtained during the experiments with natural playback frequencies (compare Figure 4.4). In the given example, the fish clearly regularized its discharge activity while following the electrically silent dummy (indicated by the grey shaded area). During the 5 Hz electrical playback sequence (Figure 4.6B), which corresponds to an IDI of 200 ms, the IDI-distribution of the responding fish was mostly unaffected, except for a few short sequences of similar discharge activity. At a playback frequency of 10 Hz (Figure 4.6C), many adoptions of this discharge rate by the following fish caused an additional mode at 100 ms, which corresponds to the playback frequency. In response to playback presentations of 15 Hz (IDI of 66 ms, Figure 4.6D) and 25 Hz (IDI of 40 ms, Figure 4.6E), *M. rume* locked their discharge activity almost entirely to the playback frequencies at which the mobile dummy emitted EODs. During the presentation of the even higher playback frequencies of 40 Hz (IDI of 25 ms, Figure 4.5F) and 80 Hz (IDI of 12.5 ms Figure 4.5G), fish were unable to sustain discharge activity at corresponding rates. Instead, they signaled with relatively regular intervals of twice the playback-IDI duration (50 ms) in response to the 40 Hz playback (IDI of 25 ms), and approximately three times the playback-IDI duration (38 ms) in response to the 80 Hz playback (IDI of 12.5 Hz). Almost immediately after the end of playback presentation, all fish returned to more irregular discharge activity with longer IDIs. This is evident both from the exemplary signaling responses between seconds 10 to 15 (right-hand side of Figure 4.6) and the corresponding IDI-histograms (grey, left-hand side of Figure 4.6), which closely resemble the IDI-

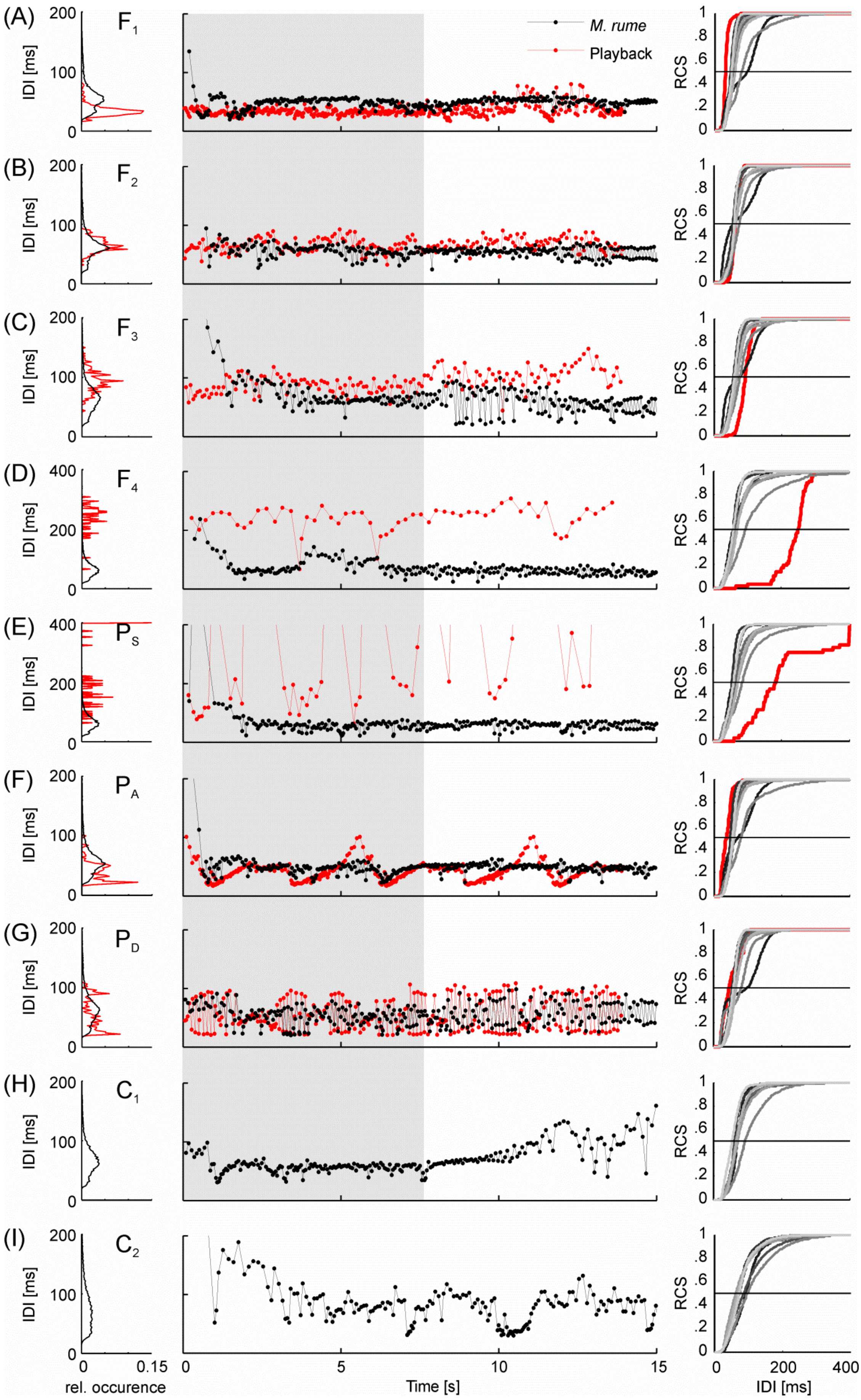
(A)
F1
(B)
F2
(C)
F3
(D)
F4
(E)
PS
(F)
PA
(G)
PD
(H)
C1
(I)
C2
M. rume
Playback
IDI [ms]
0
100
200
400
0
0.15
rel. occurence
0
5
10
15
Time [s]
RCS
0
.2
.4
.6
.8
1
0
200
400
IDI [ms]

Figure 4.4 (previous page): Representation of natural playback conditions and the electrical responses of *M. rume*. Left: Relative IDI-distribution of playbacks (red) and *M. rume* (black) pooled for all individuals per condition. Middle: Time course of electrical playback IDIs (red) with an exemplary response of fish # 2 (black). Right: Relative cumulative sums (RCS) of IDI-distributions of playbacks (red) and *M. rume* (grey, graded to distinguish between different ranks. Darker graphs represent more dominant individuals). Each curve represents data from ten trials that were recorded from an individual fish within the respective condition. The shaded area represents the duration of dummy movement. Note the different scaling in D and E.

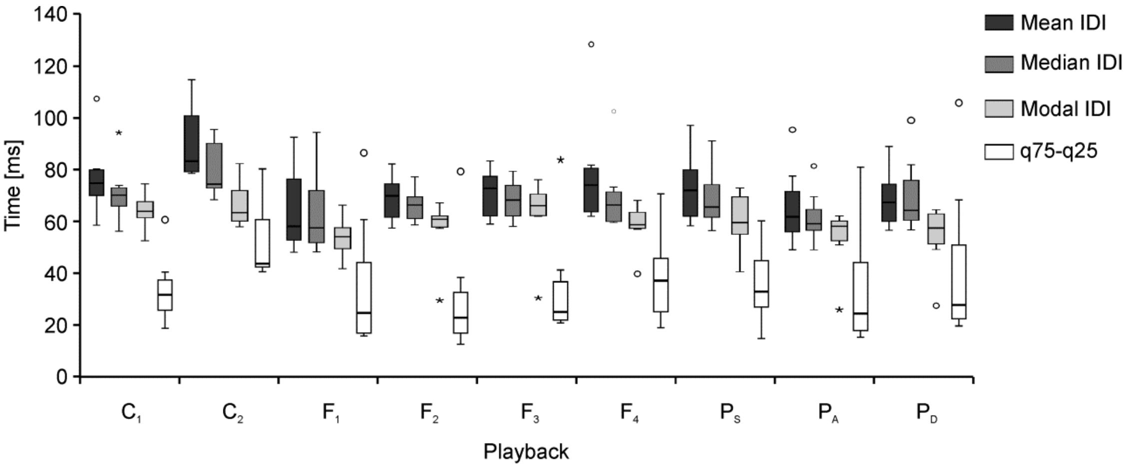

Figure 4.5: IDI-distribution patterns in response to natural electrical playback sequences. Box plots indicating means, medians, modes and inter-quartile differences (q75–q25) of the IDI-distributions of eight *M. rume* in response to different electrical playback conditions and controls.

distribution of the fish during the electrically silent control (Figure 4.6A). The adoption of discharge activity by *M. rume* in response to constant-frequency playback is summarized for individual fish in Table 4.2. It shows that the median IDI-modes of animals that were responding to constant-frequency playback of 10 Hz, 15 Hz, and 25 Hz, corresponded to the respective IDI-durations of 100 ms, 66 ms, and 40 ms, for the majority of fish. At higher playback frequencies, all but one individual discharged preferably at half the playback rate (IDI of 50 ms in response to the 40 Hz playback, which corresponds to an IDI of 25 ms) or a third of the playback rate (IDI of 38 ms in response to the 80 Hz playback, which corresponds to an IDI of 12.5 ms).

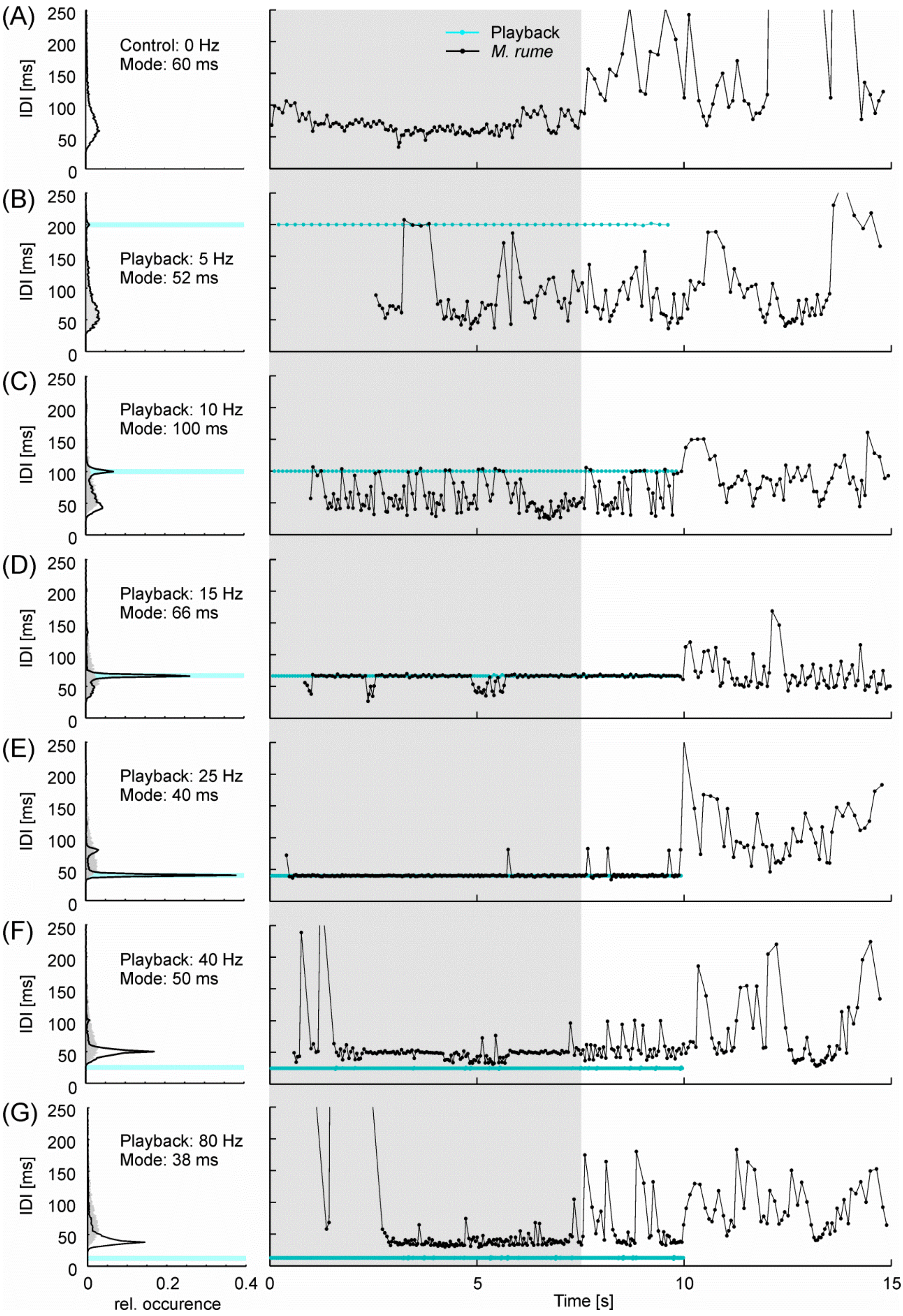

Figure 4.6: Electrical signaling responses of *M. rume* to constant-frequency playback presentation. Left: Relative IDI-distributions of playback presentations (blue bar) and the electrical signaling responses of *M. rume* pooled for all individuals per experimental condition. Histograms are separately displayed for intervals observed during the first ten seconds of the trial featuring electrical playback (black line)

and the five seconds after the playback had stopped (grey area). Playback frequencies and IDI-modes are given in the insets. Right: Time course of electrical playback IDIs (blue) with an exemplary response of a single fish (black) for each experimental condition. The shaded area represents the duration of the dummy fish's movement. Based on data from Toma (2014b).

Table 4.2: IDI-modes of single *M. rume* in response to constant-frequency electrical playback sequences. The table lists the presented playback frequencies in Hertz with the corresponding IDI-duration in milliseconds in the row below. For all eight fish that participated in this set of experiments, IDI-modes are given in milliseconds for all experimental conditions. Median IDI-modes demonstrate that most fish adopted playback frequencies of 10 Hz, 15 Hz, and 25 Hz, whereas they signaled at multiples of the playback IDI-duration in response to the higher-frequency playback presentations of 40 Hz and 80 Hz. Based on data from Toma (2014b).

	Playback [Frequency and IDI]						
	0 Hz	5 Hz	10 Hz	15 Hz	25 Hz	40 Hz	80 Hz
Fish [#]	Control	200 ms	100 ms	66 ms	40 ms	25 ms	12.5 ms
1	68	34	100	66	40	50	38
2	60	58	100	66	40	50	38
3	52	62	100	66	40	50	38
4	48	74	100	66	80	56	46
5	58	50	42	66	40	50	38
6	60	44	42	66	40	50	38
7	70	56	100	66	40	50	38
8	74	48	44	66	40	50	38
Median [IDI]	60 ms	53 ms	100 ms	66 ms	40 ms	50 ms	38 ms

A particular discharge pattern was represented by double pulses, which were sequences of alternating long and short IDIs. Figure 4.7A shows an exemplary double-pulse pattern displayed by fish #5 in response to playback P_D, which also featured double pulses (Figure 4.4G). The temporal occurrence of double pulses in response to all experimental trials featuring playback P_D is summed over the recording period of 30 s in Figure 4.7B and demonstrates a steep decline of this pattern within a few seconds after the end of playback presentation. The number of double pulses varied between the different playbacks. They were most numerous in response to the double-pulse playback P_D, differing significantly from all but the response to playback F₃ based on Fisher's LSD ($F_{(3.070,\,21.488)} = 18.35$, $p < 0.001$, $\varepsilon = 0.44$ on arcsine-square-root transformed data). A functional role of double pulses as a communication signal is supported by the fact that this pattern was virtually absent during the electrically silent control condition C_1 (Figure 4.8).

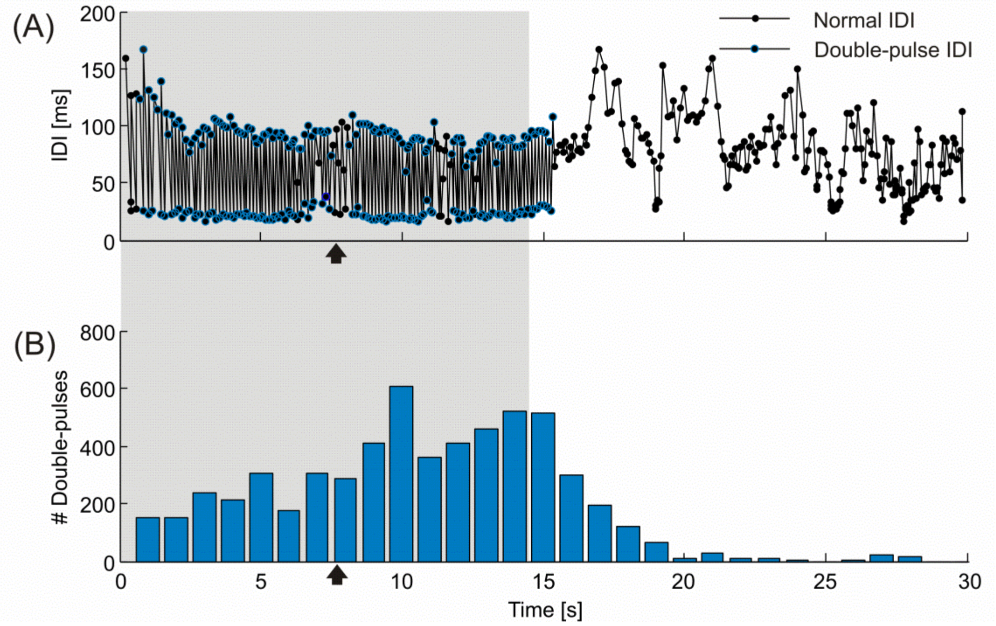

Figure 4.7: Double-pulse responses over time. (A) Exemplified electrical response of fish #5 to the double-pulse playback P_D (see Figure 4.4G) with intervals belonging to double-pulse sequences marked by blue circles. (B) The total amount of double-pulse related IDIs is pooled per second for the time course of all experimental trials with playback P_D. The shaded area represents the duration of the playback. Dummy fish movement stopped at the time point indicated by black arrows.

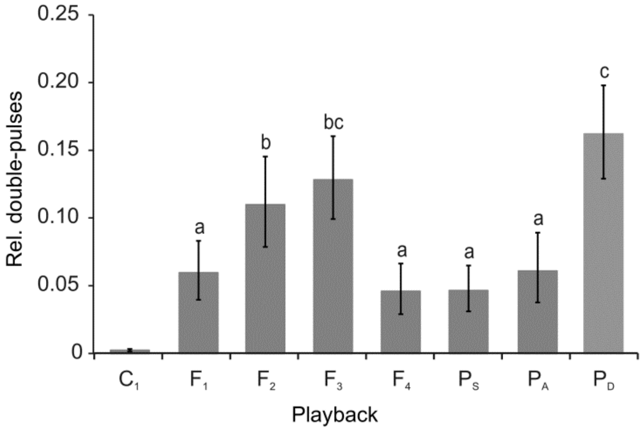

Figure 4.8: Double pulses in response to natural electrical playbacks. Relative amount (*mean* ± *s.e.*m) of double pulses emitted in response to different playback conditions and the electrically silent control (C_1). Categories not sharing a common superscript letter differ significantly based on Fisher's LSD ($\alpha = 0.05$).

Apart from differences in the number of double-pulse discharges in response to different electrical playbacks, there was also variation in double-pulse displays among individual fish. Figure 4.9A sums the total number of double pulses over time that was emitted by each *M. rume* in response to all trials featuring natural electrical playback sequences. Similar to the data presented in Figure 4.7B, double-pulse production increased in most fish over the time course of playback presentation, peaking shortly after its offset (see also Figure 4.13A) and declined to virtually zero within a few seconds afterward.

The total amount of double pulses that was displayed by an individual fish was furthermore correlated with its rank within the hierarchy. Higher-ranking individuals produced more double pulses than lower-ranking ones ($\rho_s = -0.71$, $p = 0.047$, Figure 4.9B). Also, the number of double pulses produced per trial decreased with the number of tests performed with an individual in an experimental session (Figure 4.10), indicating that this signaling pattern was subject to habituation. In response to playback F_2, the highest amount of double pulses was emitted at a distance of approximately 100 mm between *M. rume* and the dummy, and none were observed at a distance greater than 287 mm.

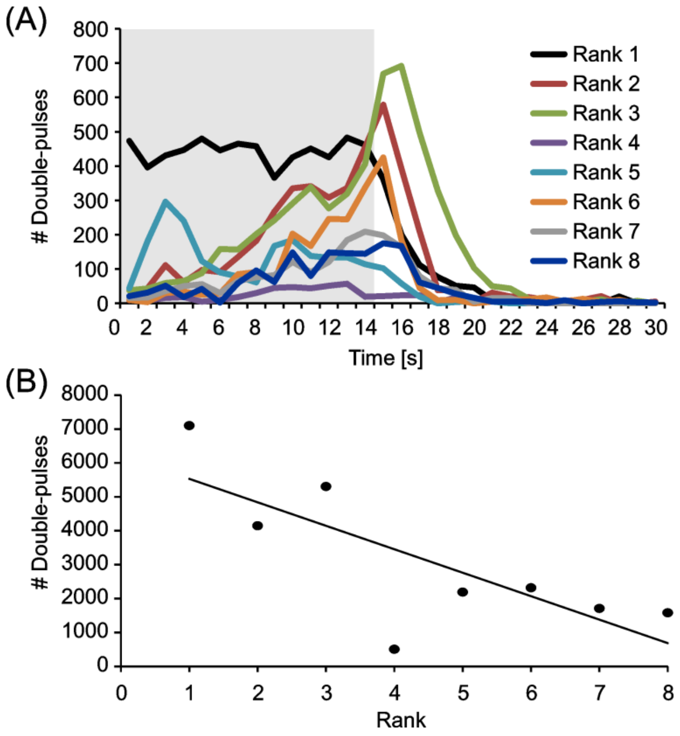

Figure 4.9: Double pulses and hierarchy rank. (A) The number of double pulses emitted per second of trial duration by each fish is summed for all trials involving electrical playback. Individual fish are color-coded according to their hierarchy rank. The shaded area represents the duration of the playback. (B) Association between double-pulse display and hierarchy rank for all tested individuals of *M. rume.*

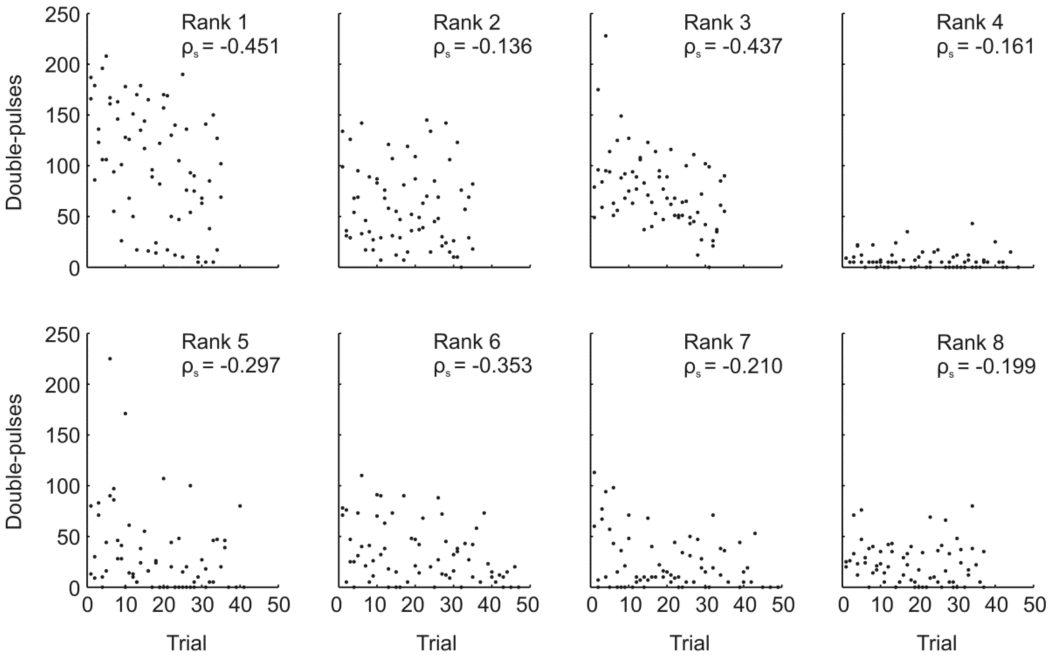

Figure 4.10: Habituation of double-pulse displays. Data show negative correlations between the number of consecutive trials performed with an individual and the number of double pulses this individual emitted in response to electrical playback. Spearman rank correlation coefficients (ρ_S) are given for each fish in the inset of the diagram.

Autocorrelation coefficients of discharge sequences were calculated to quantify discharge regularizations, with higher coefficients pointing to more regular discharge activity in *M. rume*. The average maximum amount of autocorrelation within a time frame of 200 ms over the recording period of 30 seconds was highest for playback F_1 (*mean* = 0.352, 95% CI [0.299, 0.405]) and lowest for the stationary control C_2 (*mean* = 0.265, 95% CI [0.233, 0.296]). No experimental category differed significantly from the moving control C_1 (*mean* = 0.327, 95% CI [0.272, 0.382]), based on Bonferroni adjusted *p*-values (Figure 4.11). Autocorrelation varied more clearly in response to constant frequency playback presentation (repeated measures ANOVA, $F_{(6, 42)} = 25.26$, $p < 0.001$; Figure 4.12). The longest sequences of strong regularization with autocorrelation coefficients ≥ 0.5 were observed in response to the 15 Hz playback (*mean* ± *s.e.m.* = 4.16 ± 0.59 s) and the 25 Hz playback (*mean* ± *s.e.m.* = 4.47 ± 0.54 s), whereas the shortest sequences occurred in response to the electrically silent control (*mean* ± *s.e.m.* = 0.32 ± 0.09 s) and the 5 Hz playback (*mean* ± *s.e.m.* = 0.55 ± 0.14 s).

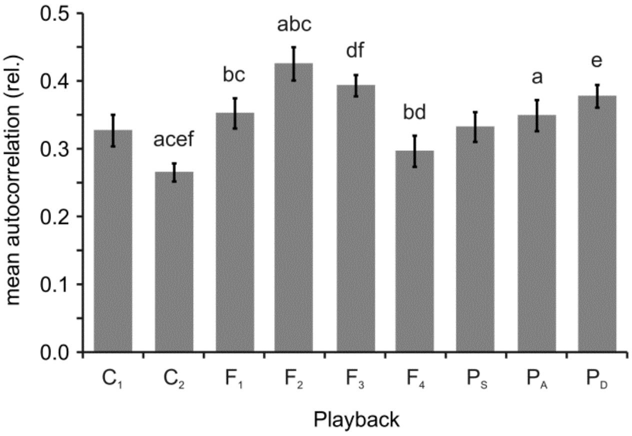

Figure 4.11: Autocorrelation of IDI-sequences in response to natural playback sequences. Average of the maximum autocorrelation within a time frame of 200 ms over a 15 s recording period for each experimental condition (*mean* ± *s.e.m.*). Categories sharing a common superscript differ based on Bonferroni adjusted *p*-values.

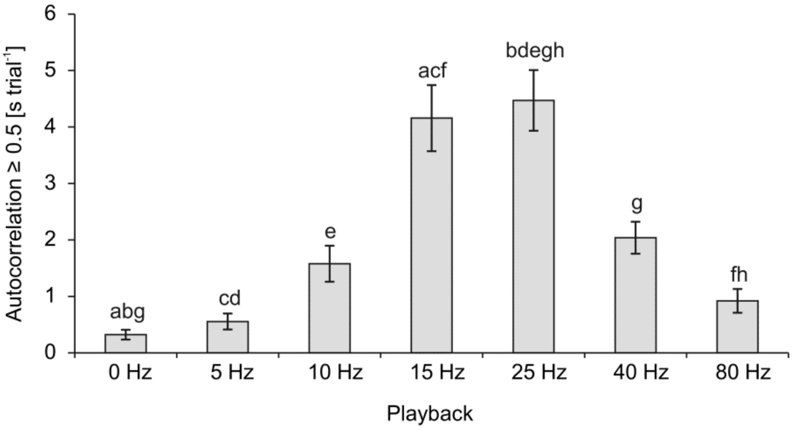

Figure 4.12: Autocorrelation of IDI-sequences in response to constant-frequency playback. Average duration (*mean* ± *s.e.m.*) of sequences with a maximum autocorrelation coefficient ≥ 0.5 extracted for each fish and playback condition from a 200 ms response-time frame. Categories sharing a common superscript letter differ based on Bonferroni corrected *p*-values. Based on data from Toma (2014b).

Figure 4.13 summarizes the quantification of autocorrelation within a signal sequence over time. An exemplary IDI-sequence of fish #3 (black) with strong regularization in response to playback F_2 (red) is depicted in Figure 4.13A. The animal responded to the offset of the playback stimulus with a short sequence of double pulses and continued to discharge with longer, less regular intervals for the rest of the recording. For the sequence depicted in Figure 4.13A, autocorrelation is quantified over time in Figure 4.13B, with correlation coefficients color-coded from −0.75 to 0.75 for the timeframe analyzed. Autocorrelation of the discharge activity of fish #3 was strong during playback presentation, and during the short sequence of double pulses that followed, and decreases abruptly after that.

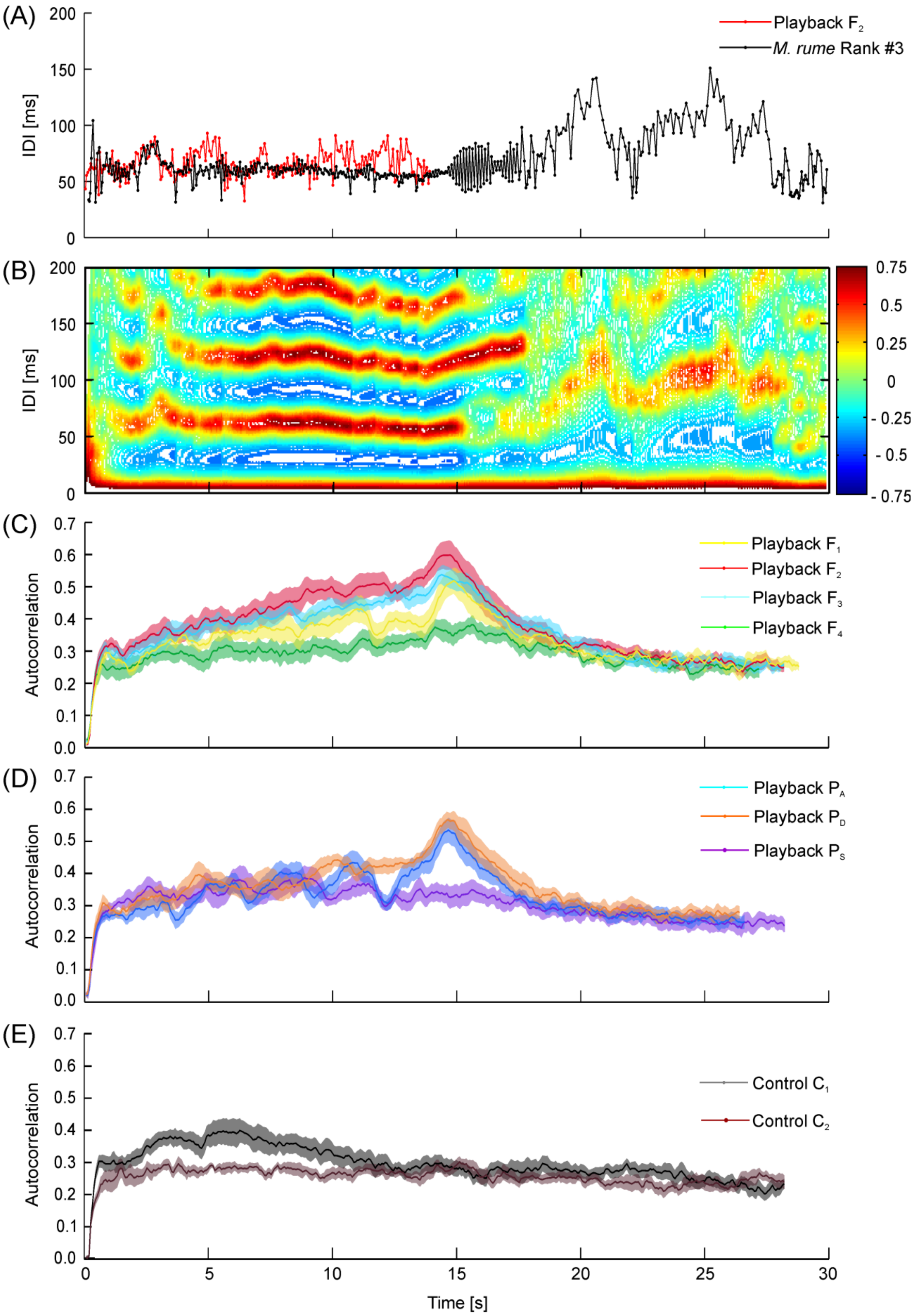

Figure 4.13: Autocorrelation of electrical discharge activity in *M. rume*. (A) Exemplary demonstration of a regular discharge pattern with high autocorrelation (compare B) of fish #3 (black) in response to playback F_2 (red). Note the typical double-pulse pattern short after stimulus offset. IDIs were longer and more variable in the second half of the recording when the dummy was silent. (B) Autocorrelation dia-

gram for the sequence shown in (A), with a color-coded representation of the correlation between the fish's current discharge activity with its own signal within the previous 200 ms. (C–E) Maximum autocorrelation for all trials averaged for all individuals of *M. rume* depicted over a period of 30 seconds. (C) Frequency-based playback trials F_1–F_4; (D) pattern-based playbacks $P_{A,D,S}$; (E) controls C_1 and C_2. Shaded areas represent the standard error of the mean.

Average time courses of regularization of all fish in response to natural playback sequences and control conditions are depicted in Figure 4.13 C–E. Data are mean values of the average autocorrelation displayed per frame by all fish in the respective experimental category, with shaded areas representing standard errors of the mean. During electrical playback presentation, correlation coefficients steadily increased, peaking shortly after the offset of the stimulus and then declined to a baseline level of approximately 0.3, similar to the value of the motionless control C_2. This effect was weaker or even absent in response to the low-frequency playbacks F_4 and P_S (Figure 4.13C, D). The moving control C_1 caused an initial short increase in regularization that declined a few seconds afterward and reached baseline levels after the dummy fish stopped moving. Quantification of the duration of coherent sequences of autocorrelation exceeding the baseline level of 0.3 revealed longer sequences in response to higher frequency playbacks as compared to the low-frequency playbacks F_4 and P_S and the controls (Figure 4.14).

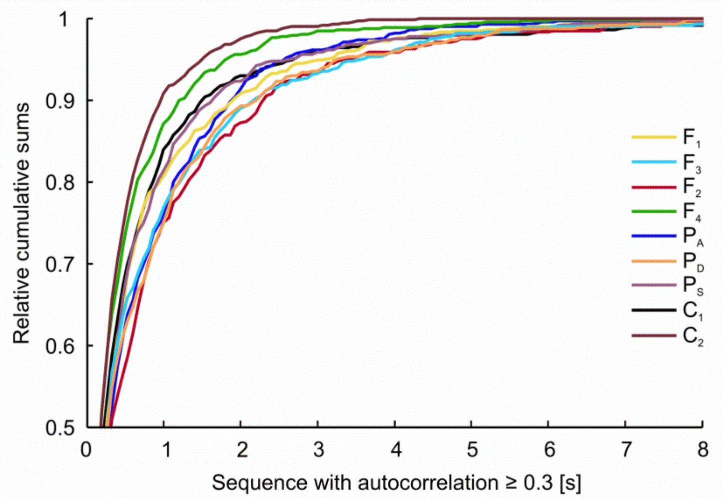

Figure 4.14: RCS of temporal sequences with an autocorrelation coefficient ≥ 0.3. Graphs indicate the proportion of sequences with high autocorrelation for a given duration depending on the playback condition.

4.3.4 Electrical discharge interactions and synchronizations

All animals showed preferred latency responses as well as latency avoidance responses to the natural electrical playback signals, i.e., the fish responded to a certain proportion of the playback EODs by emitting time-locked EODs of their own (Figure 4.15). The preferred latency, or 'echo response,' ranged from 19 to 25 ms and occurred in response to all electrical playbacks (Figure 4.16). The same was true for latency avoidance responses, which directly preceded echo responses at around 15 ms after the playback EOD (Figure 4.15). No consistent differences in the ratio between observed and expected latencies were found based on the different playback IDI-patterns, although the different shapes of the latency diagrams suggest that fish may show some individual characteristics in their echo response (Figure 4.15). Preferred latencies of the fish during the constant-frequency playback experiments were longer and lasted for 25 to 32 ms (Figure 4.17). The strongest echo responses were observed in response to the presentations of 15 Hz (Figure 4.17C) and 25 Hz (Figure 4.17D) playback sequences. Echo responses to the 40 Hz and 80 Hz playback sequences could not be faithfully displayed because the corresponding playback IDIs of 25 ms and 12.5 ms were shorter than the response latencies of the fish. This demonstrates that the echo response is well adjusted to the frequency range within which the fish are usually signaling.

Adaptive cross-correlations between the signal sequences of the playback and the fish revealed that animals frequently synchronized their discharge activity to the playback signals. This occurred preferably at a response time of approximately 20 ms, which corresponds to the latency of the echo response (Figure 4.18). The relative amount of maximum cross-correlation was on average significantly higher ($F_{(1,97)} = 171.03$, $p < 0.001$) when IDI-sequences of fish and dummy were recorded in the same trial (*mean* = 0.204, 95% CI [0.199, 0.210]) compared to randomly occurring correlations, which were calculated from fish IDI-sequences and playback patterns that were recorded during independent experimental trials (*mean* = 0.151, 95% CI [0.146, 0.157]). However, the differences in the relative amount of maximum correlation, which were detected between the responses to the different playbacks after subtraction of randomly occurring correlations in each of the playback conditions, account at most for a statistical trend ($\chi^2_{(6)} = 11.57$, $p = 0.07$). No matter which playback sequence was used, fish always synchronized a certain fraction of their EODs to the signals emitted by the dummy. This indicates that *M. rume* synchronized its discharge behavior largely independently of the current playback sequence and without adopting the actual patterns or frequency distributions of the particular playback. The duration of sequences with correlations between the signals of *M. rume* and the electrical playback exceeding 0.3, however, varied depending on the presented playback sequence (Figure 4.19). Longer runs of high correlation were elicited by playbacks F_2 (red) and P_A (dark-blue), whereas the low-frequency playbacks F_4 (green) and P_S (purple) accounted for fewer long sequences of high correlation. The influence of playback condition on the duration of periods of high correlation was statistically significant at a relative cumulative sum (RCS) of 0.75 ($\chi^2_{(6)} = 22.39$, $p = 0.001$, dotted line in Figure 4.19).

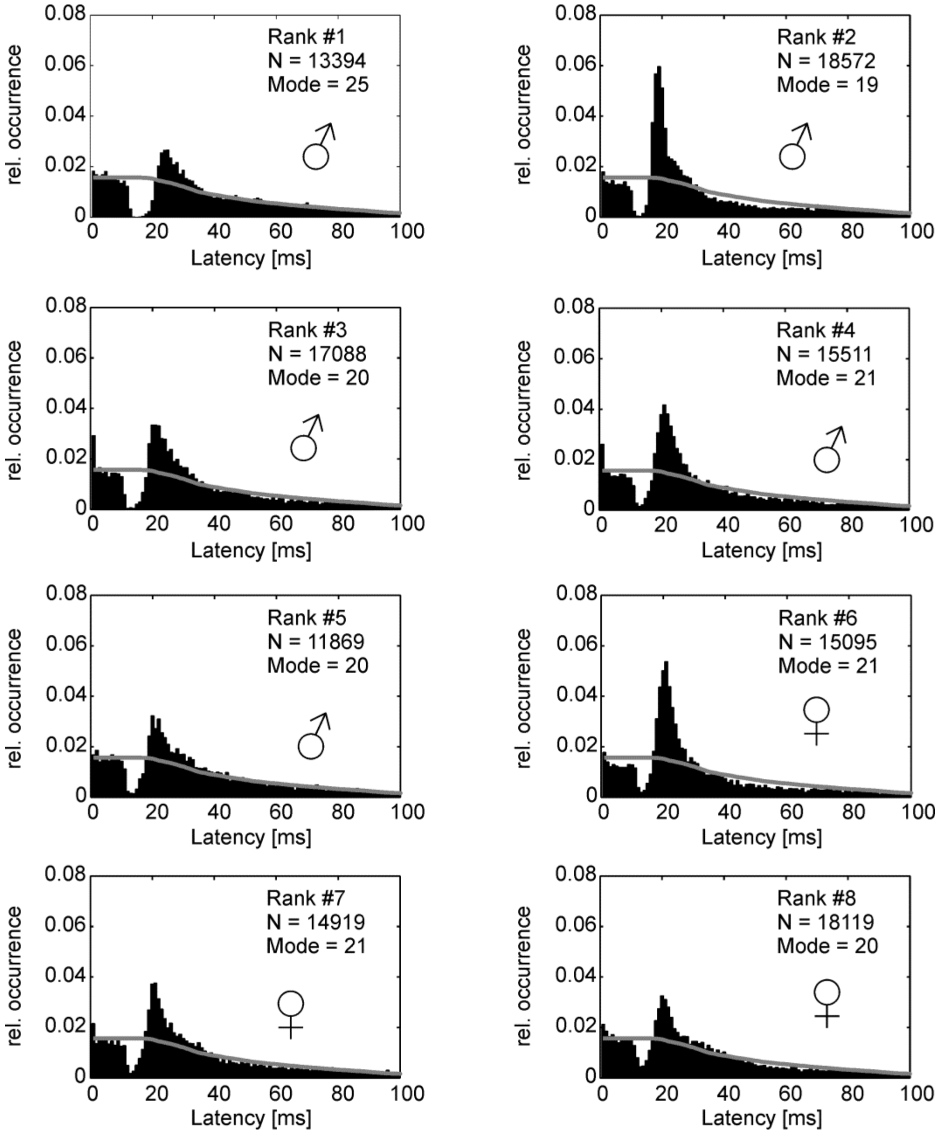

Figure 4.15: Echo responses of individual *M. rume* to all natural electrical playback sequences. A combination of preferred latencies and latency avoidance occurred in all eight individuals. Overall responses to all natural playback sequences are pooled for each individual. Insets identify individual fish according to their rank within the hierarchy. N = number of response-EODs the respective diagram is based on. Mode = duration of the most abundant latency in milliseconds. Grey lines indicate the expected latency distribution based on the playback EOD-distribution. Bin size: 1 ms.

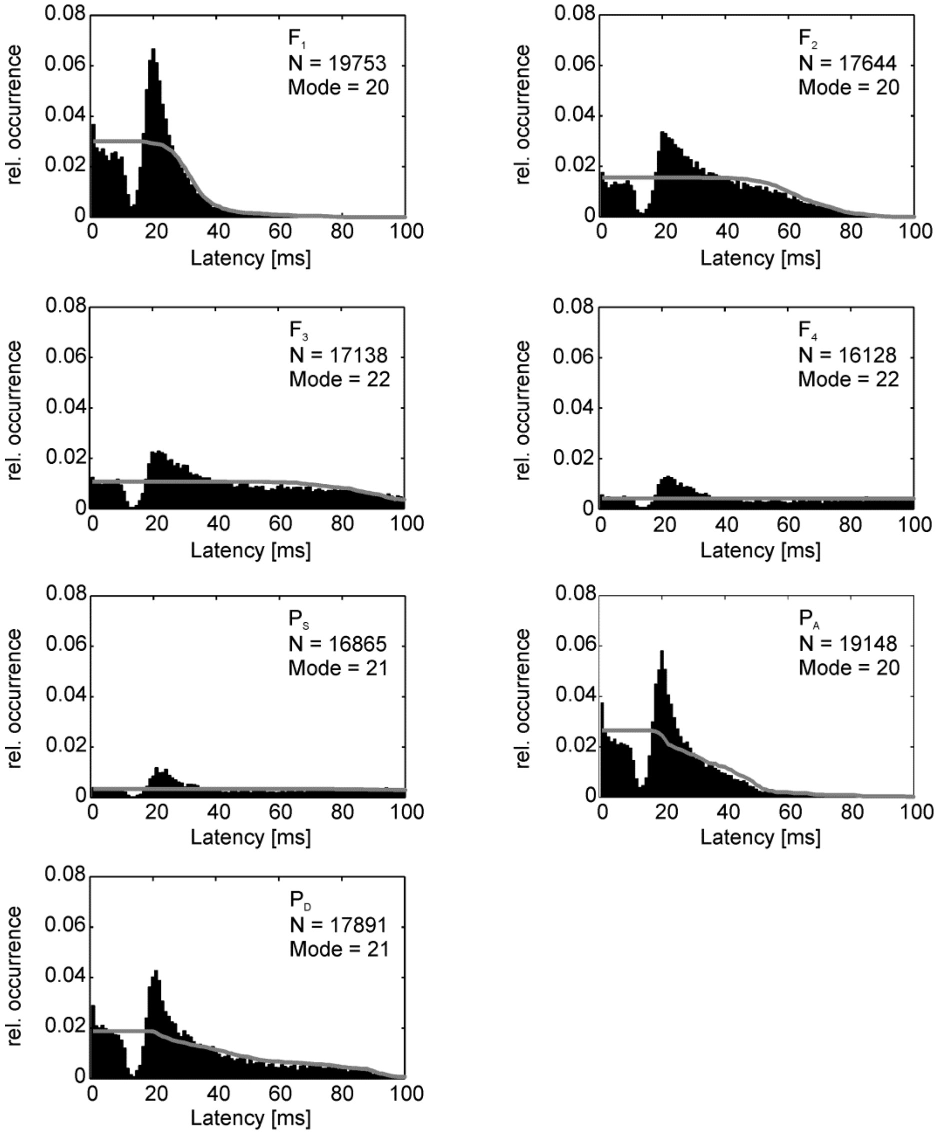

Figure 4.16: Echo responses of all *M. rume* to the different natural electrical playback sequences. A combination of preferred latencies and latency avoidance occurred in response to all natural electrical playback sequences. Overall responses of all eight fish are pooled for each playback. Insets identify the different playbacks. N = number of response-EODs the respective diagram is based on. Mode = duration of the most abundant latency in milliseconds. Grey lines indicate the expected latency distribution based on the playback EOD-distribution. Bin size: 1 ms.

4.3.5 Motor interactions with the dummy fish

To analyze the influence of electrical playbacks on interactive behaviors of *M. rume*, seven different motor patterns were quantified (Figure 4.20, see video link in Appendix A). Statistically significant differences between the experimental conditions were detected for 'cut off' (Figure 4.20A, $\chi^2_{(7)} = 14.97$, $p = 0.036$) and 'circling' (Figure 4.20B, $\chi^2_{(7)} = 15.82$, $p = 0.027$). In both cases, almost no instances of the respective motor patterns occurred in

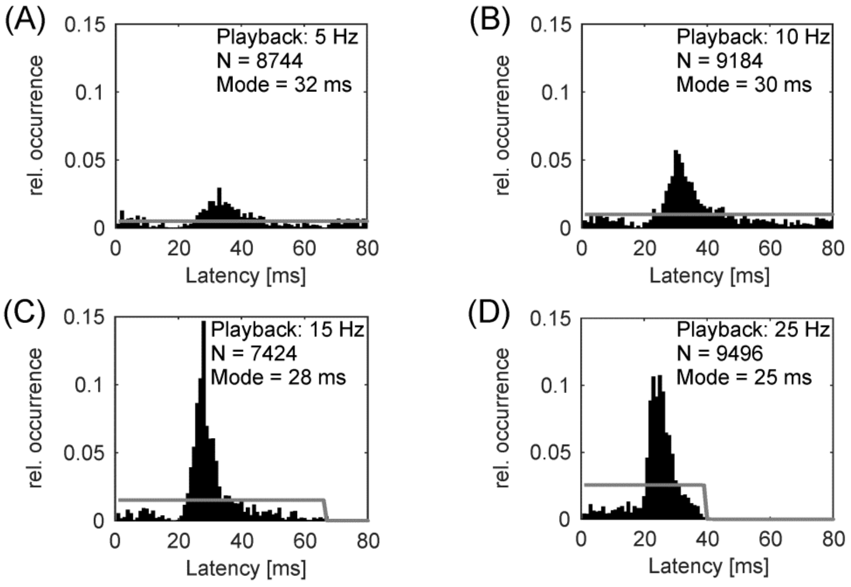

Figure 4.17: Echo responses to the constant-frequency electrical playback sequences. Preferred latencies occurred in response to all constant-frequency electrical playback sequences and were particularly abundant during the presentation of 15 Hz (C) and 25 Hz (D) playbacks. Echo responses to 40 Hz and 80 Hz could not be faithfully displayed because the IDI-duration that corresponds to these frequencies was shorter than the preferred latency with which the fish responded to the playback EODs. Insets identify the different playback frequencies. N = number of response EODs the respective diagram is based on. Mode = duration of the most abundant latency in milliseconds. Grey lines indicate the expected latency distribution based on the playback EOD-distribution. Bin size: 1 ms. Based on data from Toma (2014b).

response to the silent control condition C_1, and the vast majority was performed by the most dominant fish #1. A similar overall response pattern was detected for 'lateral probing' (Figure 4.20C), although these differences were not statistically significant ($\chi^2_{(7)} = 7.31$, $p = 0.40$). Both 'lateral-' (Figure 4.20D) and 'radial va-et-vient' (Figure 4.20E) were performed by all tested individuals, and occurred independently of the experimental condition ($\chi^2_{(7)} = 11.19$, $p = 0.13$; $\chi^2_{(7)} = 7.52$, $p = 0.38$). 'Head butts' directed at the dummy fish (Figure 4.20F) came almost exclusively from the most dominant fish #1, and most instances were observed in response to playback P_A, which featured discharge accelerations associated with aggressive behavior. Interestingly, most instances of touching the dummy fish were observed during the silent control C_1, although the overall model for 'touch' (Figure 4.20G) was not significant ($\chi^2_{(7)} = 11.14$, $p = 0.13$).

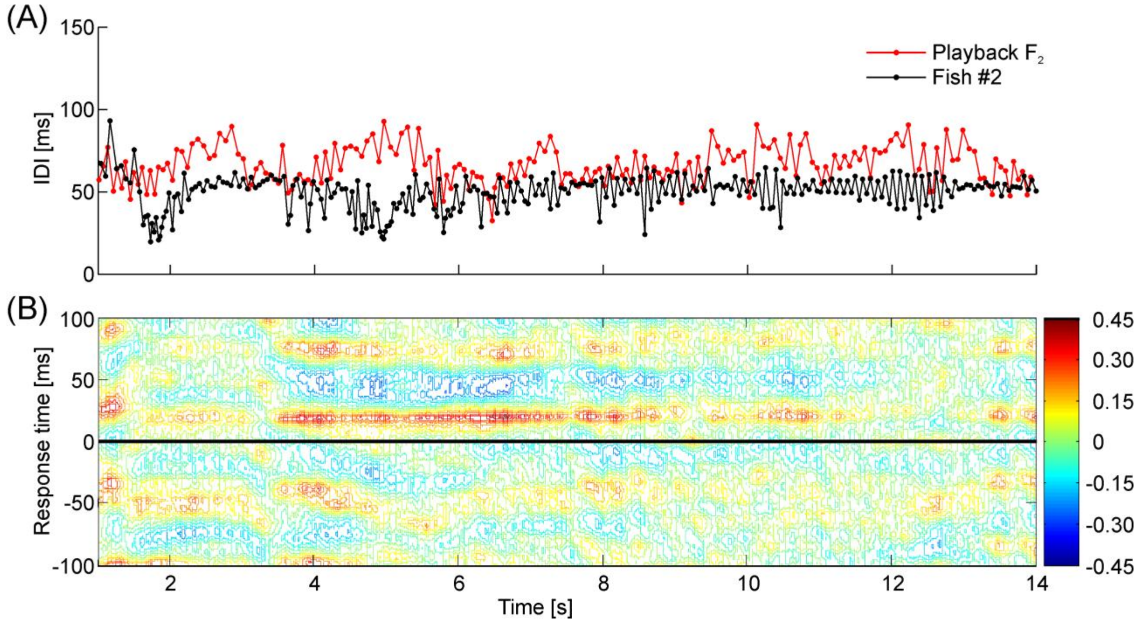

Figure 4.18: Adaptive cross-correlation analysis between pulse-sequences of playback and *M. rume*. (A) IDI-sequences of playback F_2 (red) and an exemplary response of fish #2 (black). (B) Cross-correlation diagram for the sequence shown in (A). Correlation coefficients are plotted color-coded for response times of *M. rume* ± 100 ms in relation to the playback signals over time. The red band at a response time of about 20 ms in the upper part of the diagram in (B) demonstrates a relatively high correlation between the discharges of fish #2 and the dummy at this latency and indicates that the fish synchronized its discharge activity to the playback for a period of several seconds.

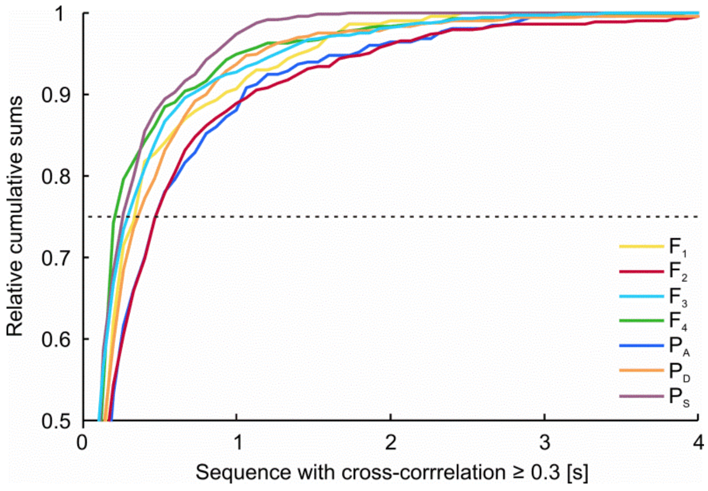

Figure 4.19: Sequences of electrical discharge interactions. Relative cumulative sums (RCS) of time periods with a cross-correlation coefficient ≥ 0.3. The graphs illustrate the proportions of sequences of a given length based on playback condition for temporal correlations between EODs of *M. rume* and the dummy. Statistical comparisons between the effects of the different playbacks were performed at an RCS of 0.75. At this value, 75% of all sequences were shorter than the x-axis intersection of their respective graph with the dashed line.

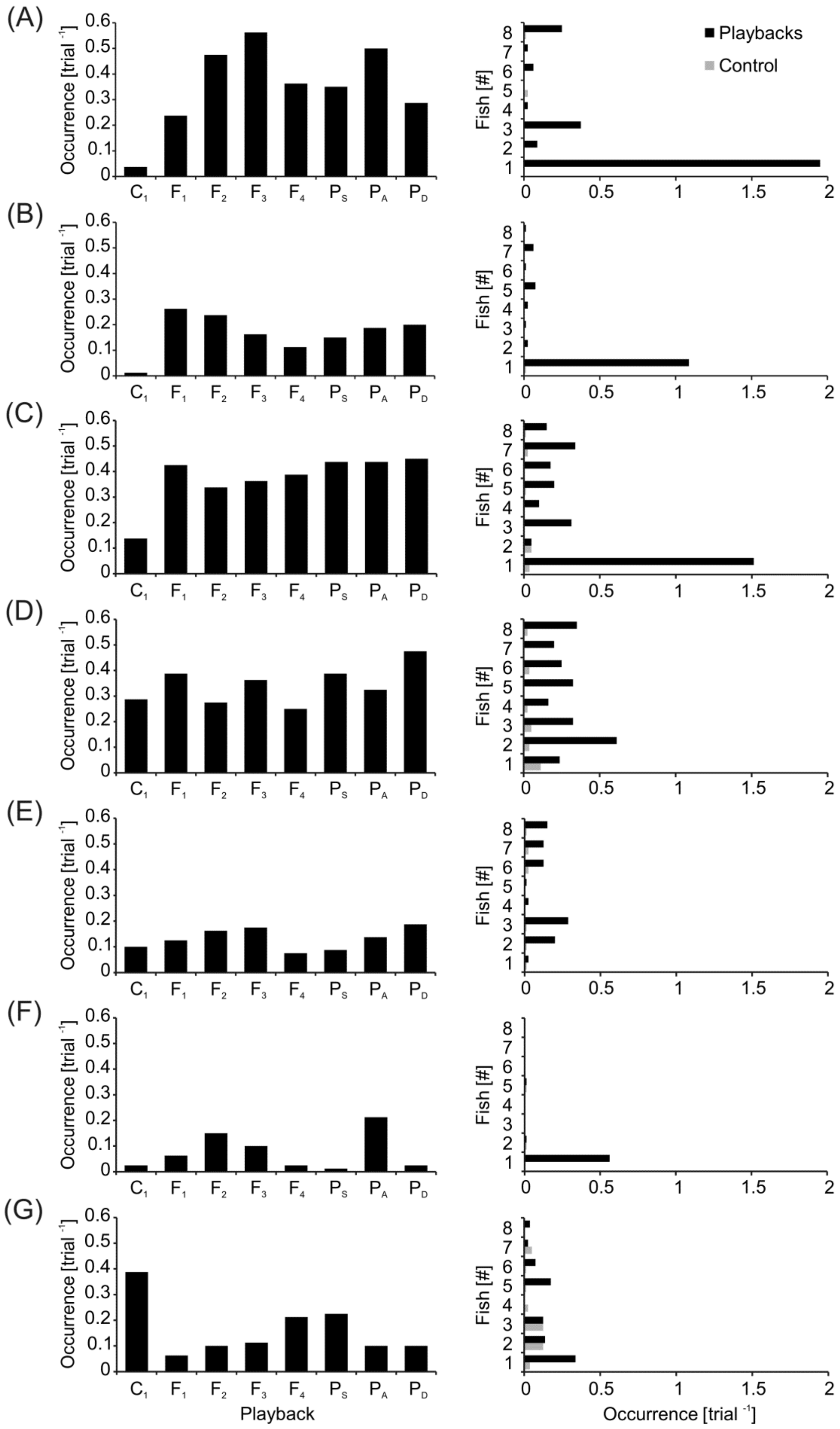

Figure 4.20: Motor behaviors in response to the dummy. (A) cut off, (B) circling, (C) lateral probing, (D) lateral va-et-vient, (E) radial va-et-vient, (F) head butt and (G) touch. The number of instances per trial depending on the test condition is shown on the left. On the right, the same number is resolved for all animals according to their hierarchy rank for all playback conditions (black) and the control C_1 (grey).

Distance and angular relationship between the dummy and the following *M. rume* were analyzed framewise over the time course of all experiments with the silently moving control C_1 and for playback F_2. The distance between the snout of the test fish and the closest point on the dummy is plotted for both conditions on the upper panel of Figure 4.21A. On average, fish followed faster (see Figure 4.3C) and closer during playback presentation compared to the control condition. Without playback presentation, the distance between fish and dummy was larger and consistently more variable, as indicated by the mean difference of standard errors in the lower panel of Figure 4.21A. After the dummy stopped moving, fish approached closer but swam away quicker during the control, whereas they stayed nearer to the dummy when it emitted electrical playback signals.

The position of the following fish from the dummy's coordinate system is visualized in Figure 4.21B by plotting the absolute angle between the dummy's direction of movement and the line connecting the centers of dummy and fish against the time course of a trial. While test fish tended to swim behind the dummy during the control condition, they followed on average more lateral and with a higher variability during playback presentation. The mean differences of means and standard errors depicted for both treatments in the lower panel suggest that these difference in following-behavior were consistent and depended on whether electrical playback signals were present or not.

Similarities and differences in following-behavior between individual fish are further emphasized by the trajectories shown in Figure 4.22. During playback presentation, the most dominant fish #1 (Figure 4.22A) showed numerous instances of circling the dummy both while it was moving and at its terminal position. Fish #1 always entered the testing area in parallel to the dummy's trajectory during the playback condition but moved along the trajectory when the dummy did not emit electrical playback. This latter behavior was particularly obvious in fish #3 (Figure 4.22B), which reproduced the dummy's trajectory quite closely during the control condition, but turned away and swam back to the living area soon after the dummy stopped moving. The lowest ranking fish #8 kept a larger distance to the dummy but approached closer during playback presentation than during the control condition (Figure 4.22C).

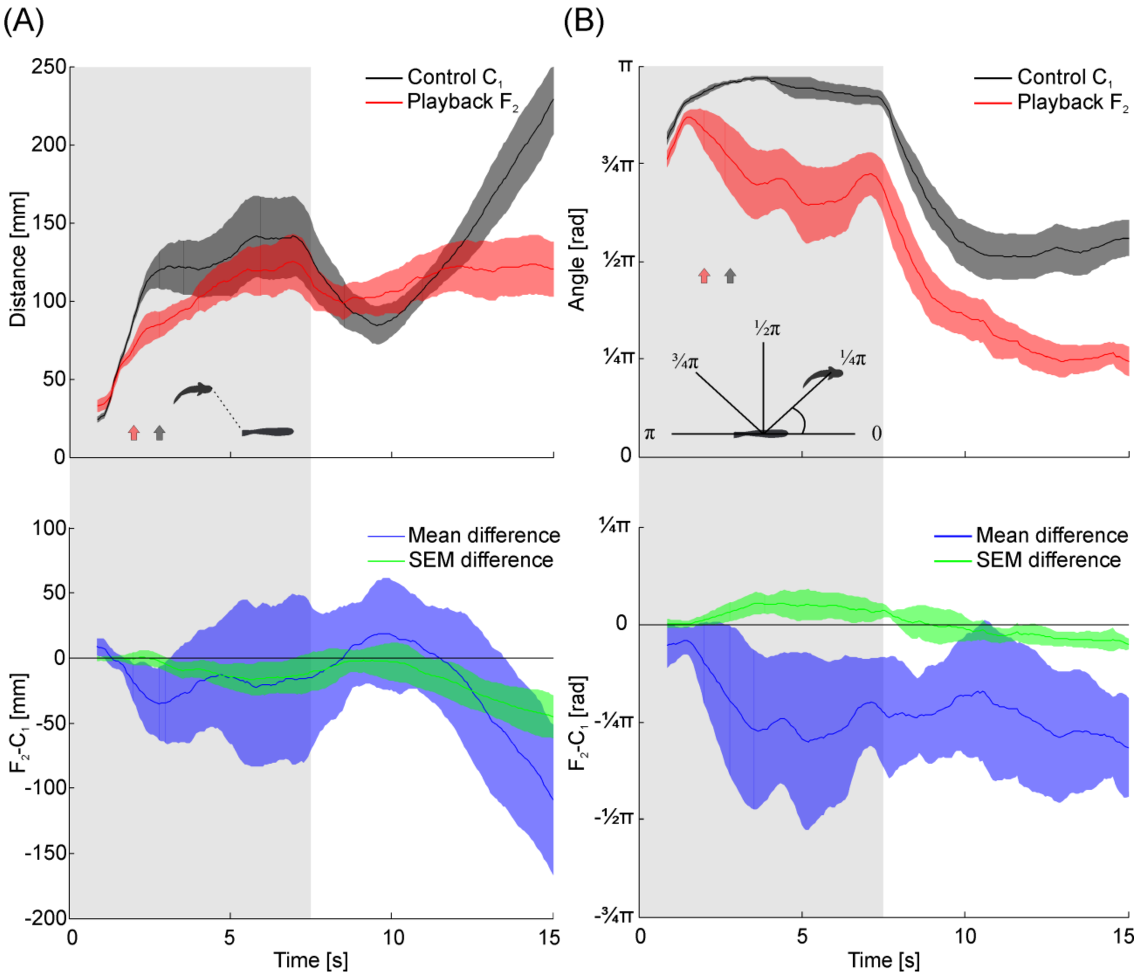

Figure 4.21: Spatial interactions of *M. rume* with the mobile dummy. (A) Distance between the focal fish's snout and the closest point on the dummy in the time course of all experiments with playback F_2 (red) and the silent control C_1 (black). Means and standard errors are depicted for all eight *M. rume* performing ten trials each. Differences between means (blue) and standard errors (green) between the two conditions are depicted in the section below, where 95% confidence intervals indicate that fish stayed longer in the vicinity of the dummy during playback presentation compared with the silent control C_1. (B) Absolute angular difference between the direction from dummy to focal fish and the dummy's orientation during the time course of all experiments with playback F_2 (red) and control C_1 (black). Mean values and the respective standard errors are depicted framewise. Differences between means (blue) and standard errors (green) between the conditions are presented with 95% confidence intervals in the section below, indicating that fish followed differently based on whether electrical playback signals were present or not. Arrows mark the average time when animals entered the testing area during playback presentation (red) and control (black). Shaded areas represent the time frame during which the dummy fish was moving.

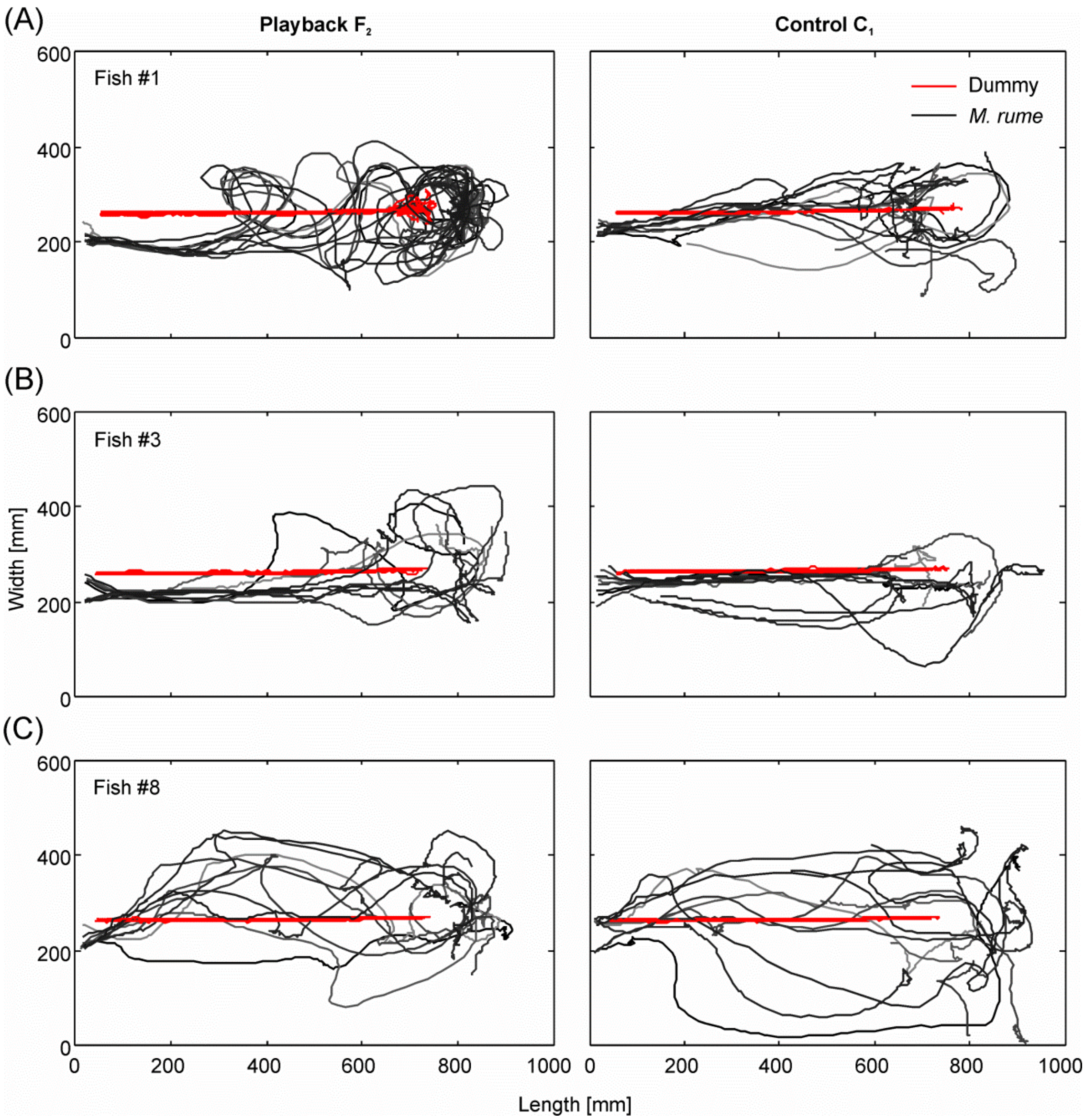

Figure 4.22: **Swimming trajectories.** Comparison of trajectories of dummy (red) and *M. rume* for ten trials (grey, graded to distinguish trajectories from different trials) per condition in three fish (#1, #3, and #8) in response to playback F_2 (left) and the electrically silent control C_1 (right).

The simultaneous recording of electrical discharges and swimming behavior allowed to associate interactive signaling activity during discharge synchronizations with the spatial parameters obtained from the swimming trajectories. The relative amount of correlation between the signals of *M. rume* and the mobile dummy was on average highest at a distance of approximately 90 mm during the presentation of playback F_2 (Figure 4.23A). The longest distance of 520 mm was recorded between fish #5 and the dummy. Correlation coefficients exceeding 0.3 occurred only up to a distance of 419 mm (fish #7, Figure 4.23B).

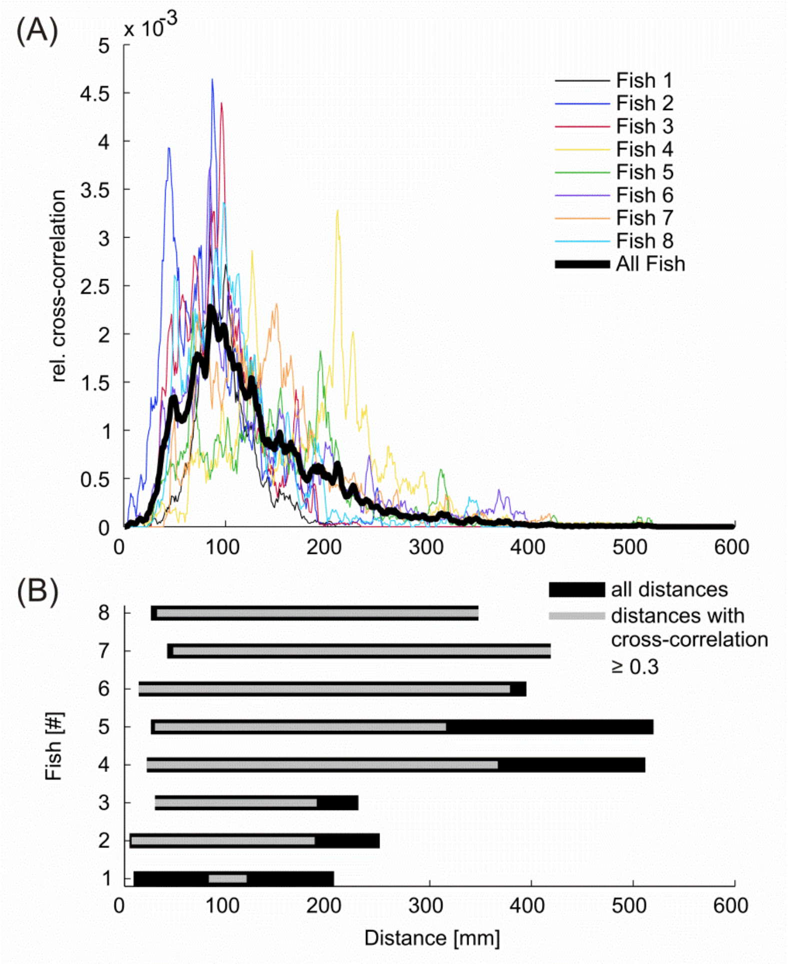

Figure 4.23: Influence of distance on interactive signaling. (A) Relative amount of correlation between discharge events of individual *M. rume* and playback F_2 depending on the distance between the animal's snout and the closest point of the dummy. On average (thick black line) correlation was highest when fish and dummy were approximately 90 mm apart. (B) The total range of distances kept between each fish and the dummy (black) is contrasted with the range of distances where correlations between the discharges of *M. rume* and playback F_2 exceeded 0.3 (grey).

4.4 Discussion

The results presented in this study provide new insights into the relationship between motor behaviors and electric signaling strategies in weakly electric fish. It was shown that a mobile fish dummy could recruit solitary individuals of *Mormyrus rume proboscirostris* from a shelter into an open area. This following-behavior was reliably observed in response to a variety of electrical playbacks with natural and artificial IDI-sequences and was, to a much smaller extent, also induced by the electrically silent control (Figure 4.3). It occurred despite the fact that visual perception of, and orientation towards the dummy were not possible because of the experimental design. During fish–dummy interactions, animals frequently displayed stereotyped motor behaviors towards the dummy, and some of these patterns were almost exclusively observed in fish that followed the dummy when it emitted electrical playback (Figure 4.20). This suggests that the playback-emitting, mobile dummy fish successfully induced an artificial social context, which can be used by scientists to reveal behavioral principles in standardized and controlled experiments on electrocommunication. The presence of electrical playback also affected the spatial relationship between *M. rume* and the mobile dummy during following-behavior, thereby providing clues concerning the sensory systems involved in the observed behavior. The shift towards a lateral following-position in the presence of electrical playback (Figure 4.21B) indicates that the perception of EODs via the knollenorgan pathway serves not only in detecting communication signals of other fish but also as an important sensory basis for spatial aspects of social interactions.

This study also demonstrates the electric signaling strategies based on discharge frequencies, patterns, and interactions that fish use when they follow an electrically signaling mobile dummy. The dummy emitted either natural electrical playback sequences or constant-frequency playbacks. The natural electric playback sequences used in this study were recorded from freely behaving individuals of *M. rume*, which were engaged in different behavioral contexts, such as aggressive interactions, hiding, foraging, slowly swimming and others. As a consequence, the sequences varied in average discharge frequencies (F_1–F_4) and temporal IDI-patterns (P_S, P_A, P_D). However, since these were exemplary recordings, one has to be careful to describe these sequences as typical for a specific behavioral context (Kroodsma, 1989; McGregor *et al.*, 1992).

Different playbacks did not lead to different inclinations of the fish to follow the dummy, with the exception of the low-frequency playback F_4. With this playback, lower-ranking individuals were less likely to be recruited, a correlation that was also observed for the electrically silent control C_1 (Table 4.1). A possible explanation for this correlation might be a potential relationship between dominance and personality traits of the tested individuals (Kareklas *et al.*, 2016). Hierarchy ranks of the fish were correlated with their size (Figure 4.2). Thus, animals with a bolder personality profile might have grown faster in a risk-free captive environment, and the same personality trait might have caused them to reacted with a stronger tendency to explore the dummy during the more subtle stimulation of the experimental conditions C_1 and F_4.

Mormyrids simultaneously employ their electrical signals for active electrolocation and electrocommunication, which means that electric signaling responses have to be discussed in both contexts. Overall discharge frequencies and IDI-distributions of the following fish were mostly unaffected by the sequence of the presented playbacks, but this was only the case during the experiments with natural discharge sequences. Similarly, interactive signaling, such as producing echo responses to the playback EODs, was observed as a response to all playbacks. However, context-dependent communication was obvious at the level of temporal pattern generation. By associating the electrical responses of the following fish with the relative dominance rank between individuals, particularly double pulses could be identified as a signaling pattern that was displayed with communicative intent.

A possible strategy for mormyrids to communicate behavioral states during electrocommunication could be to adopt a similar overall discharge behavior as a conspecific, which should become manifest in a shift of an animal's IDI-distribution towards the one emitted by the dummy. A multitude of studies on several mormyrid species has established that variations in overall IDI-distribution depend on activity level and behavioral context of weakly electric (Bell *et al.*, 1974; Gebhardt *et al.*, 2012a; Kramer, 1976a; 1978). In a study with the mormyrid *Gnathonemus petersii*, stationary playback electrodes were used to emit sequences that were pre-recorded in different behavioral contexts (aggression or resting). The receiving fish responded to these electrical playbacks with IDI-sequences of varying overall discharge frequencies (Kramer, 1979). In the current study, this was not the case when natural discharge sequences of varying average frequencies were presented but reliably occurred in response to playback of constant EOD frequencies within a naturally occurring range.

During the experiments with natural sequences, different playbacks did not lead to predictable differences in overall IDI-distribution of the following fish. Although individual differences in IDI-distribution occurred between individual *M. rume*, the resemblance of the overall distribution patterns for all fish was always closest to playback F_2, which was originally recorded from an *M. rume* following an electrically silent dummy fish (Figure 4.4). Only in the stationary control condition C_2 (Figure 4.5), there was a tendency of the fish to use longer IDIs and a broader interval distribution, suggesting a general effect of the moving dummy on discharge frequency and regularization that persisted independently of electric playback presentation during all experimental conditions involving the moving dummy. It therefore appears unlikely that in these experiments intentional communication of a particular behavioral context occurred at the level of overall discharge frequency. In all experiments, the dummy displayed a stereotypical, constant behavior of swimming in a straight line within 7.5 s from the starting to the end position and always at the same speed, regardless of the playback condition. After stopping, it continued emitting the particular playback sequence. As a consequence, a discrepancy might have occurred between the dummy's behavior and its EOD-signaling: even if the playback sequences contained information about the original behavioral context during the recordings, the behavior of the dummy was always just straight line swimming.

If the dummy were a real fish, and if its locomotor behavior corresponded to its signaling, the test fish might have also adjusted their overall discharge frequencies. Instead, they followed the dummy and emitted a typical 'following pattern,' which resembled the pattern F_2. They thus would have communicated their current behavioral state, which was 'following.' The results, therefore, suggest that IDI-distributions of the following fish were mainly determined by other needs, such as active electrolocation when following the dummy. Nevertheless, changes in overall discharge frequency may still provide eavesdropping individuals with information concerning a conspecific's current activity, which was invariable during the experiments.

Contrary to the experiments with natural playback sequences, constant playback frequencies of 10 Hz or higher had a strong influence on the IDI-modes of responding fish. IDIs corresponding to these discharge frequencies, or multiples thereof, were reliably adopted by *M. rume*, who either directly discharged at the same rate or responded with intervals corresponding to multiples of the playback frequency (Figure 4.6). This was especially apparent at frequencies of 15 and 25 Hz (Table 4.2), which is well within the range of average discharge frequencies emitted by active *M. rume* during swimming or feeding (Gebhardt, 2012). This range also corresponds to frequencies where regularizations of discharge activity occur in socially interacting *M. rume* (Kersten, 2017a; Kupschus, 2017; Pannhausen, 2017). Constant discharge frequencies between 15 and 25 Hz can thus be considered to be artificial examples of extremely strong discharge regularization and will be discussed as a potential signaling pattern (see below).

A second possible strategy in electrocommunication involves interactive signaling patterns for example in the form of echo-responses or discharge synchronizations, which could in turn also result in a similar IDI-distribution of the playback and the tested fish. All playback types elicited such interactive signaling responses in the recruited fish. The analysis of cross-correlations between playback pulses and the timing of EOD responses in *M. rume* showed that animals interacted electrically with the dummy largely independently of similarities between the IDI-distributions of fish and playback (Figure 4.18). While no differences between treatments remained after subtraction of randomly occurring correlations, and overall correlation coefficients were not very high in general, some playback patterns elicited on average longer periods of relatively high correlation compared to others (Figure 4.19). It is, therefore, possible to visualize the time course of EOD-synchronization and thereby conclude on the behavioral situations where they occurred. Most correlations were prominently found at a response time of approximately 20 ms after a playback EOD, which corresponds to previous descriptions of the latency of the echo response in *M. rume* (Gebhardt *et al.*, 2012b). In this study, preferred latency responses were observed in all tested individuals and in response to all presented playback patterns, although the degree of pronunciation was variable. In addition, all animals showed preferred latency avoidance within an interval directly preceding the echo response (Figure 4.15). This effect was not sex-specific and therefore stands in contrast to results by Lücker and Kramer (1981), who found that male and female *Pollimyrus isidori* reacted differently by displaying either a preferred latency response or preferred latency avoidance. Exhibition of preferred latency response and preferred latency avoidance has been reported to occur in both male and female *Mormyrus kannume,* although not within the same individuals (Orlov *et al.*, 2009).

The third and most obvious electrocommunication strategy in mormyrid weakly electric fish is to encode communicative intent into specific patterns within discharge sequences. Such patterns were represented in this study by discharge regularizations and double-pulse patterns. Regularizations of electric discharge activity have been suggested to increase the spatiotemporal resolution of active sensing and lead to constant sensory input at the receptor level, thus improving the performance of active electrolocation (Hofmann *et al.*, 2013; von der Emde, 1992). Regularizations have, however, also been described in a communicative context as a response to electrical stimuli (Moller, 1970) and as a reaction to stimulation with conspecific signals (Bauer, 1974; Moller and Bauer, 1973). While Moller (1970) hypothesized that regularizations improve active electrolocation, reports of regularized intervals during antagonistic behavior (Kramer and Bauer, 1976; Terleph, 2004) and during courtship and spawning (Baier and Kramer, 2007; Machnik and Kramer, 2011) suggest that this pattern may also have communicative value. IDI-regularizations were quantified using autocorrelation of intervals within a 200 ms time frame. With the exception of playback F_1, *M. rume* displayed stronger regularization in response to higher discharge frequencies contained in the natural playback sequences, which is similar to the findings by Moller (1970). The observations during the constant-frequency playback experiments, which elicited much stronger regularization compared with natural discharge sequences, emphasize this result. The fact that regularizations were strongest in response to 15 Hz and 25 Hz playbacks (Figure 4.12) suggests that fish regularized their IDIs in response to perceived regularization by a conspecific within a behaviorally relevant range.

Quantification of regularization over time allowed distinguishing between the effects of the dummy's movement and the presentation of various electrical playbacks on the propensity of *M. rume* to regularize IDIs. Since the strength of regularization peaked after the offset of playback presentation, it seems unlikely that the observed behavior is solely performed to improve active sensing. It appears therefore plausible to presume communicative intent associated with strong regularization patterns in a social context. A possible social function of discharge regularization could be an overt assessment of a potential opponent through active electrolocation (Terleph, 2004). It should be noted in this context that the presented playbacks did not include a natural regularization pattern.

The communicative nature of double pulses is less ambiguous than that of simple discharge regularizations. Double-pulse patterns have been described as alternating long and short IDIs in several mormyrid species and can be classified as a form of regularization themselves (Bauer, 1974). They have mainly been observed within antagonistic contexts and during aggressive behavior in *G. petersii* (Bauer, 1972; Bell *et al.*, 1974; Kramer and Bauer, 1976), and are considered to be aggressive threat signals, which were also displayed by nest-guarding males in two *Pollimyrus* species (Baier and Kramer, 2007). In *M. rume,* it has previously been observed that solitary individuals emitted double-pulse patterns only in response to electrical playback presentation (Gebhardt, 2012). The present study confirms this result by demonstrating that double pulses were virtually absent in response to the electrically silent control condition (Figure 4.8). Additionally, most double pulses were emitted in response to the playback pattern P_D, which also contained double pulses. Since the emission of double pulses was subject to habituation (Figure 4.10), and there appears to be no obvious advantage for active electrolocation, it is suggested that this pattern serves as a threat signal in *M. rume* as well. However, the function of this threat signal may rather relate to claiming dominance at the beginning of a sequential assessment strategy (Enquist and Leimar, 1983) than to overt aggression. This assumption is supported by the observation that higher-ranking individuals produced more double pulses than lower-ranking fish (Figure 4.9). Although the observation periods in this study were too short to observe the processes underlying hierarchy formation, these results open the opportunity to test the communicative value of this signal pattern by systematic variation of its properties in controlled playback experiments or dyadic contests.

Such experiments have in the meantime been performed by Kersten (2017a), who staged dyadic contests between similarly sized *M. rume* and quantified the animal's locomotor behavior and electric signaling activity during successive stages of agonistic encounters. In these experiments, animals competed over a single shelter for 15 minutes, which were subdivided into three periods of equal duration for analysis. Electric signaling, including regularization, discharge cessations, and double pulses were quantified for 15 seconds at the beginning of each evaluation period. In agreement with the predictions of a sequential assessment strategy, Kersten (2017a) found that the number of lateral displays engaged in by the opponents declined during the contest, whereas more aggressive displays like chasing and head butts increased in number. Electric signaling displays also changed in the course of the contest situation. Regularizations were frequent at the beginning of an encounter, whereas the number of short cessations, which were almost exclusively produced by the winner of a contest, increased towards the end. Double pulses were most frequently produced at the beginning of a contest, and the abundance of this signaling pattern declined during later stages (Figure 4.24). Interestingly, individuals who later won the contest produced significantly more double pulses in the first phase of the competition, compared with those who later emerged as losers (Figure 4.24). The number of double pulses generated at the beginning of a conflict could thus predict which individual was going to win the contest. Taken together, these findings support the hypothesis that double pulses are a conventional treat display that is used by *M. rume* to signal aggressive motivation at the beginning of the sequential assessment of a competitor.

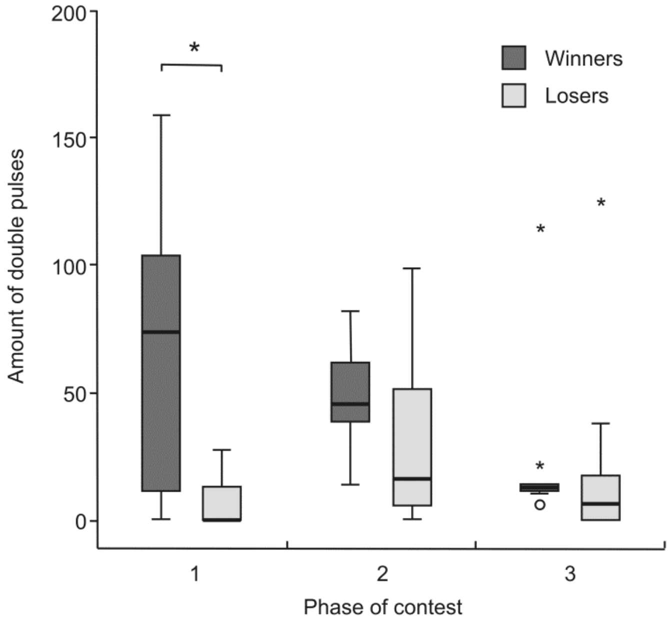

Figure 4.24: Double-pulse signaling in dyadic contests. Box plots depicting the number of double pulses generated by $n = 9$ pairs of similarly sized *M. rume* that were competing for a shelter during three phases of equal duration. The number of double pulses decreased in the course of the encounter. Individuals who later won the contest (dark boxes) generated significantly more double pulses at the beginning of the contest than those who later lost (light boxes). Wilcoxon signed-rank test, $Z = -2.31$; $p = 0.021$. Modified from Kersten (2017a).

Communication displays ultimately aim at triggering behavioral responses of the receiving party and may thereby initiate some form of physical interaction between signaler and receiver. The motor patterns displayed by *M. rume* towards the mobile dummy fish lend further support to the idea that interactive behaviors between live fish and a playback emitting dummy can be used as a proxy for the investigation of social behavior in mormyrids under controlled experimental conditions (see also Moller and Serrier (1995)). Trajectory 'cut offs,' complete 'circling,' and 'head butts' were hardly directed at the electrically silent dummy. This shows that appropriate locomotor communication behaviors are only provoked by dummies emitting EODs (Figure 4.20). Both 'head butts' and 'circling' have previously been described in social interactions between mormyrids (Kramer, 1976a). The results of this study thus provide a framework for further studies involving interactive playback patterns, as well as more complex trajectories (Worm *et al.*, 2014) (see chapters 5 and 11).

The inter-individual distance at which weakly electric fish produce a signal may also allow to conclude on whether its function relates to active electrolocation or electrocommunication. The active range of electrocommunication has been inferred from experiments (Moller and Bauer, 1973; Moller *et al.*, 1989) and extends beyond the limits of active electrolocation due to the high sensitivity of the knollenorgan receptors (Bennett, 1971c). Double pulses and high correlations with the playback signals emitted by the mobile dummy occurred up to a distance of 287 and 419 mm (Figure 4.23), respectively, which is approximately within the range where discharge cessations were observed in response to an approaching conspecific in *Brienomyrus niger* (Moller *et al.*, 1989) (see chapter 9 for similar results involving double pulses in *M. rume*). The highest amount of both signaling types, however, was most prominent at a distance of 90–100 mm, which corresponds to the outer limit of active electrolocation (Moller, 1980).

The ability to locate the source of a signal is crucial if the objective of communication is to initiate social interactions. Similar studies aiming at manipulating the behavior of other, non-electric fish species by using mobile dummy fish have mainly relied on visual cues, or at least made no explicit assumption concerning the sensory systems involved in triggering the observed behavior (Butail *et al.*, 2013; Faria *et al.*, 2010; Ruberto *et al.*, 2016; Ward *et al.*, 2008) (see Bierbach *et al.* (2018) for a recent exception). Since all experiments with *M. rume* in this study were performed in darkness with only infrared illumination, vision can be excluded to have mediated following-behavior (Ciali *et al.*, 1997; Kreysing *et al.*, 2012).

Although not much is known about its efficiency, mormyrids also possess a functional lateral line system (Szabo, 1965) (see Schumacher (2017) for anatomical evidence for a reduced functionality in *G. petersii*). However, the fact that *M. rume* tended to follow right behind the mobile dummy during the silent control C_1, often reproducing the dummy's trajectory (Figure 4.22), suggests an involvement of hydrodynamic cues in following-behavior (Pohlmann *et al.*, 2001). Lateral line information has been demonstrated to play a role in shoaling behavior (Partridge and Pitcher, 1980; Pitcher, 1979), and hydrodynamic cues produced by robotic fish have been shown to influence swimming preferences in individual fish (Marras and Porfiri, 2012; Polverino *et al.*, 2013). In the present study, animals also had their active electric sense at their disposal, which could have been used to detect the dummy within the range of active electrolocation (von der Emde *et al.*, 2010). The fact that fish were following the EOD-emitting dummy mainly in a lateral position (Figure 4.21B) suggests that electric signals may be a natural determinant of spacing between mormyrids (Moller *et al.*, 1982), and that passive electroreception, i.e., the perception of the EODs of a conspecific, may be more relevant for following than hydrodynamic sensing and active electrolocation. This assumption is supported by the findings of Schluger and Hopkins (1987), who demonstrated that weakly electric fish navigate along the electrical field lines to approach an electrical dipole source such as a conspecific individual emitting EODs. This ability will be investigated in greater detail in chapter 9.

Given the many overlaps of electric signaling behaviors and motor response patterns that are directed either at inanimate objects during active electrolocation or towards conspecific individuals during social encounters, it may on many occasions be neither possible nor reasonable to attempt assigning a particular behavior exclusively to either active electrolocation or electrocommunication. Lateral probing during active electrolocation and circling during social interactions may not be fundamentally different behaviors (Crockett, 1986), and it is easy to conceive how regularization patterns, which may have evolved to improve active sensing, take over some communicative function (see also section 6.2). A similar transition from a pure electrolocation feature to a system involving a communicative function could have occurred for interactive signaling patterns. Echoing, which can be a means to avoid the jamming of an animal's sensory perception during active electrolocation (Heiligenberg, 1976), also leads to synchronized bursts between individuals and thus may serve in mutual recognition and group coherence (Arnegard and Carlson, 2005). Synchronization of EOD timing with a conspecific may, therefore, be a means to address another individual without impairing the functionality of active electrolocation in the process. In section 6.3, this argument will be expanded based on the results of chapters 5 and 11.

Communication systems can develop over evolutionary time when sensory cues, inadvertently generated by animals without communicative intent, allow conspecific individuals to predict the behavior of the animal generating the cue by exploiting pre-existing sensory systems (Bradbury and Vehrencamp, 2011). Although encoding 'conventional signals' (Guilford and Dawkins, 1995; Maynard Smith and Harper, 1988) into IDI-sequences appears plausible from a theoretical point of view, the actual amount of distinct signal patterns that can be produced may be limited due to the properties of the nuclei involved in central pattern generation in the mormyrid brain (Carlson and Hopkins, 2004a). The difficulty in isolating unequivocal communication features from overall IDI-distributions, as well as the sometimes gradual transition between electrolocation and electrocommunication signals, emphasize the dual nature of electrical signaling in weakly electric fish. Similarly, in bats, dual functions of vocalization for both echolocation and social communication have recently been reported (Bohn and Smotherman, 2015; Jones and Siemers, 2011; Knörnschild *et al.*, 2012). Between simple eavesdropping, during which individuals could deduce a conspecifics behavior by monitoring its discharge rate and encoding conventional information into stereotyped IDI-patterns with communicative intent, electrocommunication may rely on more subtle interactions whose true significance has yet to be uncovered.

5. Project 2: Evidence for Mutual Allocation of Social Attention through Interactive Signaling in a Mormyrid Weakly Electric Fish

This chapter, as well as some of the arguments outlined in section 6.3, are the basis for:

Worm, M., Landgraf, T., Prume, J., Nguyen, H., Kirschbaum, F. and von der Emde, G. (2018). Evidence for mutual allocation of social attention through interactive signaling in a mormyrid weakly electric fish. *Proceedings of the National Academy of Sciences* (Accepted).

5.1 Introduction

Mormyrid weakly electric fish produce series of electric organ discharges (EOD) for active electrolocation of their environment (von der Emde, 1999) and electrocommunication with nearby conspecific individuals (Hopkins, 1988). Inter-discharge intervals (IDI) between EODs are variable and can be modified to spontaneously improve the temporal resolution during active sensing (Post and von der Emde, 1999) and to encode signaling patterns into discharge sequences that are associated with characteristic behavior patterns and serve in intra-specific communication (Carlson and Hopkins, 2004b).

Apart from spontaneous changes in discharge frequency and temporal patterning, mormyrids can also produce interactive IDI-sequences. By responding to a conspecific's EODs with a preferred latency of only a few tens of milliseconds, they generate so-called echo responses, which, if mutually engaged in by two individuals, lead to synchronization episodes with time-locked signaling sequences between individuals (Gebhardt *et al.*, 2012a; Kramer, 1974; Russell *et al.*, 1974). Although echoing is a behavior consistently observed across mormyrid species, the underlying neural pathways are unresolved, and its behavioral significance remains speculative. Echo responses have been interpreted either as a form of jamming avoidance behavior during active electrolocation (Heiligenberg, 1976; Schuster, 2001) or as a communication strategy, possibly by functioning as a social signal enhancing group integration and affirmative interactions (Arnegard and Carlson, 2005; Gebhardt *et al.*, 2012b; Russell *et al.*, 1974).

Systematic investigation of the implications of echoing for social communication is impeded by the difficulty to assign EODs to the respective sender individual in experiments involving more than a single freely moving fish, as well as by the lack of control over the behavior of the fish that invokes echo responses from a conspecific. Here, both problems were solved by using a freely moving, mobile dummy fish capable of emitting predefined or dynamic sequences of electrical playback EODs in an interactive behavioral experiment with single individuals of the weakly electric fish *Mormyrus rume proboscirostris*.

Robotic fish dummies have been successfully employed to investigate the features determining attraction between individual fish (Abaid *et al.*, 2012; Landgraf *et al.*, 2016; Marras and Porfiri, 2012; Polverino *et al.*, 2013), as well as collective decision making and internal dynamics in shoals of different sizes (Bonnet *et al.*, 2018; Bonnet *et al.*, 2016; Butail *et al.*, 2013; Cazenille *et al.*, 2018; Faria *et al.*, 2010; Kruusmaa *et al.*, 2016; Landgraf *et al.*, 2014; Swain *et al.*, 2012). Similar experiments have demonstrated that mormyrids are attracted to follow and interact with a mobile dummy fish based on playback of electric signaling sequences (Donati *et al.*, 2016; Worm *et al.*, 2017) (see chapters 4 and 8). Such interactions can in principle rely solely on passive electroreception (Worm *et al.*, 2018) (see chapter 9). Electrical playback signals are a convenient way to experimentally control electric signaling properties with respect to EOD-waveform, temporal discharge patterns and latency relationships. This allows to assign such signaling attributes to a behavioral context, and thus to uncover the significance of electrical signaling for communication (Hanika and Kramer, 2005; Kramer, 1979; Schuster, 2001; Teyssedre and Serrier, 1986).

In this study, a freely moving dummy was used, which could not only interact physically with live fish but was also able to produce echo responses to the fish's EOD. Closing the feedback loop at the two levels of spatial interactions and electrical signaling made it possible to isolate the effect of electrical signaling on attraction and interactive behavior. On the spatial level, following-behavior was primarily mediated by electrical signaling but occurred independently of playback type. However, electric signaling interactions of *M. rume* with the mobile dummy were enhanced when the playback allowed synchronization of discharge sequences through artificial echo responses, indicating that interactivity has some intrinsic communicative value as a signal. Based on the observation that such synchronizations were associated with following-behavior and approach configurations, it is suggested that echoing provides a relatively simple electromotor mechanism to address another individual electrically. Synchronizing discharges through mutual echo responses in mormyrids may thus serve a communicative function in integrative behavioral contexts, and constitute a strategy to mutually allocate of social attention.

5.2 Materials and methods

5.2.1 Animals

A total of 23 *Mormyrus rume proboscirostris* were kept in 50–200 L holding tanks under tropical conditions with temperatures around 25°C, a water conductivity of approximately 100 µS cm^{-1}, and a light/dark cycle of 12/12h. Animals measured between 6.4 and 11.4 cm (standard length) and had previously been used in the experiments described in (Worm *et al.*, 2018) (chapter 9). Within each holding tank, two or more individuals were confined to separate compartments providing a shelter. These compartments were divided by water permeable barriers that prevented physical contact but allowed electrocommunication. Food was provided at least five times a week in the form of defrosted *chironomid* larvae. All experiments were carried out in accordance with the guidelines of German law, with the animal welfare regulations of the University of Bonn, and with the 'Guidelines for the treatment of animals in behavioural research and teaching' (ASAB, 2006).

5.2.2 Experimental setup

Experiments were performed in a 120 cm x 100 cm x 20 cm tank, which was mounted on top of a metallic support frame, leaving the base area accessible from below (Figure 5.1). The tank walls were covered from all sides with white cardboard, and the base plate was covered with white self-adhesive foil on the inside. The water within the tank was filtered and heated to a temperature of 26 ± 1°C, and water conductivity was adjusted to $100 \pm 5\ \mu S\ cm^{-1}$ for all experiments. Filter and heater were removed before every experimental session, and the water level within the tank was kept at 15 cm. The frame supported a second plane 16 cm below the tank, which was made from a 10 mm Plexiglas board. A wheeled robot (Figure 5.2A) (Landgraf *et al.*, 2012a) was placed on the level below the tank, where it could be manually steered from a PC to move on arbitrary trajectories using custom-written software (Hai Nguyen, Freie Universität Berlin) via a wireless connection. A dummy fish was made from an 8 cm fishing bait that was mounted on a small base plate with a small magnet glued underneath. The dummy was provided with a pair of carbon electrodes inserted into the rubber at the snout and the rear end, as well as a pair of silver electrodes, which stuck out dorsally along the longitudinal axis (Figure 5.2B). From the center of the robot, a neodymium magnet was held up to the bottom of the tank, where it coupled to the magnet glued underneath the base plate of the dummy. Thus, the dummy fish within the tank reproduced the trajectories of the remote controlled moving robot underneath the tank.

A multi-electrode array consisting of five pairs of carbon electrodes was evenly distributed inside the tank and recorded all electrical activity independently of fish position. Signals were recorded using differential amplification (Brownlee Precision Model 440, Palo Alto, CA), digitized (CED Power 1401, Cambridge Electronic Design, Cambridge, UK), and recorded to disk using Spike2 software (Version 5.21, Cambridge Electronic Design, Cambridge, UK). All behavior within the tank was simultaneously recorded at 15 fps using an infrared sensitive video camera (DMK 23FM021 FireWire Camera with Vari Focal T4Z2813CS-IR CCTV Lens, The Imaging Source, Bremen, Germany) and the Spike2 Video Recorder. Illumination was provided indirectly using a LED floodlight resulting in 1.5 lux of visible light intensity (Light ProbeMeter™, 403 125, Extech Instruments) at the water surface in the center of the tank. Camera vision was enhanced by additional illumination with infrared spotlights (850 nm, IR Illuminator Model SA1-60-C-IR, Itakka, Wattens, Austria).

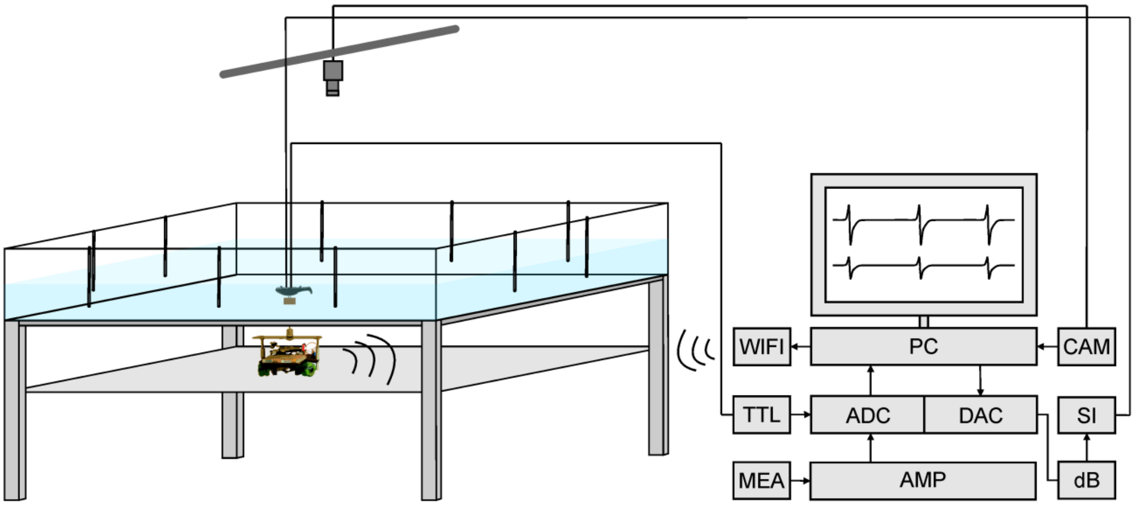

Figure 5.1: Illustration of the experimental setup and technical components. The robot moved on the level underneath the tank, thus defining the trajectory of the dummy via a magnetic coupling. Electric signals and motor behavior were recorded by an array of five pairs of electrodes in the tank and a video camera from above. MEA: Multi-electrode array. AMP: differential amplifier. ADC: analog to digital converter. TTL: Trigger box generating a TTL pulse for each EOD registered at the dummy's recording electrodes. CAM: video camera. PC: computer for data acquisition, playback output and control of the robot. WIFI: wireless control of the robot's trajectory. DAC: digital to analog converter. dB: attenuator. SI: analog stimulus isolation unit powering the electrical playback signal.

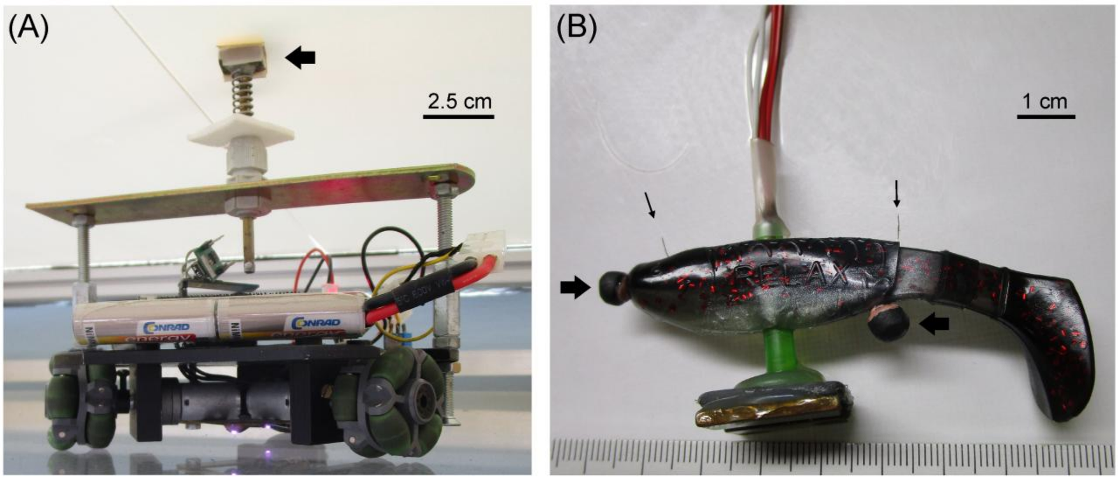

Figure 5.2: Components of the mobile dummy fish. (A) Remote controlled robot on the plain underneath the experimental tank. (B) Dummy fish mounted on a magnetic base plate, incorporating a pair of playback electrodes (thick arrows) and a pair of trigger electrodes (thin arrows). Wires connect the electrodes to the battery-driven SI-unit (red) and the trigger box (white).

The silver electrodes of the dummy were used to record signals of the fish when it came into close range of the dummy. These signals were amplified differentially using a custom build trigger-box (University of Regensburg), which generated a TTL pulse for each signal exceeding a threshold determined by amplification. The TTL-output of the trigger-box was connected to the event input of the CED 1401 and signals were used to generate interactive electrical playback involving the mormyrid echo response in real-time via the Spike2 sequencer (see below).

The dummy's carbon electrodes were used for playback generation and were connected to a stimulus-isolation unit (model 2200, A-M Systems Inc., Carlsborg, WA) as a power supply. Playback signals were output via the Spike2 sequencer, converted from digital to analog using the CED 1401, and attenuated (dB-attenuator, University of Regensburg) to match the EOD-amplitude of a living fish of medium size. The key components of the experimental setup are illustrated in Figure 5.1.

5.2.3 Playback sequences

Two types of electrical playback sequences were generated using a pre-recorded template EOD that was averaged from 50 EODs of an *M. rume*, which were recorded head-to-tail (high-pass: 1 Hz) and digitized at a sampling rate of 50 kHz. Static random playback sequences were generated using a custom-written script for Matlab (Version R2013b, The MathWorks Inc. Natick, MA), which concatenated template EODs to sequences of 15 seconds. IDIs were randomly selected within two standard deviations around the mean (67 ms) of a distribution with a mode of 60 ms that was obtained from a similar experiment and contained a total of 17644 IDIs. Random playbacks were repeated three times to obtain a 45-second stimulus protocol, and a new sequence was designed for every trial. Dynamic echo playbacks were generated by programming the Spike2 sequencer to produce playback signals at intervals greater than 60 ms in the absence of a trigger signal but respond with a latency of 21 ms to the detection of a fish's EOD by the trigger electrodes of the dummy. A refractory period was included to prevent the program from echoing to its own signals. The flowchart (Figure B.1) in Appendix B illustrates the routine for dynamic playback generation.

5.2.4 Experimental protocol

Individual fish were fetched from their holding tanks and placed inside a 22 cm x 14 cm opaque start box inside the experimental tank. The dummy was then moved on random trajectories within the tank for 3 minutes to habituate the fish to any disturbances associated with the movement of the dummy and the robot on the level below. Test fish were then released from the start box and confronted with the mobile dummy in three consecutive trials featuring either static playback with random intervals, dynamic playback imitating the mormyrid echo response, or no playback as a control. The order in which these conditions were presented were pseudo-randomized. The dummy was moved by the experimenter on arbitrary trajectories that were designed to approach the fish and entice it to follow into the open area of the tank. Each presentation started with a 10 s period without electrical playback, followed by three 15 s episodes where the respective condition was repeated, resulting in a total of 55 s of recorded data. Episodes were marked by a 100 ms infrared light flash that assured synchrony between video recording and waveform data. Alternatively, the mobile dummy was removed after the habituation period, and the behavior of the fish after release from the start box was recorded according to the time points defined for playback presentation without any interference. This second control was performed with all individuals in a separate experimental session on a non-consecutive day. Half of the animals were subjected to this control in the first session, while the other half were first confronted with the moving dummy. The experimental protocol also involved trials with groups of two, three, and four individuals of *M. rume*. The results of these experiments will be presented in chapter 11.

5.2.5 Data analysis

Videos were rectified to compensate for radial distortion and tracking was performed using Ctrax (The Caltech Multiple Walking Fly Tracker, version 5.0, (Branson *et al.*, 2009)) as well as the provided FixErrors GUI for Matlab. Real world distances were calculated from pixel differences using a known distance within the tank as a reference. The center distance between fish and dummy, their difference in orientation, as well as the relative position of the fish from the dummy's coordinate system (and vice versa) were calculated using the BehavioralMicroarray toolbox for Matlab provided with the Ctrax software. This relative position was defined by the angular deviation between the dummy's direction of movement and the connecting line between the centers of dummy and fish. Swimming speeds of dummy and fish were calculated from the real world coordinates and smoothed using a sliding average of three adjacent values to mitigate artifacts from tracking at a relatively low frame rate of 15 fps. A characterization of swimming speeds can be found in Appendix B (Figure B.2). Distances from the snout of the fish to the closest wall of the tank, as well as the closest distance to the dummy were manually assessed every three seconds using ImageJ (version 1.46r, National Institutes of Health, USA). The resulting 15 values were averaged to obtain a single value per fish for further statistical analysis. The number of turns performed by the dummy was counted manually from video recordings, and the proportion of turns that were followed by the fish was calculated for each condition. Fish were counted as following a turn only if they had been swimming in the same direction as the dummy both before and after the latter changed its direction of movement. All videos were renamed and randomized for this analysis to leave the experimenter blind to the experimental condition in question.

Spike2 waveform data were transformed into time series by marking the occurrence of all EODs, which were subsequently assigned to either the playback sequence or the tested fish. IDIs were calculated separately for the two resulting time series to visualize the respective signaling sequences and for comparison of interval distributions across experimental conditions. IDIs of all fish were pooled into a single histogram per condition for visualization, using a bin size of 2 ms. Distribution modes were extracted for each fish and test condition for further statistical analysis.

Echo responses displayed by the fish were analyzed by using the EOD-sequence of the playback as a reference and calculating the latencies with which the fish generated EODs in response to the stimulus until the occurrence of the next playback EOD. Results were visualized by plotting the relative occurrence of all observed latencies. The latency distribution that would be expected if both IDI-sequences were independent time series was obtained by inverting the relative cumulative histogram of the stimulus IDI-distribution. The definition of stimulus and response signals was also switched to check for echo responses of the playback sequence to the fish's signals. Echo responses were quantified according to (Kramer, 1974) by calculating the ratio of observed latencies at the mode of the latency distribution to the amount of EODs that would be expected at that latency if assuming no dependency between the IDI-sequences of playback and fish. This ratio was obtained for all fish and compared statistically between the two playback conditions.

Adaptive cross-correlations for a response window of ± 100 ms were calculated between two IDI-sequences each, using the playback signals as reference values. Calculations were performed according to the procedure described in Gebhardt *et al.* (2012a) (see also section 4.2.7). The IDI-sequences of playback and fish were transformed into high-resolution time series comprising a value for each millisecond of a trial. The intensity of temporal synchronization between IDI-sequences was quantified over the time course of the experiment via correlation coefficients and was visualized using color-coded contour plots. For each of the high-resolution time points, the maximum correlation value within the 100 ms response-time frame was extracted for correlations of the fish's signals with the IDI-sequence of the playback, as well as for correlations of the playback signals with the IDI-sequence of the fish. The average of these maximum values over the 45-second period was calculated for each experiment and used for further statistical analysis. As a control for randomly occurring correlations, cross-correlation analysis was performed for both playback types by using the IDI-sequence the fish had emitted independently of the playback during the control trial involving the electrically silent dummy in the same session.

Values of maximum correlation from the high-resolution time series were averaged to obtain single values matched to the corresponding video frames. For both the static random playback and the dynamic echo playback, sequences of successive frames, for which the assigned value of correlation of the fish's electric signaling response reached or exceeded 0.3, were quantified and used to calculate the length of synchronization episodes. Relative cumulative histograms were used to determine the duration of synchronization sequences at a proportion of 0.75 for statistical comparison of the effect of the two playback conditions on the duration for which the fish synchronized their electrical discharge activity to the respective playback.

Simultaneous tracking and electric signaling data were used to associate the linear and directional relationships between *M. rume* and the dummy to the amount of discharge synchronization the ish engaged in at a given time defined by the frame rate of video recording. This analysis was only performed for the static random playback because electric signaling sequences of the dummy during the dynamic echo playback cannot be considered independent of the signaling activity of the fish.

Statistical analyses were performed using IBM SPSS (Version 22.0, IBM Corp., Armonk, NY). Normality of the data was assessed by Shapiro-Wilk's test, and parametric or non-parametric tests for repeated measurements were used accordingly. Circular-linear correlations between the magnitude of EOD-synchronization and the angular relationships of fish and dummy were calculated using the CircStat toolbox for Matlab provided by Berens (2009). Statistical significance was accepted at the $\alpha = 0.05$ level.

5.3 Results

All animals were highly attracted by the mobile dummy fish and showed interactions both by following the dummy's trajectories, as well as by synchronizing their electrical discharge activity to the playback sequences through echo responses to the playback EODs. The attraction was particularly strong when the dummy emitted electrical playback. Swimming behavior of *M. rume* in the absence of the mobile dummy was very stereotyped. After the start box was removed, animals spent the majority of time closely following the tank walls (Figure 5.3A), inspecting the multi-electrode array, and frequently swam backward during the whole 45-second observation period. Wall-following was also observed during the control trials with the electrically silent dummy, but animals occasionally also approached and followed the dummy (Figure 5.3B). During electrical playback presentation of static random IDI-patterns (Figure 5.3C) and interactive echo responses (Figure 5.3D), *M. rume* virtually abandoned wall-following behavior and instead followed closely on trajectories resembling those of the moving dummy.

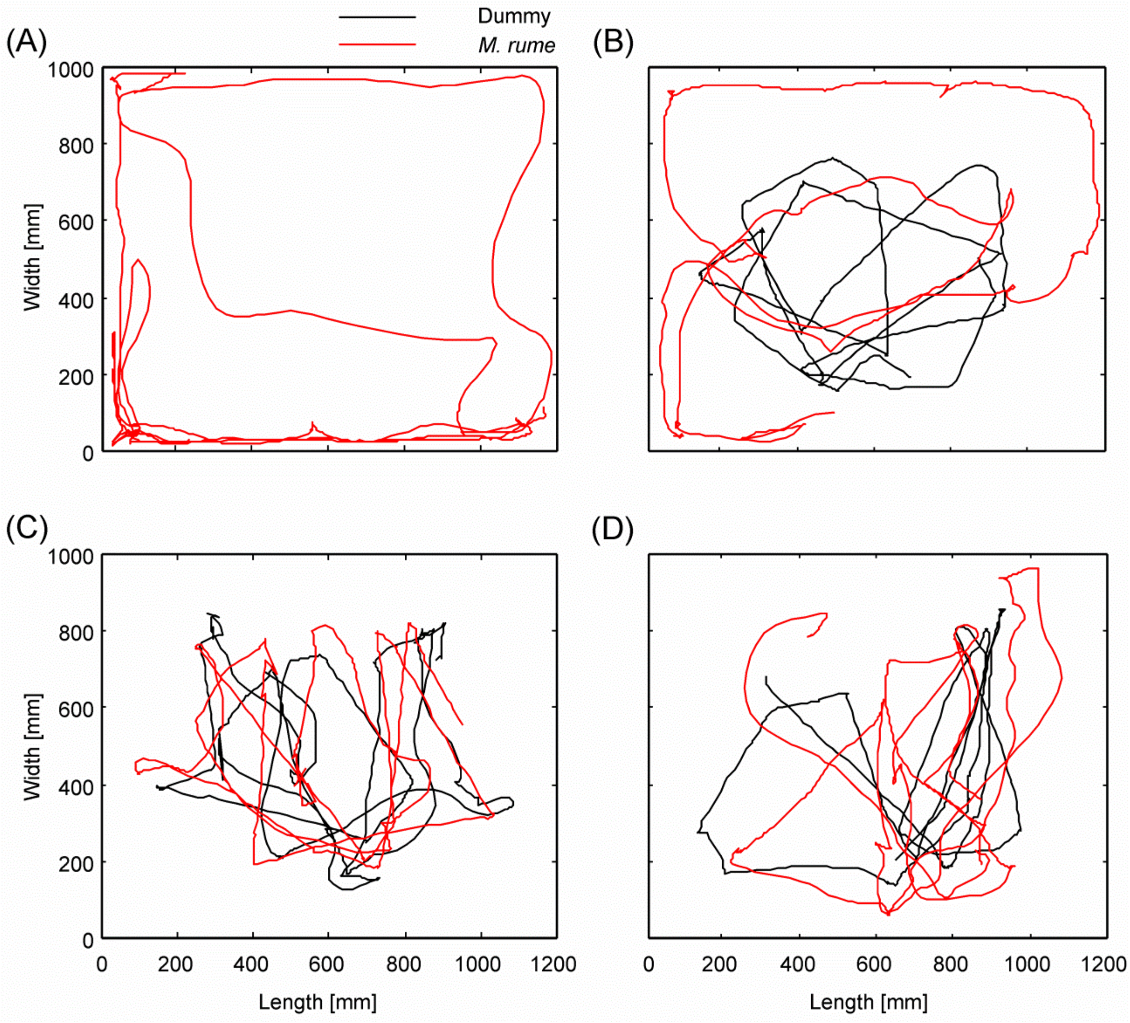

Figure 5.3: Swimming trajectories and following-behavior. Exemplary trajectories of the mobile dummy (black) and a single individual of *M. rume* (red) covering 45-second experimental trials. (A) With no dummy present, fish spent most of the time in proximity to the tank walls. (B) Control trials with an electrically silent dummy evoked occasional interest and following of the dummy fish trajectory by the test fish in addition to wall-following. Wall-following was virtually absent during trials with the static random playback (C) and the dynamic echo playback (D). Here, animals mostly followed the mobile dummy and roughly reproduced its trajectories.

The attractiveness of the moving dummy to individual *M. rume* could thus be quantified by the willingness of the fish to abandon wall-following behavior, the distance they kept to the dummy, and the accuracy with which they followed the dummy's trajectories (Figure

5.4). The influence of the dummy on wall-following behavior was highly significant (repeated-measures ANOVA with Greenhouse-Geisser correction: $F_{(1.837, 40.422)} = 247.26$; $p < 0.001$, $\varepsilon = 0.61$, Figure 5.4A). Without the dummy, the average distance between *M. rume* and the closest tank wall was 49 ± 3 mm (*mean* ± *s.e.m.*). The introduction of the dummy resulted in an increased average distance between fish and tank wall, indicating a decrease in wall-following behavior. Based on Bonferroni-corrected pairwise comparisons, the effect of the electrically silent dummy significantly increased the distance to 150 ± 11 mm (*mean* ± *s.e.m.*). This was still significantly closer than during the presentation of the static random playback (*mean* ± *s.e.m* = 260 ± 6 mm) and the dynamic echo playback (*mean* ± *s.e.m.* = 259 ± 5 mm). Playback type did not influence the distance between fish and tank walls. The distance between dummy and fish was strongly influenced by electrical playback (repeated-measures ANOVA: $F_{(2, 44)} = 144.44$; $p < 0.001$), showing that electrical signaling was the main attractive feature of the dummy (Figure 5.4B). Based on Bonferroni-corrected pairwise comparisons, electrical playback led to a significant decrease in distance from an average of 301 ± 14 mm (*mean* ± *s.e.m.*) during the electrically silent control condition to 104 ± 10 mm (*mean* ± *s.e.m.*) during the static random playback, and 89 ± 8 mm (*mean* ± *s.e.m.*) during the interactive echo playback, respectively. Evaluation of the relative amount of the dummy's turns that were followed by the fish confirmed this pattern (Figure 5.4C). The absolute amount of turns performed by the dummy during the 45 second experimental period ranged from 9 to 18 (*median*: 14). While the fish only followed a ratio of 0.16 ± 0.03 (*mean* ± *s.e.m.*) of these turns during the silent control condition, the relative amount of followed turns was considerably higher in response to the static random playback (0.80 ± 0.05; *mean* ± *s.e.m.*) and the dynamic echo playback (0.84 ± 0.03; *mean* ± *s.e.m.*; Friedman test: $\chi^2_{(2)}$ 34.795; $p < 0.001$). Again, adjusted pairwise comparisons revealed significant differences between both playback conditions and the control, but not between the two playback conditions.

Electrical playback was thus crucial to attract individual *M. rume* to the mobile dummy, especially with respect to following-behavior, but the type of playback did not influence spatial interactions of the fish with the dummy. This was not the case for electric signaling responses and interactions with the different playbacks. Figure 5.5 shows the IDI-distributions pooled for all fish for the four experimental conditions with box plot insets depicting the range of IDI-modes exhibited by individual fish during each condition. Median IDI-modes differed significantly across different test conditions (Friedman test: $\chi^2_{(2)} = 31.05$; $p < 0.001$). Both the static random playback (Figure 5.5A) and the interactive echo playback (Figure 5.5B) evoked significantly shorter IDIs from the fish compared with the control condition without the dummy, where median IDIs were longest (62 ms; Figure

5.5D). The static random playback led with a median IDI-mode of 30 ms to the strongest decrease in interval length by the fish, but there was no sign of adjustment to the IDI-distribution of the playback (Figure 5.5A). This contrasts with the results from the dynamic echo playback, which led to a leveling of intervals resulting in median IDI-modes of 52 ms for both playback and (Figure 5.5B). Here, playback IDIs were subdivided into a distribution at 60 ms and higher for the randomly emitted signals, and one with shorter IDIs for signals triggered by EODs of the fish.

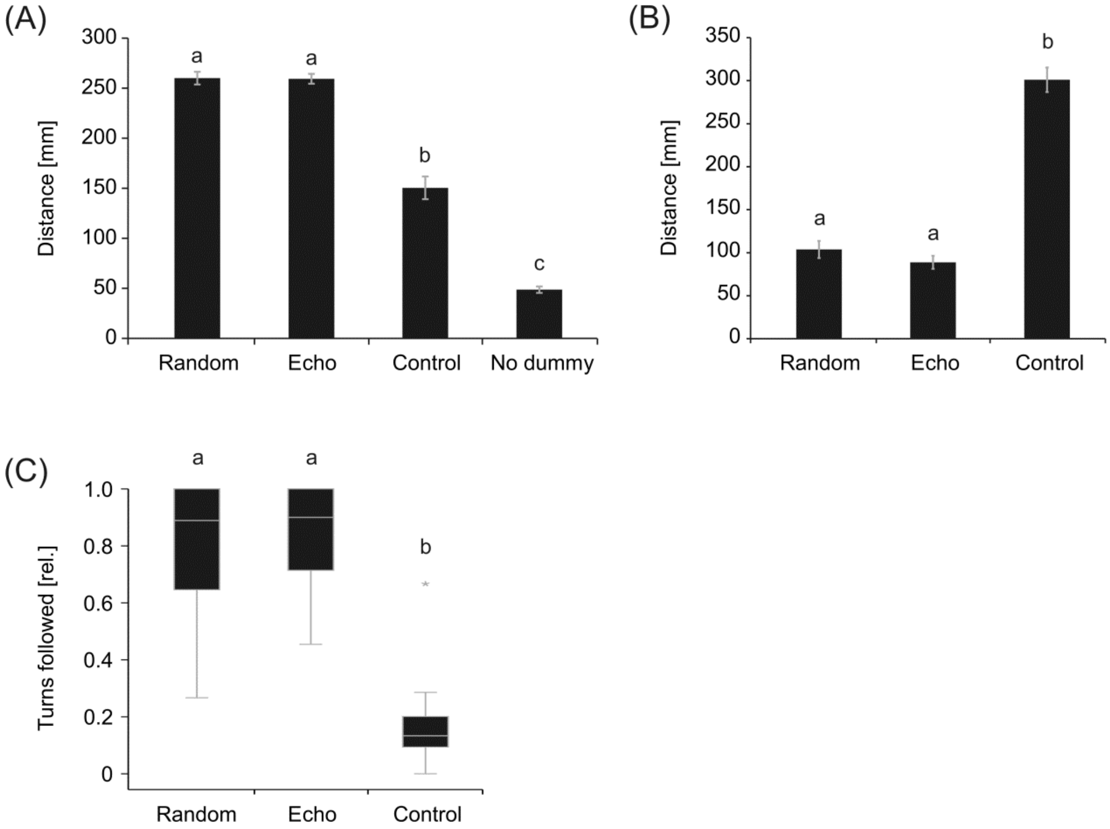

Figure 5.4: Influence of the mobile dummy on swimming behavior. (A) Mean distance between the fish and the closest tank wall. (B) Mean distance between the snout of the fish and the nearest point of the dummy. Error bars represent the standard error of the mean. (C) Relative amount of the dummy's turns that were reproduced by the fish. Categories not sharing a common superscript letter differ based post hoc adjusted *p*-values. Random: static random playback; Echo: dynamic echo playback; Control: electrically silent control; No dummy: trial in which fish were observed without interference from the dummy.

Echo responses represent an interactive signaling strategy and occur when a fish responds with an EOD at a preferred latency more often than would be expected by chance. All tested fish produced echo responses with preferred latencies ranging from 15 to 19 ms both to the static random playback and to the dynamic echo playback. Exemplary results from fish 9 for the four possible latency relationships are shown in Figure 5.6. This fish showed

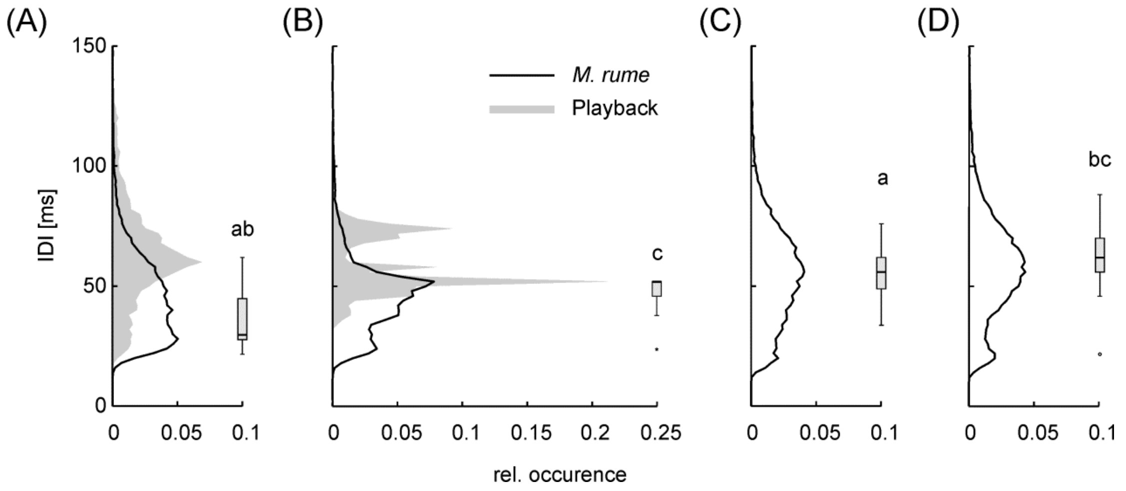

Figure 5.5: IDI-distributions in response to different experimental conditions. Histograms (black) represent the relative occurrence of interval lengths displayed by all fish in response to (A) the static random playback, (B) the dynamic echo playback, (C) the electrically silent control and (D) the control condition without the mobile dummy fish. Grey shaded histograms in (A) and (B) represent playback intervals of the respective conditions. Box plot insets summarize the distribution of IDI-modes observed across individuals. Shared superscript letters indicate a difference in IDI-distribution between the conditions based on distribution modes. Bin size: 2 ms.

distinct echo responses to the static random playback with a mode at a preferred latency of 15 ms (Figure 5.6A), as well as a slightly more pronounced response to the dynamic echo playback with a mode at a preferred latency of 16 ms (Figure 5.6B). The static random playback generated EODs at intervals independent of the test fish's signals. Consequently, the latencies with which the playback followed after the fish's EODs conformed to the expected distribution of random latencies (Figure 5.6C). During the dynamic echo playback, EODs emitted within the sensitivity range of the dummy's trigger electrodes elicited echo responses at a latency of 21 ms (Figure 5.6D).

To quantify the relative amount of echo responses produced by *M. rume* in response to the two playback conditions, the ratio of the relative occurrence of observed latencies at the preferred latency, and the corresponding value of the random distribution at that latency was calculated as an echoing quotient for each fish (Figure 5.7). The average echoing quotient in response to the static random playback was 4.53 ± 0.20 (*mean* ± *s.e.m.*), indicating an increase of responses at the preferred latency by that factor. The interactive echo playback evoked more echoing responses, resulting in a quotient of 5.31 ± 0.21 (*mean* ± *s.e.m.*) that was significantly higher than for the static random playback (paired-samples *t*-test: $t_{(22)} = -5.38$; $p < 0.001$). This suggests that echo responses induce echoing by fish who receive echoes to their EODs.

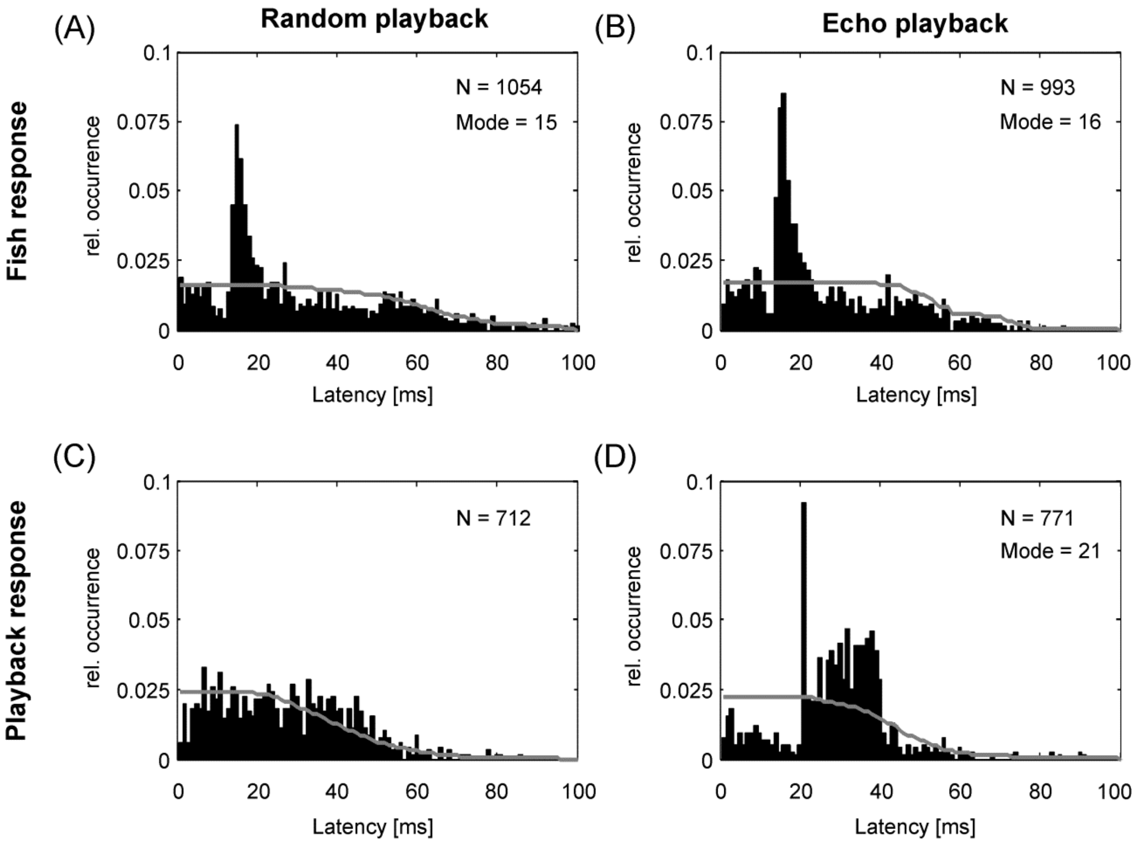

Figure 5.6: Echo responses by *M. rume* and interactive playback generation. Exemplary depiction of the relative occurrence of EOD response latencies displayed by *M. rume* and the dummy in the experiments with fish 9. Observed latencies are represented by black bars, while the grey line delineates the latency distribution that would be expected if there was no dependency between the reference IDI-sequence and the analyzed IDI-sequence. (A) Response latencies of the fish to the static random playback with a prominent mode at a preferred latency of 15 ms. (B) Response latencies of the fish to the dynamic echo playback with a prominent mode at a preferred latency of 16 ms. (C) Latencies of the static random playback to the fish's EODs conform to the expected random distribution. (D) Echo responses with a latency of 21 ms were generated during the interactive echo playback, which was triggered by EODs of the test fish in proximity to the mobile dummy. N = number of latencies evaluated for the trial. Bin size: 1 ms.

Analysis of simultaneously recorded IDI-sequences of the test fish and their interactions with the respective playback sequence over time demonstrated that interactive signaling does not necessarily require complete time-locking to entire signaling patterns, and allowed a more detailed characterization of electric signaling responses by *M. rume* to the different playback conditions. Figure 5.8 and Figure 5.9 depict exemplary results of complete 45-second experimental trials with the static random playback and the dynamic echo playback, respectively. Sections A_1 each show plots of overlaid IDI-sequences of the

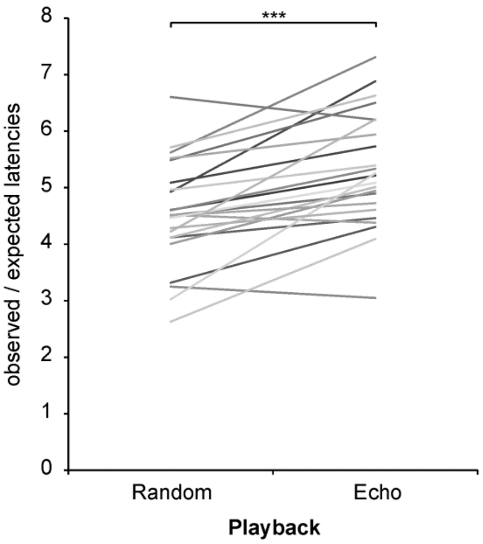

Figure 5.7: Ratio of observed to expected latencies at the echo response. The quotient allows assessing how many times more often than expected a preferred latency occurs during echoing. This proportion was higher in response to the dynamic echo playback for almost all of the *n* = 23 tested individuals (5.31 ± 0.21; *mean* ± *s.e.m.*) compared with the static random playback (4.53 ± 0.20; *mean* ± *s.e.m.*), suggesting that echo responses induce echoing by fish who receive echoes to their EODs.

respective playback condition and the responding fish. Adaptive cross-correlations calculated between both of the two pairs of sequences are shown in the panels A_2 below. Correlation coefficients for a response time of ± 100 ms are color-coded, with higher values at positive response times representing synchronization of the fish's discharges to the playback sequence, and high values at negative response times representing synchronization of the playback sequence to the fish's discharges. Maximum correlation coefficients within the 100 ms response time, representing responses of *M. rume* to the playback and vice versa, are plotted in panels A_3 of Figure 5.8 and Figure 5.9.

The IDI-sequence emitted by fish 1 in response to the static random playback (Figure 5.8A_1) illustrates the general observation that animals consistently discharged at shorter intervals in response to this playback type (compare Figure 5.5A). The cross-correlation analysis in Figure 5.8A_2 reveals consistent synchronization of electrical discharge activity by the fish to the static random playback that occurred at the response time representing the echo response. Maximum correlations frequently exceed the 0.3 correlation threshold indicative of relatively strong synchronization (see below). Since the static random playback generated artificial EODs by default independently of the signals emitted by the tested fish, all synchronizations between the random playback sequence and the electrical signaling behavior of the fish have to be considered to occur at random. Consequently, maximum correlations of this comparison hardly reach the 0.3 threshold for relatively

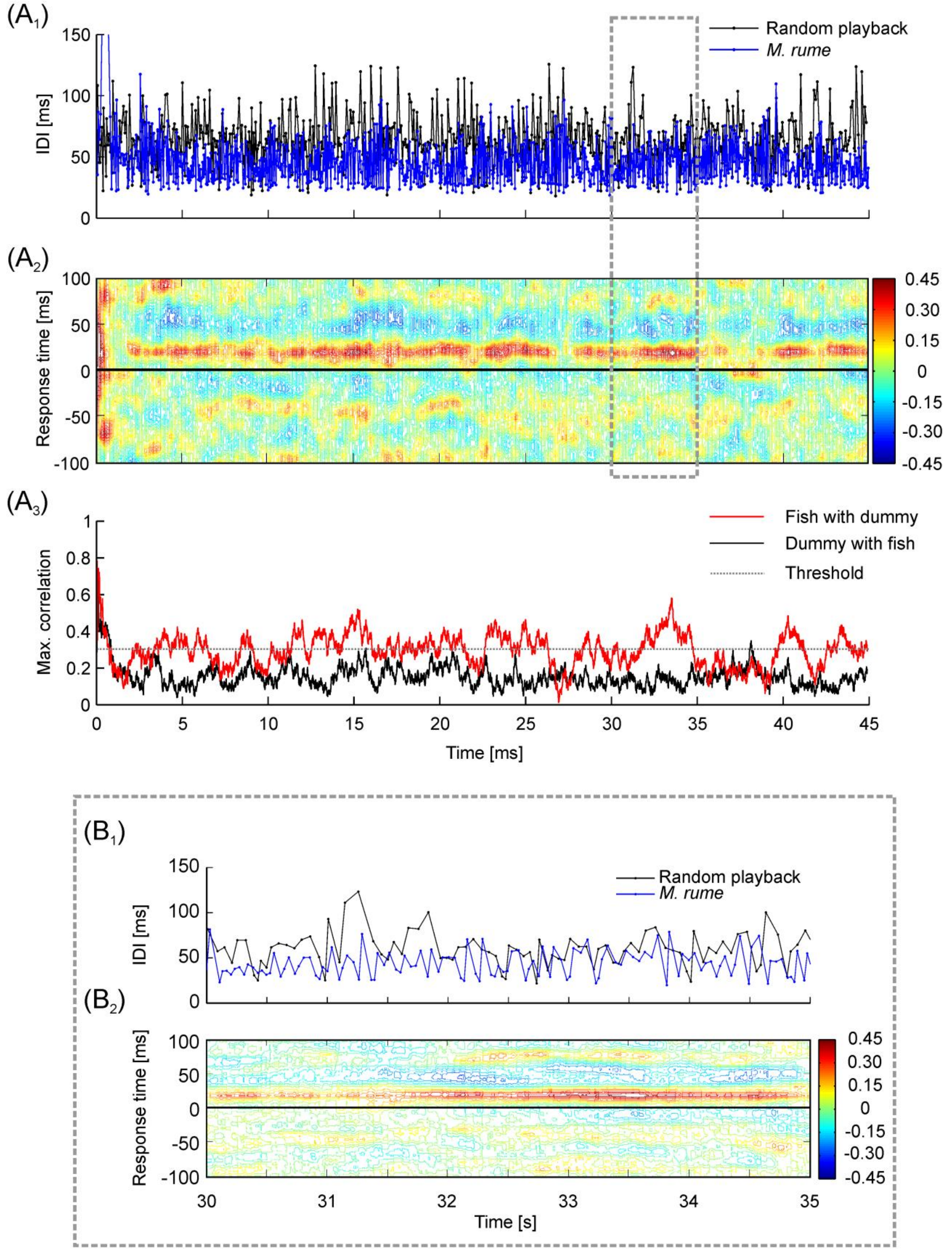

Figure 5.8: Synchronization of electrical discharge activity with the static random playback. Exemplary trial performed with fish 1. (A_1) IDI-sequences of the random playback (black) and the fish (blue) over the 45-second time course of the experiment. (A_2) Cross-correlation diagram calculated for the two IDI-sequences displayed in (A_1). Correlation coefficients of the fish's signals with the playback signals are color-coded for response times of ± 100 ms over the time course of the experiment. High correlation coefficients at positive response times thus represent synchronization of the animal's signaling behavior

to the playback at a latency defined by that response time. High correlation at negative response times would represent synchronization of the playback with the signaling behavior of the fish. This can only occur randomly in response to a static playback pattern. (A_3) Maximum correlation values indicating synchronization of the fish with the playback signal (red) as well as for the playback signal with the fish (black). These values were extracted from the data underlying subplot A_2. The dotted grey line represents the 0.3 threshold indicative of relatively high correlation. (B_1–B_2) Magnification of the section outlined by the dashed grey rectangle in (A_1–A_2).

strong synchronization. Magnifications in Figure 5.8B_1 and B_2 demonstrate that discharge synchronization does not necessarily require exact copying of discharge sequences. Signaling interactions of fish 1 with the dynamic echo playback led to more regular IDI-sequences with intervals around 50 ms (Figure 5.9A_1, compare Figure 5.5B). The cross-correlation analysis of fish 1's response to the interactive echo playback again shows synchronizations at the response time corresponding to the echo response, persisting over the majority of the time course of the experiment (Figure 5.9A_2). Since the echo playback was designed to dynamically respond to EODs emitted close to the dummy with an echo latency of 21 ms, correlations at that response time before the fish's EOD represent synchronization of the electrical playback sequence to the signaling sequence of the fish. Consequently, maximum correlations of both comparisons exceeded the 0.3 threshold and discharge synchronization by the fish extended to even higher values compared with the response to the static random playback (Figure 5.9A_3). How the adjustment of time-locked IDI-duration leads to mutual discharge synchronization is illustrated in more detail by the magnification in Figure 5.9B_1 and B_2.

The maximum correlation calculated for the 100 ms response time was averaged over the trial period to quantify discharge synchronizations between *M. rume* and the IDI-sequences of the two playback conditions (Figure 5.10). The same analysis was additionally performed for correlations of the playback sequences with signaling sequences of the fish that were acquired independently during the electrically silent control condition as a control for randomly occurring correlations. Averaged maximum correlations varied statistically highly significantly (repeated-measures ANOVA with Greenhouse-Geisser correction: $F_{(2.152,\,47.349)} = 192.0$; $p < 0.001$; $\varepsilon = 0.31$) and confirmed the general observation from exemplary trials as well as the general conclusions derived from the analysis of echo responses. Responses of *M. rume* to the static random playback (i) resulted in an average correlation coefficient of 0.31 ± 0.011 (*mean* ± *s.e.m.*), thus justifying the 0.3 threshold set to define relatively strong synchronization. The occurrence of incidental correlations of the static random playback with the IDI-sequence of the fish (ii) was with 0.16 ± 0.002

(*mean* ± *s.e.m.*) significantly lower than that of the fish with the playback. These incidental correlations were statistically indifferent to the randomly occurring correlations (both 0.16 ± 0.002; *mean* ± *s.e.m.*) observed for the independently recorded control sequences to the random playback (iii) and vice versa (iv).

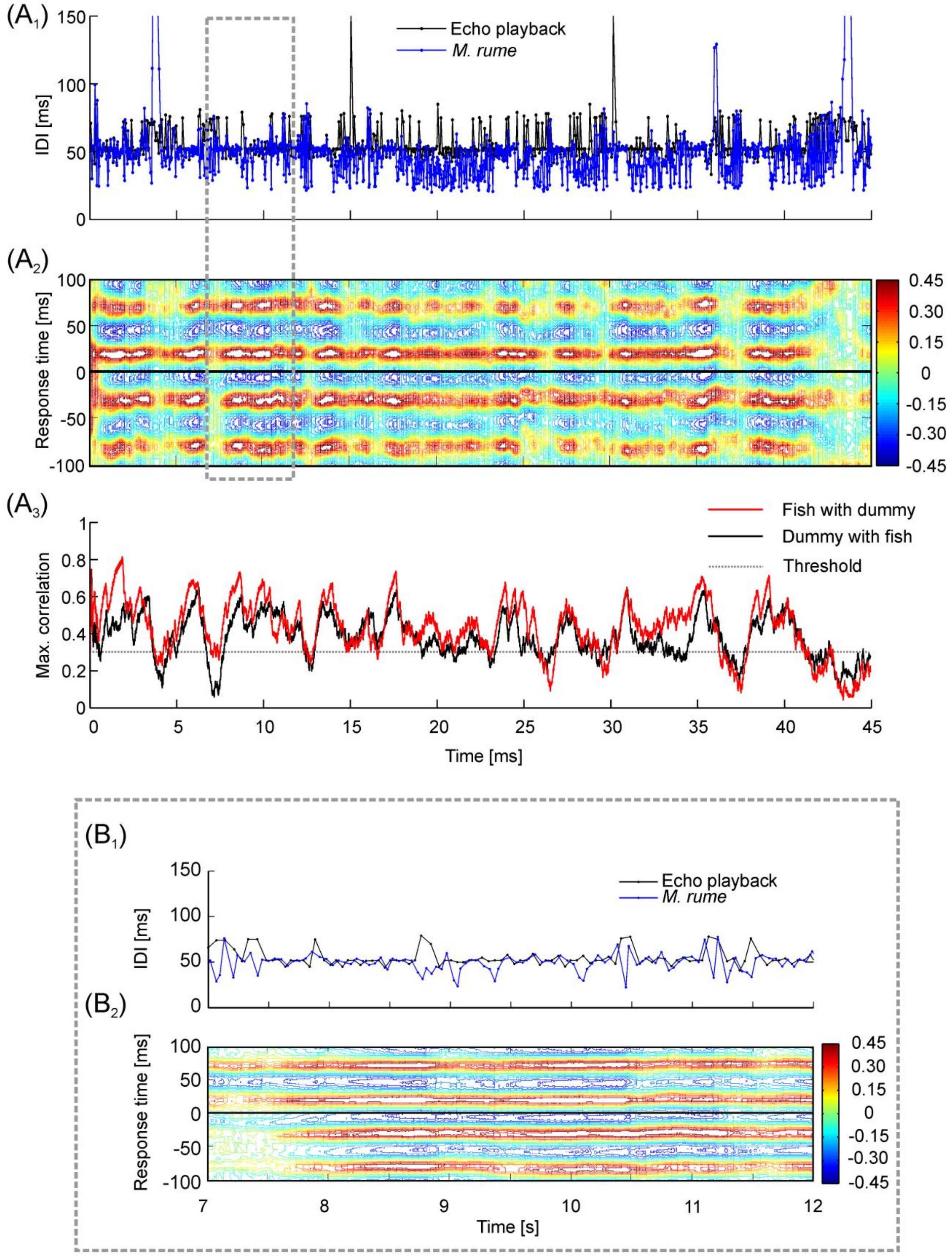

Figure 5.9 (previous page): Synchronization of electrical discharge activity with the dynamic echo playback. Exemplary trial performed with fish 1. (A_1) IDI-sequences of the echo playback (black) and the fish (blue) over the 45-second time course of the experiment. (A_2) Cross-correlation diagram calculated for the two IDI-sequences displayed in (A_1). Correlation coefficients of the fish's signals with the playback signals are color-coded for response times of ± 100 ms over the time course of the trial. High correlation coefficients at positive response times represent synchronization of the animals signaling behavior to the playback at a latency defined by that response time. High correlation at negative response times represents synchronization of the playback with the signaling behavior of the fish through an interactive echo playback triggered by EODs of the fish, which were emitted in proximity to the dummy. (A_3) Maximum correlation values are indicating synchronization of the fish with the playback signal (red) as well as for the playback signal with the fish (black). These values were extracted from the underlying data of subplot (A_2). The dotted grey line represents the 0.3 threshold indicative of relatively high correlation. (B_1–B_2) Magnification of the section outlined by the dashed grey rectangle in (A_1–A_2).

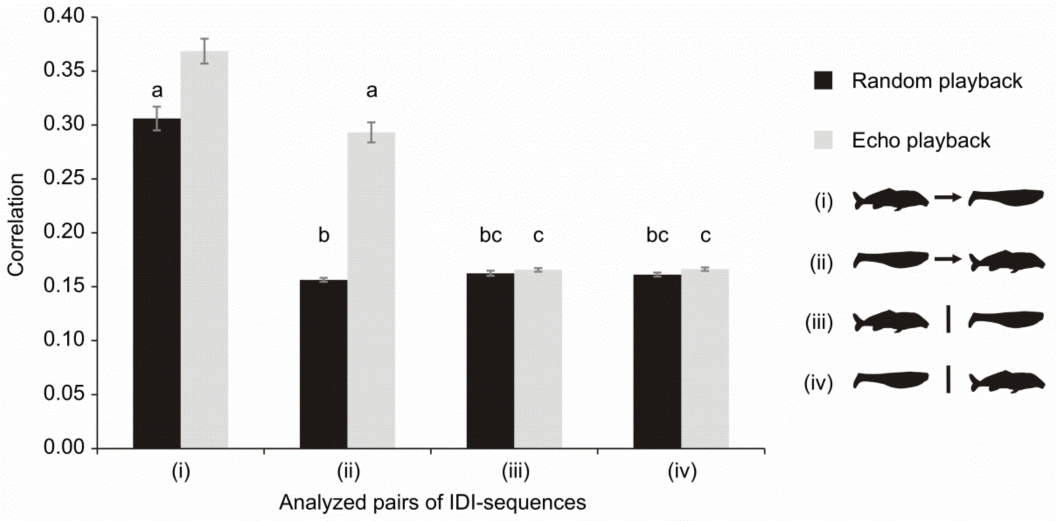

Figure 5.10: Synchronization of electrical discharge sequences. Mean of the averaged maximum cross-correlation values for trials with all fish during the static random playback (black) and the dynamic echo playback (grey). High correlation coefficients indicate stronger synchronization of electrical discharge behavior to the corresponding IDI-sequences of either playback or fish. (i) Synchronization of the electrical discharge behavior of *M. rume* to the playback sequence. (ii) Synchronization of the playback sequence to the electrical discharge behavior of *M. rume*. (iii) Synchronization of electrical signaling activity of *M. rume* during the silent control condition to the playback IDI-sequence used in the same session. (iv) Synchronization of the playback sequence to the IDI-sequence generated by *M. rume* during the silent control condition of the same session. Analyses of (iii) and (iv) were performed as a control for randomly occurring correlations and to establish a baseline value for synchronization between independent IDI-sequences. Error bars represent the standard error of the mean. Categories not sharing a common superscript letter differ significantly based on Bonferroni corrected *p*-values.

Responses of *M. rume* to the dynamic echo playback (i) resulted on average in significantly more discharge synchronization (correlation coefficient: 0.37 ± 0.012; *mean ± s.e.m.*) than during the static random playback, confirming the results obtained for the echo quotient (Figure 5.7). The respective synchronization response of the echo playback to the signals of *M. rume* (ii) was with 0.29 ± 0.009 (*mean ± s.e.m.*) statistically indifferent to the fish's response to the static random playback (i). This confirms the comparability of the dummy's interactive echo playback with the interactive signaling behavior of live fish. The independent control responses to the dynamic echo playback (iii and iv) were statistically indifferent to those obtained for the static random controls (both correlation coefficients: 0.17 ± 0.002). This confirms that statistical differences in synchronization responses to the playbacks were not due to general differences between the two playback types.

Temporal aspects of synchronization were further analyzed by quantifying the duration of sequences with correlation coefficients of 0.3 or higher. Relative cumulative histograms pooled for all fish (Figure 5.11) show that more extended sequences of relatively strong synchronization occurred in response to the dynamic echo playback compared with the static random playback. At a proportion of 0.75, the median sequence length of high-synchronization episodes to the dynamic echo playback (1.68 s) significantly exceeded the duration of high-correlation sequences during the static random playback (1.17 s, Wilcoxon signed-rank test: $Z = 2.42$; $p = 0.016$; see box plot inset of Figure 5.11).

Simultaneous recording of electrical signals and the motor behavior displayed by the fish allowed to associate the two components of interactive behavior in *M. rume* at any given time during an experiment. Tracking data allowed to analyze linear distances as well as directional relationships with regard to discharge synchronization of *M. rume* with the static random playback, and to identify the constellations they most frequently occurred in. Averaging maximal correlation values across the distances observed during all trials with the static random playback revealed that fish synchronized most strongly at a range of around 100 mm (Figure 5.12A). This range corresponds approximately to the maximum distance up to which fish are able to perform active electrolocation, i.e., the distance up to which they can detect objects by using their active electric sense (von der Emde, 1999). The median distance where relatively strong synchronizations of 0.3 or higher were observed was with 289 mm considerably shorter compared to the median maximum distance of 473 mm observed in all trials (Figure 5.12B).

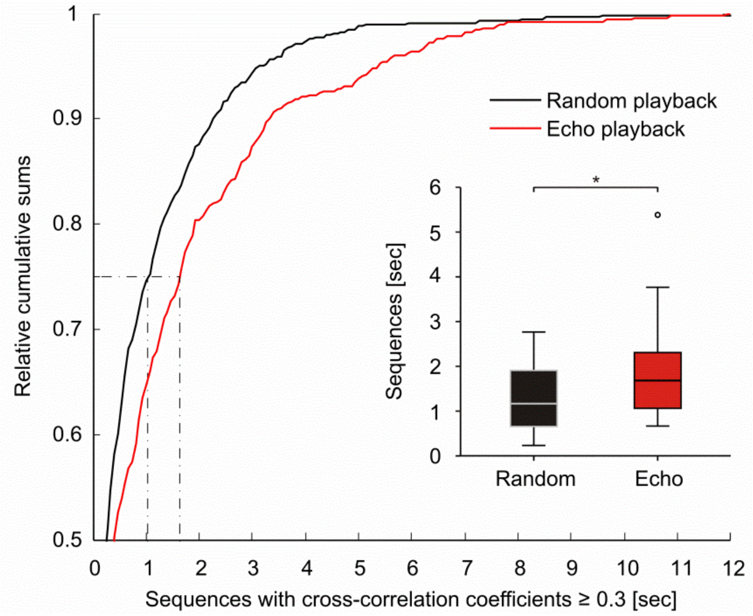

Figure 5.11: Duration of synchronization episodes. Relative cumulative sums of the lengths of sequences during which fish synchronized their EODs to the IDI-sequences of the random playback (black) and the echo playback (red) with a cross-correlation coefficient of 0.3 or higher. A statistical comparison between the effect of both playback types on the duration of synchronization episodes by *M. rume* was made at a proportion of 0.75 (dashed line). At this value, echo playback led to a significantly longer duration of synchronization events compared with the random playback (box plot inset).

The average intensity of electrical discharge synchronization was also affected by directional interrelations between the mobile dummy and the fish. Figure 5.13 illustrates three such angular relationships and summarizes their influence on discharge synchronizations engaged in by *M. rume*. These were the position of the dummy from the perspective of the fish (Figure 5.13A(i)), the position of the fish from the dummy's coordinates (Figure 5.13B(i)), and the angular difference between dummy and fish (Figure 5.13C(i)). Discharge synchronizations are expressed in Figure 5.13A–C(ii) by absolute values of maximum correlation per video frame, plotted into polar coordinates representing the angular relationships illustrated in line (i). Mean vectors express central tendencies for each fish (green) as well as for the whole group (magenta). In conjunction with the relative correlation coefficients calculated per degree (Figure 5.13A–C(iii)), these data suggest that the fish spent most of the time oriented towards the dummy (Figure 5.13A(iii)), swimming behind it (Figure 5.13B(iii)), and adopted the same orientation (Figure 5.13C(iii)). When the data were made independent of the total frequency of occurrence by averaging correlation coefficients into bins of 1° (Figure 5.13A–C(iv)), the magnitude of synchronization engaged in by *M. rume* was correlated with the dummy's position relative to the perspective of the fish ($\rho_p = 0.64$; $p < 0.001$), the fish's location relative to the coordinates of the dummy

($\rho_p = 0.43$; $p < 0.001$), and the difference in orientation between the two ($\rho_p = 0.68$; $p < 0.001$). The direction of mean vectors of averaged correlation data within polar coordinates, therefore, suggest that animals synchronized their discharge activity most intensely when swimming towards the dummy (Figure 5.13A(iv)), approaching it from behind (Figure 5.13B(iv)) with a similar orientation (Figure 5.13C(iv)). These observations are consistent with a situation where the following fish addresses a conspecific by engaging in synchronization with the discharge sequence of the individual swimming ahead.

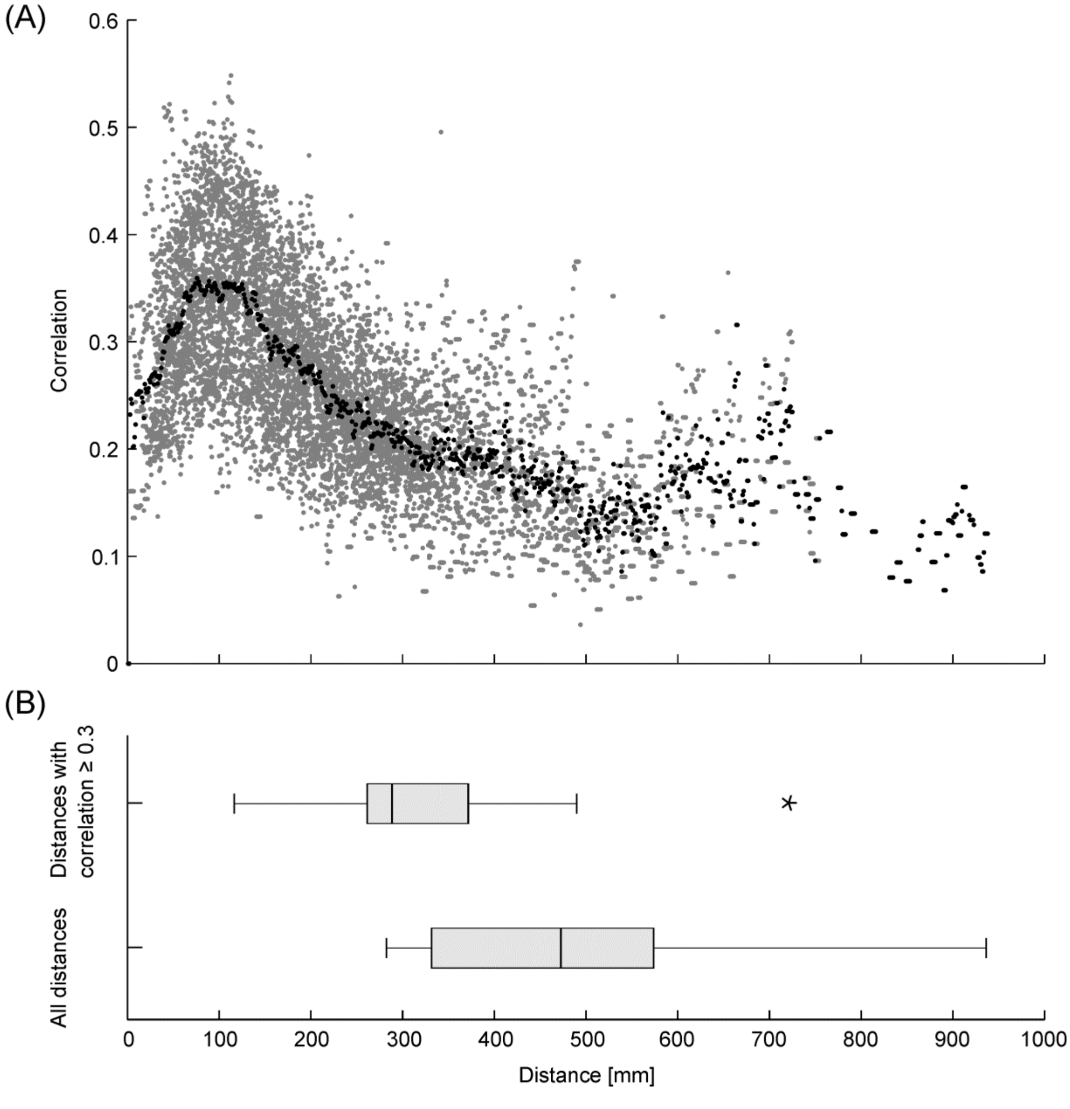

Figure 5.12: Influence of distance on electrical discharge synchronization. (A) Cross-correlation coefficients of the IDI-sequences of *M. rume* with the static random playback for all $n = 23$ animals plotted against the distance between dummy and fish observed at the respective time (grey dots). Average values per distance (black dots) show that synchronization was strongest at approximately 100 mm. Bin size: 1 mm. (B) Box plots summarize the upper limit synchronizations with cross-correlation coefficients of 0.3 or higher were observed (upper plot) and the maximum observed distance the fish kept to the dummy during the presentation of the electrical playback (lower plot).

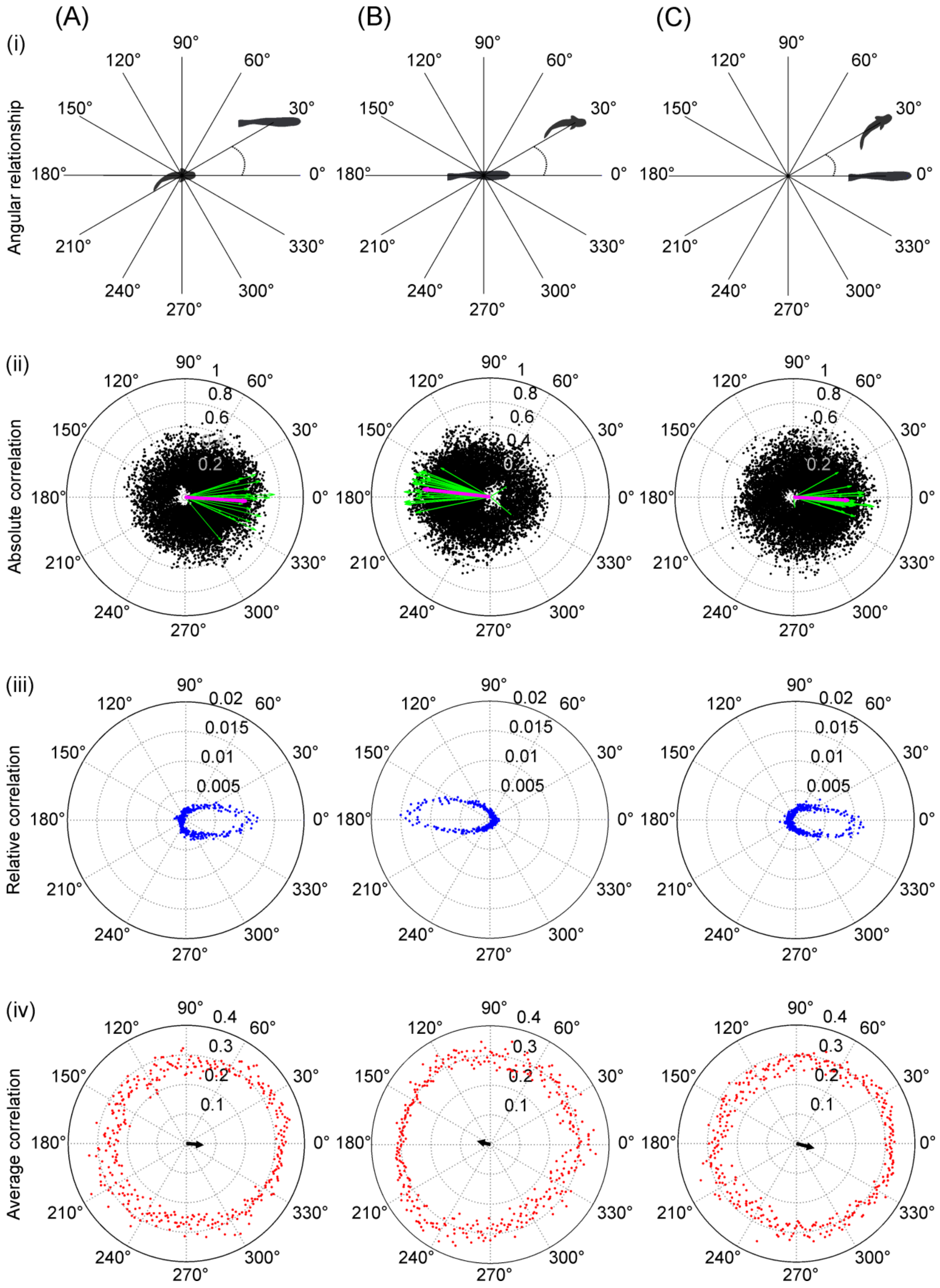

Figure 5.13: Dependency of electrical discharge synchronization on angular relationships between dummy and fish. (A) Position of the dummy from the fish's perspective. (B) Position of the fish from the dummy's coordinates. (C) Difference in orientation between dummy and fish. (i) Illustration of angular relationships between fish and dummy for angles of 30°. (A(i)) Illustration of the angle between the fish's swimming direction and the connecting line between the centers of fish and dummy. At 0°, the fish would swim towards the dummy, whereas at 180° it would swim away from the dummy. (B(i)) Illustra-

tion of the angle between the dummy's swimming direction and the connecting line between the centers of dummy and fish. At 0° the fish would swim in front of the dummy, whereas at 180° it would be positioned behind. (C(i)) A difference of 0° represents the parallel orientation of dummy and fish, whereas anti-parallel orientation represents a 180° orientation difference. (ii) Polar plots of cross-correlation coefficients as a function of the angular relationship between dummy and fish. Higher coefficients are plotted further away from the circle center. Green arrows depict the mean vector for each fish. The magenta arrow represents the mean vector for the data obtained from all fish. (iii) Relative amount of correlation per 1° for all observed angular relationships. (iv) Average cross-correlation coefficients per 1° for all observed angular relationships. The black arrow represents the mean vector.

5.4 Discussion

Determination of the key stimuli that trigger the release of social behaviors lies at the heart of behavioral biology (Tinbergen, 1948) and is a crucial prerequisite for using robotic dummies for the investigation of behavioral responses in controlled experimental designs (Mondada *et al.*, 2013). Ethorobotical experiments with various fish species have shown that mainly visual and hydrodynamic cues mediate interactions between real animals and mobile fish dummies. These include size and aspect ratio, coloration and patterning, morphological features such as realistic eyes, swimming speed and dynamics, as well as tail-beat movement and hydrodynamic wake generation (Abaid *et al.*, 2012; Kruusmaa *et al.*, 2016; Landgraf *et al.*, 2016; Marras and Porfiri, 2012; Phamduy *et al.*, 2014; Polverino *et al.*, 2013; Ruberto *et al.*, 2016). In mormyrids, the importance of electrical signaling for mediating social behaviors is well established (Khait *et al.*, 2009; Moller, 1976; Moller *et al.*, 1982). Studies using mobile dummies, which emit electrical playbacks, suggest that the EOD is a critical stimulus, which allows using dummies as a proxy for conspecifics in controlled experiments on electrocommunication (Donati *et al.*, 2016; Worm *et al.*, 2017; Worm *et al.*, 2018). The present study supports this interpretation by demonstrating locomotor and electromotor interactions of *M. rume* with a mobile dummy moving on arbitrary trajectories while emitting different types of electrical playback sequences.

In this study, locomotor and electromotor responses of individual *M. rume* to a mobile dummy, which emitted electrical playback EODs either as a static random IDI-sequence or in a dynamically interacting echo paradigm, were investigated. Social interactions and following-behavior were reliably induced in all individuals by electric playback generation. Following-behavior was quantified by examining the animals’ willingness to abandon wall-following behavior, by measuring fish–dummy distances, and by counting the number of the dummy's turns that were followed by the fish (Figure 5.4). While the electrically silent dummy had some influence on wall-following behavior, the effect of the playback emitting dummy was much more pronounced, and the evaluation of turns suggested that real following-behavior was mainly a response to electrical playback. It turned out that attraction was not influenced by the type of playback which the dummy emitted. In contrast, the fish's electromotor behavior was strongly influenced by playback type.

Mormyrids are capable of encoding distinct signaling patterns into IDI-sequences (Carlson and Hopkins, 2004b) and behavior related signaling patterns have previously been described in *M. rume* (Gebhardt *et al.*, 2012a; Worm *et al.*, 2017). In addition, it has been suggested that the general sequence of intervals may be of importance for communication (Teyssedre and Serrier, 1986). Teyssedre and Serrier (1986) found *Gnathonemus petersii* to be more attracted to original IDI-sequences compared to randomly rearranged sequences, and Kramer and Kuhn (1994) demonstrated that *Campylomormyrus rhynchophorus* preferred its own species' IDI-sequences over those of related *C. tamandua*, although vice versa this was not the case.

Both playback types presented in the current study were of artificial nature. A preference for either of the two types of sequences based on locomotor behavior could not be observed, and interactive signaling responses were also exhibited by *M. rume* towards the unpredictable random IDI-sequences. At the level of electric signaling interactions, the fish's reaction depended on whether the playback sequence was static or dynamic, i.e., whether the dummy responded to the electromotor behavior of the fish. While the static playback caused an increase in discharge frequency by *M. rume* and led to an IDI-mode that was shorter compared with the control condition, a common IDI-mode at an intermediate value was assumed during interactive signaling with the dynamic playback sequence (Figure 5.5). Echo responses were reliably elicited by the random playback (Figure 5.6A), but the relative amount of playback pulses that were responded to by echoing was higher in response to the interactive playback (Figure 5.7). This led on average to a higher degree of discharge synchronization (Figure 5.10) sustained over longer periods of time (Figure 5.11). Although it was not possible to design an interactive playback sequence that matched the random sequence regarding variability and average frequency whether or not the fish chose to interact with the dummy, the observed difference in interactive signaling by the fish did not depend on general differences between the playback types. This was demonstrated using independently recorded sequences as a control (Figure 5.10). The higher amount of interactive signaling by *M. rume* seems, therefore, to result from the interactivity of the dynamic playback sequence, which means that an animal that receives echoes reacts by responding with more echoes of its own. This in turn leads to more intense discharge synchronization between individuals. Similar findings were reported for

G. petersii, who generated echo responses to playback signals from both resting and aggressively behaving conspecifics, but synchronized their discharges more intensely to an interactive playback sequence that responded with echoes to the EODs of the test fish (Prume, 2015a). Since there was no obvious effect on locomotor behavior in the current study, these results support the notion that echoing the EODs of conspecifics serves as an important function during electrocommunication in mormyrids.

Echoing may, however, also serve as a jamming avoidance strategy. Heiligenberg (1976) demonstrated that *Brienomyrus niger* needed at least 4–8 successive EODs that were undisturbed by overlapping conspecific EODs for optimal performance during active electrolocation of an approaching object. Schuster (2001) proposed a mechanism by which an after-effect is caused in the command nucleus by the first EOD of a train of foreign signals, which can cause mormyrids to echo preferentially to the last pulse within a sequence. This mechanism may help to avoid signal overlaps caused by echoing in groups of more than two fish. The jamming-avoidance hypothesis stands in contrast to the observation by Schumacher *et al.* (2016b) that *G. petersii* was not impaired in the ability to perform an object discrimination task during jamming conditions, neither by a conspecific nor by high-frequency electric playback of EODs. These fish did not respond with echoes to the jamming EODs. Instead, the jamming fish echoed the test fish.

Jamming avoidance strategies are also known from other active sensory systems such as active electrolocation in gymnotiform pulse-type (Westby, 1979) and wave-type electric fishes (Heiligenberg, 1980; Watanabe and Takeda, 1963), and echolocation in bats (Gillam *et al.*, 2007; Takahashi *et al.*, 2014; Ulanovsky *et al.*, 2004), but see Götze *et al.* (2016). The necessity for a jamming avoidance strategy is more apparent in gymnotiform weakly electric fish because they lack a reafferent neuronal pathway, which enables mormyrids to distinguish between their own EODs and those generated by other individuals (Bell, 1981; Bell and Grant, 1989; Zipser and Bennett, 1976). Nevertheless, also in gymnotiforms jamming avoidance strategies have been linked to social communication, in particular concerning identity information and dominance relationships. The otherwise very stereotypical jamming avoidance response in wave-type *Eigenmannia lineata* was found to vary between male and female, as well as between juvenile and adult individuals (Kramer, 1987). Westby (1979) demonstrated that the more dominant of two resting *Gymnotus carapo* adopted the more efficient jamming avoidance strategy, and thereby more likely jammed the subordinate individual. *Gymnotus carapo* exhibits a strongly increased threshold for electroreception for about 12.5 ms after an EOD, thus limiting sensitivity to the time window where their own discharge will occur (Westby, 1975). In an interactive electrical playback protocol, *G. carapo* preferentially discharged after half of the average playback IDI, i.e., within the time window during which the receptors of the conspecific represented by the playback would have been sensitive to the response of the fish. This observation led Forlim and Pinto (2014) to hypothesize that these animals may choose to discharge within or outside the refractory period of a conspecific, depending on whether they intend to communicate.

Jamming avoidance can serve an essential function during communication by providing signaling individuals with a strategy to emphasize their signals and reduce obstructing overlaps with the signals of competitors. Strategic adjustments of signal generation have been described in calling insects (Murphy *et al.*, 2016), frogs (Zelick, 1986), and songbirds (Benichov *et al.*, 2016), and a dual role of echoing is also conceivable for mormyrids.

A third reason why the sensory perception of mormyrids may benefit from echoing is that it ensures compatibility of active and passive electrolocation during social interactions. Mormyrids were shown to use the information provided by knollenorgan electroreceptors to approach a dipole source, representing a conspecific, from outside the range of active electrolocation (Hopkins, 2005; Schluger and Hopkins, 1987). Thus, a conspecific's EODs also provide spatial information during social interactions (Worm *et al.*, 2018) (chapter 9). Since afferent information generated by the stimulation of knollenorgans is inhibited at the level of the hindbrain by a corollary discharge during the generation of an animal's own EOD (Bell and Grant, 1989), echoing the EOD of a conspecific would guarantee that active electrolocation does not impair passive sensing performance in a social context. Echoing will be perceived by the individual that is approached, and could have ritualized into a communication display where the approached individual echoes as well and thereby signals that it is aware that it has been detected. In the current study, the median value of the largest distance, at which relatively strong synchronization of *M. rume* with the dummy's playback occurred, corresponded to the 30 cm range for echoing determined by Russell *et al.* (1974). Additionally, synchronization was strongest at the outer limit of active electrolocation at around 10 cm (Figure 5.12) (von der Emde, 1999). At this distance, passive sensing may be the most reliable source of information available about conspecifics during the nocturnal activity period of mormyrids (Moller *et al.*, 1979).

Echoing of the EODs of conspecifics has been observed in several mormyrid species, but reports of associations between behavioral displays and echo responses do not point towards a specific behavioral context. Echo responses have been observed during agonistic encounters in *G. petersii* (Bell *et al.*, 1974; Terleph, 2004) and their occurrence was negatively correlated with aggressiveness (Kramer, 1974). However, an unambiguous dependency between echoing and a subsequently established dominance relationship could not be confirmed (Bell *et al.*, 1974). Observations from other species reported a reduction or even absence of echo responses during aggressive encounters compared with other behaviors like foraging or resting (Gebhardt *et al.*, 2012b). Echo responses by resting individuals are consistent with the proposition of Russell *et al.* (1974) that echoing may mark the intrusion of a territory by a conspecific. However, echoing is not confined to resting individuals, and *M. rume* generated echo responses as a reaction to a variety of playback sequences that differed in pattern and average discharge frequency (Worm *et al.*, 2017). The finding of Lücker and Kramer (1981) that preferred latency responses and preferred latency avoidance constitute a sex difference in *Pollimyrus isidori* could not be confirmed for other species (Orlov *et al.*, 2009; Worm *et al.*, 2017). The fact that echo responses can be observed in a variety of behavioral contexts as diverse as agonistic encounters, foraging, and resting suggests that echoing may serve a more general signaling purpose, which is not necessarily linked to an activity-dependent behavioral context. Arnegard and Carlson (2005) described discharge synchronizations through mutual generation of echo responses in group-hunting *Mormyrops anguilloides* and interpreted echoing as a pack-cohesion signal promoting mutual acknowledgment of individual recognition.

The corollary discharge, which is generated by the command nucleus in the mormyrid brain each time the EOD is initiated, results in inhibitory postsynaptic potentials of up to 10 ms. Theses potentials are measurable in the cells of the nucleus of the electrosensory lateral line lobe, where the afferent fibers of the knollenorgans project to (Bell and Grant, 1989). Thus, the echo response might assure that the sender places its EOD between the end of this refractory period and before of the next EOD of the receiver. The EOD will, therefore, be registered by the receiver and may thereby signal notification of the sender and eventually mutual attention through discharge synchronization in a variety of behavioral contexts. The observation that the correlation of the directional relationships between *M. rume* and the mobile dummy with the amount of synchronization engaged in by the fish was most pronounced when fish attended to the dummy by following its trajectory supports this integrative interpretation of the purpose of echoing.

In summary, there are three possible and mutually not exclusive functions of the echo response in mormyrids. Echoing may have originally been a strategy for jamming avoidance during active electrolocation of the environment, as well as during passive electrolocation of conspecifics via the knollenorgan pathway. Based on these functions, it may have ritualized into a communication display that ensures signal transmission between individuals and allows them to affirm mutual attention during social interactions.

6. Discussion: Communication

Animals communicate to coordinate behaviors that are relevant to many vital aspects of their lives. Adaptive signaling strategies have evolved for the net benefit of senders and receivers all across the animal kingdom, resulting in intricate communication systems and behavioral displays that become particularly evident during the contexts of intraspecific competition and reproduction (see chapter 3). Fishes, especially teleosts, are no exception to this rule. While many fishes communicate primarily using vision and olfaction, most modalities from their rich repertoire of sensory systems can also assume some function during communication (see section 3.3). This is also the case with the active electrosensory systems that evolved independently in South American gymnotiform and African mormyriform weakly electric fish. In fact, communication may have been the key innovation for which active signal generation evolved in these two teleost lineages, whose ancestors had previously reacquired a passive electrosensory system (Arnegard *et al.*, 2010b).

Electrocommunication in mormyrids is based on the waveform of their EOD and the temporal variation of intervals within series of successive EODs (see section 3.5 and Figure 1.5B). While the adaptive potential of waveform-based communication in weakly electric fishes is relatively well understood (see sections 1.4 and 3.5.1), this is not the case with IDI-based electrocommunication in mormyrids. Few authors have given much consideration to precise and objectively quantifiable definitions of signaling displays (see Carlson and Hopkins (2004b) for an exception). Although the link between mormyrid behavior and IDI-variation is undeniable and has been investigated many times both in solitary fish and socially interacting individuals (see section 3.5.2), most work on pattern-based electrocommunication is rather descriptive with respect to the actual information content of such signaling displays. Especially the mechanisms that make these signals reliable sources of information for conspecifics (Maynard Smith and Harper, 1995) have not received adequate attention. This situation encourages anthropocentric interpretations of the observed signaling displays but explains very little of their adaptive value.

This lack of knowledge, persisting in spite of a vast number of studies on electrocommunication, can in part be explained by the fact that electrical signals from solitary mormyrids are easily recorded and interpreted, while the assignment EODs from two or more individuals to the respective sender is time-consuming and prone to errors. This assignment problem is particularly challenging if the signals result from unrestrained social interactions. However, such unrestrained interactions are required if one intends to observe the full repertoire of signals used by a species to communicate (Guariento *et al.*, 2016).

In Part One of this thesis, mobile dummy fish that can generate playback of electric signaling sequences were introduced as experimental tools for the investigation of IDI-based electrocommunication. This approach allowed to circumvent the difficulty in assigning EODs to their respective sender because the playback sequence is easily subtracted from the data to obtain the response sequence emitted by the fish. Additionally, a mobile dummy can be a closer mimic of a conspecific individual when compared with a stationary playback electrode, and may, therefore, provoke more natural behavior displays by the responding fish.

Two different setups were designed to investigate electromotor and locomotor behaviors of *M. rume* in response to playback of natural and artificial IDI-sequences that varied in overall discharge rate, patterning, or interactivity with the EODs emitted by the test fish. The setup that was developed and introduced in chapter 4 permitted only linear movement of the dummy. It allowed exact replication of test conditions, but, although the fish initiated the experimental trials, the dummy's movement and playback generation proceeded in an open loop that was not affected by the subsequent behavior of the fish. The setup used in chapter 5 allowed to close the feedback loop between the electro-communicating dummy and the test fish on two levels. The experimenter was able to modify the swimming trajectories of the dummy in real-time based on live video recordings of the experiment, and the dummy was able to emit playback EODs with a latency that corresponded to the echo response of *M. rume* each time the fish generated an EOD in proximity to the dummy.

In both cases, the mobile dummy reliably induced following-behavior in *M. rume* (Figure 4.3, Figure 5.3, and Figure 5.4). This behavior was maintained even after several turns and despite the fact that the dummy moved into open areas of the tank, which would usually be avoided by the fish. Following-behavior was to a significantly lesser extent also induced by the electrically silent dummy, partly even under non-visual conditions. The sensory cues a mobile dummy must provide to attract live *M. rume*, as well as the sensory systems mediating their behavioral responses, will be investigated in more detail in Part Two of this thesis.

Differential attraction of *M. rume* towards the mobile dummy based on variations of electric signaling sequences was, with a single exception (Table 4.1), hardly observed during the experiments. This invariance suggests that electrical communication signals already constituted a strong social stimulus independent of the exact sequence of their presentation (but see Teyssedre and Serrier (1986)). Unrewarded alternative-choice experimental designs, which require that the tested fish express a preference for one of two alternative signaling sequences, may, therefore, be better suited to uncover how subtle differences in IDI-patterning provide meaningful information about a signaling individual (Machnik and Kramer, 2008b).

Nevertheless, the analysis of the electromotor responses of the fish to the mobile dummy made it possible to identify electrical signaling strategies used by *M. rume* during electrocommunication. The results of the studies presented herein, as well as other work that has meanwhile been done on this subject, allow a better understanding of electric signaling strategies and enable interpretations concerning their potential function in an evolutionary adaptive context. The most apparent electrocommunication strategies of *M. rume* observed in this study were based on discharge regularization and double-pulse IDI-patterns, as well as on interactive discharge synchronizations mediated by the mormyrid echo response.

6.1 Double-pulse patterns

In *Mormyrus rume proboscirostris*, double pulses were first described as a social display by Gebhardt (2012), who interpreted them as a 'peaceful signal.' The social nature of double-pulse sequences in *M. rume* could be confirmed several times. Double pulses were generated only in response to either conspecifics or playback of electrical communication signals (Figure 4.8 and Figure 9.7) (Kersten, 2017a; b; Worm *et al.*, 2017; Worm *et al.*, 2018). However, the results presented in this thesis advocate an alternative explanation concerning the function of double pulses during electrocommunication. In response to playback of electrical communication signals, individuals with a higher rank within the social hierarchy responded by generating more double pulses (Figure 4.9), and most double pulses were produced in response to a double-pulse playback (Figure 4.8). The latter result could not be reproduced by Kersten (2017b), who did not observe higher amounts of double pulses in response to long duration double-pulse playbacks, possibly due to a research design that did not compare within-subject variation between the playback conditions.

Kersten (2017a) provided evidence that agonistic encounters between similarly sized pairs of *M. rume* were resolved in agreement with the predictions of the sequential assessment model for conflict resolution (Enquist and Leimar, 1983; Enquist *et al.*, 1990) and that they involve successive rounds of increasingly costly fighting displays. Here, double pulses were most abundant during the first phase of an encounter, and individuals that later won the contest produced significantly more double pulses at the beginning (Figure 4.24) (Kersten, 2017a). Double pulses are therefore suggested to be conventional signals of aggressive motivation that are displayed at the beginning of the sequential assessment of two opponents and have no additional signaling costs associated with their production

(Guilford and Dawkins, 1995). Behaviorally, they should be differentiated from discharge accelerations, which signal overt aggression in many mormyrids including *M. rume* (Bell *et al.*, 1974; Carlson and Hopkins, 2004b; Gebhardt *et al.*, 2012a; Kramer, 1976b). Accelerations were associated with direct attacks that occurred during later stages of a competitive encounter (Kersten, 2017a; Kupschus, 2017).

During noncompetitive encounters in groups of three individuals, Kupschus (2017) found no significant differences in double-pulse generation, neither at different time points of the encounter nor in relation to the relative dominance ranks of the group members. Similarly, in a comparison of natural and mixed groups of *M. rume*, there was no significant difference in the number of double pulses produced by individuals that differed in relative size or the order in which they left a shelter. There was, however, a tendency that larger individuals generated more double pulses in mixed groups, in which the formerly ahead swimming fish was replaced by a mobile, playback-emitting dummy (Pannhausen, 2017).

The criterion that was introduced to quantify double pulses in section 4.2.7, and which has been used during the analysis performed in all subsequent studies, is based on a simple threshold and thus cannot provide an in-depth characterization of double-pulse sequences. Double-pulse displays can take a variety of forms, and a more detailed analysis of this variation should provide further insights into the functions of this signal during electro-communication. For instance, if there is a correlation between the longer of the two alternating intervals and the size of the signaling fish, double pulses could contain reliable information concerning the relative fighting ability of the individual that generates the signal. Double pulses can be messy and appear somewhat uncoordinated (Figure 9.4B), but also highly regular with both the long and the short IDIs being remarkably constant over time (Figure 9.4A). Preliminary observations suggest that regular double pulses are generated by individuals approaching a stationary playback source (chapter 9, Kersten (2017b)), whereas double pulses of individuals that follow a mobile playback source or are engaged in agonistic interactions are more irregular (chapter 9, Kersten (2017a)). If the generation of regular double-pulse patterns during physical interactions is more difficult to coordinate for an individual, regularity of double-pulse sequences during social interactions could provide reliable information concerning a senders quality and might be perceived to be more attractive to reproductive females than irregular double-pulse displays. Finally, the duration of both long and short IDIs of a double-pulse sequence can be modulated dynamically, generating a higher-order pattern in the process (Figure 4.13A). Such patterns could have more subtle functions during electrocommunication and have not been differentiated by the broad definition of double pulses used in research studies so far. Additionally, information may also be contained in, or even be dependent on, the order of distinct social signaling patterns, particularly also regularizations, accelerations or discharge cessations. The order in which acoustic playbacks were presented to Japanese great tits (*Parus minor*) influenced the reaction of these birds to communication patterns that each contain a distinct message when presented independently, which led the authors to suggest the presence of compositional syntax in this animal communication system (Suzuki *et al.*, 2016).

Similar to the function proposed for double pulses in *M. rume*, 'scallops' have been suggested to be an IDI-pattern that is used by *Brienomyrus brachyistius* during electrocommunication in dominance-related situations (Carlson and Hopkins, 2004b). It would, therefore, be interesting to find out whether double pulses are generated centrally by the same neuronal nuclei that were shown to be responsible for the initiation of 'scallops' in *B. brachyistius* (Carlson and Hopkins, 2004a) (see section 1.3, Figure 1.6 and Figure 1.7). It would also be conceivable that individual *M. rume* differ in their style of double-pulse signaling, which could provide identity information analogous to the function proposed for 'scallops' in *B. brachyistius* (Baker *et al.*, 2016). Functional similarity of two signaling displays that differ among mormyrid species is also supported by behavioral observations by Worm *et al.* (2018) (chapter 9) in *M. rume,* and Moller *et al.* (1989) in *B. niger*. In both cases, fish that were approaching a source of electric communication signals fell electrically silent for several seconds before engaging in characteristic electrical 'rebound activity,' which was represented by very regular double-pulse patterns in the case of *M. rume*.

6.2 Regularization of discharge sequences

A sensory function of discharge regularizations during active electrolocation has been demonstrated several times (Arnegard and Carlson, 2005; Hofmann *et al.*, 2014; Toerring and Moller, 1984; von der Emde, 1992). Regularizations were, however, also interpreted as an important discharge pattern in the context of electrocommunication, because they occur in social situations and in response to electrical signals (Bell *et al.*, 1974; Moller and Bauer, 1973; Moller *et al.*, 1989; Terleph, 2004). The results presented in the preceding two chapters support this interpretation for *M. rume*. Figure 4.6 and Figure 4.12 show that discharge regularizations by the fish were particularly strong in response to playback of constant discharge frequencies within a range that corresponds to the discharge rates of freely behaving *M. rume* during social interactions (Kersten, 2017a; Kupschus, 2017; Pannhausen, 2017). The fact that animals regularized their intervals at the exact frequency of these exaggerated, artificial regularization patterns (or multiples thereof), and did not regularize at some individual discharge rate, suggests an involvement of echoing in the production of regularization displays. Indeed, long sequences of discharge synchronizations were observed at a response latency corresponding to the echo response in *M. rume* (Toma, 2014b) (Figure 4.17). It also suggest that both regularizations and echoing are important signaling strategies during electrocommunication. In *Gnathonemus petersii*, who displays a very sharply delineated echo response at a preferred latency of 12 ms (Russell *et al.*, 1974), artificially generated echoes resulted in strongly regularized discharge patterns at an IDI of 24 ms (Prume, 2015a). While this may have resulted from an innate preference of *G. petersii* to regularize their discharge activity at the corresponding frequency, experiments with modified echo latencies suggested that echoing is involved in mutually synchronized regularization displays of two fish (Kersten, 2016).

During the experiments with *M. rume*, fish always responded with a certain degree of discharge synchronization that exceeded correlations that would be expected for independent IDI-series. On the other hand, constant playback frequencies led to an almost mechanical locking of discharges by the fish to the playback. Echoing of a conspecific's EOD might therefore be of special importance during discharge regularizations that are displayed during the assessment of a potential opponent. The idea that discharge regularizations are important during an early stage of sequential assessment is supported by their association with lateral displays, and by the observation that both discharge regularizations and lateral displays occurred significantly more often at the beginning of a dyadic contest in *M. rume* compared with later stages of the encounter (Kersten, 2017a). A similar decline of the two displays was also observed in noncompetitive encounters in groups of three *M. rume* (Kupschus, 2017). Discharge regularizations may thus have ritualized from a mechanism that guarantees a high temporal resolution during active electrolocation into a social assessment strategy (Terleph, 2004). They may in this respect be compared with the assessment of dominance relationships through visual staring in humans (Kalma, 1991), which also gathers information while simultaneously sending a signal.

During discharge regularizations in mormyrids, the high temporal resolution of active electrolocation should facilitate the assessment of an opponent's size and fighting ability. Since discharge regularizations are frequently observed in association with lateral displays (Bell *et al.*, 1974; Terleph, 2004), competing individuals are likely close enough to impair each other's active electrolocation ability through jamming (Heiligenberg, 1977). Mutual synchronization of regularized discharges mediated by the echo response would therefore not only facilitate mutual assessment through active electrolocation, but also ascertain that the respective other individual is aware that it is being assessed (see section 6.3). The duration of regularized discharge sequences during confrontations with a potential opponent could thereby reflect the confidence of a signaling individual to win an escalated fight over a resource. It is therefore suggested, that discharge regularizations represent an advanced, but still early stage of sequential assessment. This hypothesis would be supported, if more dominant individuals maintained discharge regularizations for longer periods of time, or if the duration of regularization periods could predict the outcome of a contest, which was not the case during the contest experiments performed by Kersten (2017a). However, in the second phase of these experiments the designated winner of the contest regularized their discharge activity significantly more strongly to the signals of the later loser than vice versa. This could have reflected a situation, where the subordinate animal already refrained from engaging in discharge synchronizations that lead to mutually regularized intervals.

Prolonged periods of regularized discharge activity could also provide clues to another individual's physical condition because of the higher energy expenditure required for signaling at higher rates (Markham *et al.*, 2016). This relationship may explain the occurrence of discharge regularizations in the context of reproduction in several mormyrids (Baier and Kramer, 2007; Bratton and Kramer, 1989; Werneyer and Kramer, 2005), during which it could serve as a reliable signal that is constrained by a signaler's physical condition.

6.3 Echo responses and discharge synchronizations

During the playback experiments with *M. rume* in chapter 4, all animals responded with a characteristic combination of a preferred latency of about 20 ms and a preceding period of latency avoidance (Figure 4.15). This means that the fish generated echoes to the playback EOD, and this occurred in response to all playback sequences (Figure 4.16). Consequently, echoing could not be assigned unequivocally to a particular behavioral context based on the different playbacks that were emitted by the dummy.

Echoing of a conspecific's EOD has been considered to be a jamming avoidance strategy that prevents signal overlap of nearby individuals during active electrolocation (Heiligenberg, 1977; Schuster, 2001). However, since the magnitude of electric dipole fields diminishes with the third power of distance, active electrolocation is restricted to a radius of about one body length around the fish (von der Emde, 1999). The inter-fish distances at which jamming could occur is thus far shorter than the 30 cm radius that defines the range at which echoing starts to occur (Russell *et al.*, 1974). Due to the short duration of the EOD in relation to the IDI, extended series of overlapping EODs, which would actually impair the faculty of active electrolocation (Heiligenberg, 1977), seem rather unlikely. In fact, *G. petersii* was not impaired in its active electrolocation ability when jammed by a conspecific (Schumacher *et al.*, 2016b). Social functions of echoing have therefore been considered by several authors (Arnegard and Carlson, 2005; Kramer, 1974; Lücker and Kramer, 1981; Russell *et al.*, 1974). Social situations that require a high temporal resolution of active electrolocation are the competitive lateral displays that are accompanied by strongly regularized and mutually synchronized discharge displays (see section 6.2). In these situations, competing individuals are spatially close enough to impair each other's capability to actively electrolocate, and a correct assessment of each other's fighting potential would be of mutual interest because it allows settling a conflict without an escalated fight (Maynard Smith and Price, 1973).

The results presented in this thesis suggest that social functions of echoing and jamming avoidance during active electrolocation are not mutually exclusive. An essential feature of social signaling is to communicate a sender's location. It was shown by Schluger and Hopkins (1987) that mormyrids can use their electrosensory modality to approach a signaling conspecific despite the fact that electric dipole fields do not provide unequivocal directional information that could be used by the fish for a direct approach (Hopkins, 2005). The results presented in chapter 9 of this thesis expand these findings to a moving signal source and provide evidence that passive electric sensing via the knollenorgan pathway is an important sensory basis also for spatial interactions between mormyrids during electrocommunication (Worm *et al.*, 2018). Echoing of a conspecific's EOD may consequently not only avoid jamming of the active sensory system but also make sure that a conspecific's signals do not coincide with the inhibition of knollenorgan inputs through corollary discharges during active signal generation (Bell and Grant, 1989). Echo responses may thus also constitute a strategy to avoid jamming of the passive electrosensory modality that detects social signals via the knollenorgan pathway.

At the same time, echoing will also guarantee that a sender, who generates echo responses, places its EOD after the end of the period during which a receiver's knollenorgan afferents are blocked, but before the next signal generated by the receiving individual. In this respect, echoing may be very similar to the concept of the 'electrosensory refractoriness avoidance response' proposed by Guariento *et al.* (2014) for South American gymnotiform *Gymnotus*. In addition to jamming avoidance in the sender's active and passive electrosensory systems, refractoriness avoidance through echoing might guarantee that the other individual will detect the signal generated by the sender. This refractoriness avoidance entails the possibility that the receiving individual will notice the fact that it is subject to social intentions by the fish that produces echo responses. In other words, echoing avoids jamming of the knollenorgan pathway of an approaching individual, while simultaneously assuring that the other individual realizes that it is being approached. Since this works in both directions, the approaching individual will also be aware of being detected once the approached individual starts generating echo responses of its own. The result would be a closed feedback loop in an electromotor 'action-response' communication system (compare Figure 3.1, Hurd and Enquist (2005)), which may allow individuals to mutually allocate social attention during electrocommunication.

This interpretation is consistent with the findings from chapter 5, where it was shown that artificially generated echo responses evoked more echoes in *M. rume* compared with electrical playback of random pulse sequences (Figure 5.7). Similar results have also been obtained in experiments with *Gnathonemus petersii* (Prume, 2015a). Interactions of the electromotor behavior of two individuals thus seem to be a fundamental signaling strategy during electrocommunication. This notion is further supported by the observation that the magnitude of electrical discharge synchronizations was correlated with geometric interrelations between the synchronizing fish and could be associated with social interactions and approach configurations. When *M. rume* encountered a mobile dummy that emitted static playback sequences with random intervals between EODs (chapter 5), the discharge synchronizations the fish engaged in were on average strongest when it followed the dummy from behind, in a parallel orientation (Figure 5.13), and at a distance corresponding approximately to the outer limit of active electrolocation (Figure 5.12). In experiments involving more than one fish in addition to the mobile dummy (chapter 11), episodes of relatively strong discharge synchronization by *M. rume* frequently occurred in behavioral situations during which the individual that initiated synchronization approached either the dummy or a conspecific in the process (Figure 11.14 to Figure 11.20). It is thus suggested that echoing provides a means to address a particular individual electrically by placing EODs into the sensitive window of the designated receiver. This ability might be particularly useful during electrocommunication in groups, where electrical noise is imposed on dyadic social interactions by the signaling activity of conspecifics. It has, however, also implications for the complexity of social interactions and their dynamics in electrocommunicating mormyrids.

Mormyrids have been shown to be capable of individual recognition based on the waveform of an individual's EOD (Hanika and Kramer, 2005), and were also shown to possess relatively advanced cognitive abilities. This latter assessment is based both on the amount of resources devoted to their relatively large brains (Nilsson, 1996; Sukhum *et al.*, 2016), as well as their performance during various kinds of discrimination tasks involving associative learning by conditioning (Schumacher *et al.*, 2016a; von der Emde and Fetz, 2007).

While extended brain size can in part be attributed to the requirements of processing actively acquired electrosensory information (Finger *et al.*, 1981), the ecological constraints of navigating complex environments at night (see section 1.4) may also have favored cognitive flexibility in mormyrids.

Both *M. rume* and *G. petersii* are invertivores that mostly prey on insect larvae (Kouamélan *et al.*, 1999; Nwani *et al.*, 2011). However, field reports from predatory *Mormyrops anguilloides* have shown that these mormyrids gather in relatively stable groups and hunt in packs for small cichlids (Arnegard and Carlson, 2005). Based on their observations, Arnegard and Carlson (2005) hypothesized that mutual synchronization of bursts through echoing allows 'mutual acknowledgement of recognition' between individuals of the group. Jamming avoidance in the knollenorgan pathway could in these situations facilitate undisturbed mutual identification of individuals based on differences in EOD-waveform, or the exchange of dominance related waveform information (see section 3.5.1) between unfamiliar individuals to determine hierarchy ranks without fighting (Parker, 1974). It would in this respect be interesting to test whether there is a relationship between dominance and EOD duration in *M. rume* as well, and if so, whether individual fish are more likely to attack a dummy playing back EODs that are shorter than their own signals, compared with a dummy emitting longer EODs.

Collective, coordinated and collaborative hunting strategies of varying degrees of complexity have been documented for several species of fish (Arnegard and Carlson, 2005; Bshary *et al.*, 2006; Herbert-Read *et al.*, 2016; Lönnstedt *et al.*, 2014; Merron, 1993; Strübin *et al.*, 2011), and it becomes increasingly evident that such capabilities are no unique feature of mammalian predators (Brosnan *et al.*, 2010; Bshary *et al.*, 2014; Dinets, 2017). The observation of nocturnal pack-hunting in *Mormyrops anguilloides* involving burst synchronization has interesting implications for the significance of echoing during mormyrid social behavior. The ability to synchronize electric signals with conspecifics, combined with the capability to recognize individuals based on the waveform of their EOD, may have served as a foundation for the evolution of cognitive capacities and promoted some form of 'Machiavellian intelligence' (Bshary, 2011) in mormyrids. Echoing may thus enable mormyrids to perform social behaviors otherwise restricted to animals with more advanced cognitive capacities. One such capacity is vocal imitation, which is quite rare among animals (Fitch, 2000; Hauser *et al.*, 2002). Exceptions are dolphins and parrots, who are widely believed to possess advanced cognition and can use learned vocal labels to address specific individuals by imitating their calls (Balsby *et al.*, 2012; King and Janik, 2013). In analogy, mormyrids would need to be able to imitate either EOD-waveforms or IDI-sequences specific for different individuals, a behavior for which there is no evidence. However, echoing allows to match signaling sequences of another individual with high temporal precision, and the echo response may thus enable mormyrids to address another individual within a group without the necessity of a capacity for imitation. Although the neuronal correlates of echoing have not yet been identified in detail, the involvement of more than a few synapses is unlikely because of the short latency between stimulus and response EOD (Russell *et al.*, 1974). The echo response may, therefore, be a simple mechanism that allowed the evolution of complex social interactions not frequently observed at the taxonomic level of fish.

In conclusion, the preceding sections provided substantial evidence for communicative functions of double pulses, regularizations, and discharge synchronizations mediated by echoing of a conspecific's EODs. Double pulses may represent the first stage of sequential assessment during which they communicate aggressive motivation to a potential competitor. Discharge regularizations, in conjunction with lateral displays, and aided by echo responses, seem to constitute an advanced but still early stage of a competition, during which opponents assess each other's fighting potential through active electrolocation. Echoing may also allow performing such interactions within a group by addressing a specific individual and may thus be an electromotor basis for complex social interactions by enabling mutual allocation of social attention in mormyrid weakly electric fish.

III. Part Two: Biomimetics, Ethorobotics, and Mixed Societies

7. Introduction: Ethorobotics

Nature has stimulated human creativity for centuries and served as a source of inspiration that triggered advances in science and technology. Living organisms evolved specialized adaptations and strategies that allowed them to develop ecological niches and survive in challenging environments. Many of these adaptations represent natural solutions to specific problems that organisms had to solve on an evolutionary timescale, and which can be applied to technical problems or inspire technological innovation. An early example is Leonardo da Vinci's (1505) famous—but unsuccessful—attempt to translate his studies on the flight of birds into a flying machine actuated by human muscle power. Nevertheless, the systematic investigation of biological principles and adaptations to solve technical problems is a relatively recent endeavor, which eventually originated the interdisciplinary field of bionics. As a research discipline, bionics aims to apply the results of basic research on biological structures, mechanisms, and processes to technical problems by using them as an inspiration for innovative technology applications and solutions (Nachtigall, 1998).

Bionic principles can be applied to the development of structures and mechanisms, sensory systems and information processing, as well as behavioral adaptations and strategies. The intense study of the nanostructure of water-repellent biological surfaces has provided a detailed understanding of the principles underlying superhydrophobicity, and allowed the design of biomimetic self-cleaning and drag-reducing surface materials (Barthlott *et al.*, 2016). Similarly, the combination of strong adhesion and easy separation of the gecko's feet, which allows these animals to navigate even upside down on smooth surfaces, has inspired researchers to understand the structural and mechanic principles underlying this ability and to create materials with similar properties (Autumn and Puthoff, 2016).

Nature has originated a tremendous wealth of sensory systems and information processing strategies, many of which allow the detection of stimulus qualities alien to human perception. Biological sensory systems are the result of hundreds of millions of years of fine tuning by natural selection and have evolved to perform highly specific tasks, thus bearing a great potential to be translated into technical applications (Barth *et al.*, 2012). Examples are infrared detectors based on the infrared-sensitive sensilla of pyrophilous beetles (Schmitz and Bousack, 2012), detection and processing of hydrodynamic information by artificial lateral line systems (Bleckmann *et al.*, 2012), and the adaptation of the principles of active electrolocation of weakly electric fish (von der Emde *et al.*, 2009).

Bionics can also utilize various natural behavioral strategies and adaptations. An extensive field of research is the investigation of natural locomotion strategies and their control, which is frequently studied by constructing biomimetic robots that are designed to navigate environments on land, in the air, or underwater. Examples are the design and control of six-legged locomotion by insect robots (Dürr *et al.*, 2004), and the biomimetic quadruped robot inspired by a running cheetah (Seok *et al.*, 2015). Other robots were designed to mimic movement strategies of animals that cope with challenging environments, like snakes that master the ascend of sandy slopes (Marvi *et al.*, 2014), or salamanders that can walk, crawl, and swim in wetland environments (Crespi and Ijspeert, 2009). In the air, flapping wings can today actuate robotic birds (Mackenzie, 2012) and insects (Ma *et al.*, 2013). Biomimetic underwater locomotion can be driven by carangiform oscillations similar to swimming fish (Barrett *et al.*, 1999). But roboticists have also adapted the locomotion strategies of floating jellyfish (Guo *et al.*, 2007), anguilliform lampreys (Stefanini *et al.*, 2012), jetting octopuses (Sfakiotakis *et al.*, 2015), as well as the fin-undulations of manta rays (Li *et al.*, 2017) and cuttlefish (Wang *et al.*, 2011) for robotic actuation. Such biomimetic and bioinspired research not only considers alternative ways of propulsion and locomotion that are potentially suitable for performing useful tasks in remote and inaccessible environments, but it also originates innovative and energy efficient technical components, materials, and procedures for technical applications. Other behavioral strategies with potential for technical implementations are related to communication and group dynamics. The study of animal flocks, herds, and swarms (see chapter 11) has inspired ambitious scientific endeavors such as the interdisciplinary CoCoRo-project, which aims at creating a self-aware swarm of autonomous underwater robots (Schmickl *et al.*, 2011).

7.1 Electric fish as a source of bioinspiration

Electric fishes have long been a source of inspiration for technical inventions (Bleckmann *et al.*, 2004; Caputi, 2017; von der Emde *et al.*, 2009). While the original invention of the electric battery was inspired by strongly electric fish, its design was not based on a real understanding of bioelectricity (Finger and Piccolino, 2011a). Today, electric eels (*Electrophorus electricus*) continue to be a model for biocompatible sources of electricity that may power next-generation medical implants (Schroeder *et al.*, 2017). The principles of active and passive electrolocation have been adapted to endow mobile robots and sensory probes with the capacities for object detection, navigation, and orientation (see section 8.2.2). Active sensing strategies of weakly electric mormyrid fish *Gnathonemus petersii* have inspired the development of a catheter system that could be used to detect and classify atherosclerotic plaques in human blood vessels (Gottwald *et al.*, 2017b). The capacity to locate moving prey using actively generated electric signals also involves specialized locomotion strategies. Gymnotiform knife fish (*Apteronotus albifrons*) exhibit high maneuverability even at low speed due to an undulating ribbon-fin, which may equip robotic underwater vehicles with similar locomotor flexibility (MacIver *et al.*, 2004).

7.2 From biomimetics and bioinspiration to ethorobotics

The cooperation of engineers and roboticists with behavioral biologists, enabled by technological advances in miniaturization and computation, has originated the novel and interdisciplinary research field of ethorobotics. The use of robotic devices in behavioral biology bears great potential for the study of complex aspects of animal behavior, which cannot be reliably controlled in live individuals (Krause *et al.*, 2011). But researchers from technological and biological disciplines are confronted with different challenges and may approach this collaboration with different motivations. For an engineer, the implementation of bioinspired mechanisms and the solution of associated technical problems constitute major challenges, whereas biologists may be more interested in understanding the biological principles and mechanisms underlying a certain behavior. Engineers may rely on knowledge acquired through biological research to adopt a particular capability of a model organism and implement this capability into technical devices and applications. On the other hand, robotic devices can be useful tools to develop, test, and refine hypotheses in behavioral biology (Klein *et al.*, 2012; Webb, 2000). From a scientific point of view, even failed attempts to implement a particular bioinspired performance into a technical device of similar size as the natural model, can lead to new discoveries and a better understanding of the physical constraints an animal is faced with in its natural habitat, because roboticists have to solve similar problems when designing a replica as did evolution (Webb, 2000). In this respect, robotics research provides an advantage compared with theory-driven research and simulations, because roboticists get the 'laws of physics included for free' (Mitri *et al.*, 2013), which provides them with a testing ground for feasibility and efficiency of their models. Additionally, it is always clear what is hard-wired and thus 'innate' in a robot (Webb, 2000). A robot 's behavior is under the experimenter's control at any time, assuring exact repeatability and standardized conditions, particularly in behavioral experiments where robots are used to trigger responses from live animals (Krause *et al.*, 2011). Thus, robots can be used to test hypotheses regarding the control of animal behavior under realistic conditions (Webb, 2000), but they may also serve as research tools in explorative studies that eventually lead to the development of new hypotheses, which can subsequently be tested using real organisms (Mitri *et al.*, 2013).

As a biological research discipline, ethology is concerned with the study of animal behavior and aims at unraveling the key stimuli and principles that trigger innate, stereotypical behavioral responses in animals. For this purpose, scientists have long used simple lures and decoys as dummies to set up behavioral experiments, and the systematic reduction of a replica is a well-established method to identify releasing mechanisms for innate behavior patterns (Lissmann, 1932; Tinbergen, 1948). Particularly useful tools for such experiments are playbacks of behavioral displays, which can be presented for example as acoustic, visual, or electrical stimuli, and allow to restrict stimulus presentation to a particular sensory channel (Kramer, 1979; McGregor, 2000). Robots offer the opportunity to design interactive and multimodal experimental designs, which allow determining how cues from different sensory systems contribute to a particular behavior (Krause *et al.*, 2011).

Robotic animal models have for example been used to study multimodal courtship signals in túngara frogs (*Engystomops pustulosus*) (Taylor *et al.*, 2008), adjustment of male courtship displays to the response of female bowerbirds (*Ptilonorhynchus violaceus*) (Patricelli *et al.*, 2006), and to test alternative hypothesis regarding the female preference for synchronous courtship waving in fiddler crabs (*Uca mjoebergi*) (Reaney *et al.*, 2008). In other projects, robotic models were used to study multisensory contributions of visual and acoustic signals to the alarm behavior of wild tree squirrels (*Sciurus carolinensis*) (Partan *et al.*, 2009), the cross-modal integration of visual and acoustic cues that elicit aggression in male dart-poison frogs (*Epipedobates femoralis*) (Narins *et al.*, 2005), and to disentangle the meaningfulness of visual displays for different receivers with regard to aggression or mate choice in southern sagebrush lizards (*Sceloporus graciosus*) (Martins *et al.*, 2005).

Fish are popular model organisms in studies on animal-robot interactions because of the tendency of many species to form shoals (Pitcher and Parrish, 1993) and the vast amount of stereotyped behavioral displays that have already been described (Oehlert, 1958; Simpson, 1968; Tinbergen, 1952a). Species such as zebrafish (*Danio rerio*) or sticklebacks (*Gasterosteus aculeatus*) are widely recognized model organisms and are frequently kept in the laboratory (Cianca *et al.*, 2013; Faria *et al.*, 2010). Scientific interest in the behavior of other fish species may also be warranted by commercial considerations (Kruusmaa *et al.*, 2016). More examples of ethorobotical research projects involving fish are referenced in sections 4.1, 5.1, 8.1, and 11.1.3.

7.3 Ethorobotical concepts for the manipulation of animal behavior

The results of ethorobotical research projects can in many instances be used for secondary applications involving commercial interests, or to further promote scientific understanding of behavioral principles. Profound knowledge of the mechanisms underlying behavior patterns can be exploited to control animal behavior in various contexts, such as fishing or farming, conservation, or during scientific experiments. Researchers have proposed innovative ways to manage livestock using robotic sheepdogs (Vaughan *et al.*, 2000), virtual fences (Butler *et al.*, 2006), or social control of animal groups by manipulation of individual group members with wearable devices (Correll *et al.*, 2008). Similarly, Rossi *et al.* (2013) explored the potential of using robotic dummies to control the swimming patterns of farmed fish. So-called Robirds, which are remote-controlled models of the peregrine falcon, are successfully used for bird control at airports because birds do not habituate to the combination of moving silhouette and flapping wings that is exhibited by the robotic birds of prey (Folkertsma *et al.*, 2017). Biomimetic aerial or underwater vehicles may also provide access to complex or hostile environments, for instance, to gather military intelligence (Mackenzie, 2012; Rufo and Smithers, 2011). In the laboratory, biomimetic robot animals may be used to develop standardized behavioral paradigms, for example, to evaluate the effects of pharmacologically active substances on zebrafish (Cianca *et al.*, 2013; Spinello *et al.*, 2013) and rats (Shi *et al.*, 2010).

An interesting development from ethorobotics experiments is the concept of a mixed society consisting of real animals and artificial agents, the latter of which can infiltrate the animal group and are programmed to dynamically interact with the animals (Mondada *et al.*, 2013). This methodology enables closed-loop experiments with more sophisticated behavioral sequences compared with simple dummies, because robots can react to input generated by animals (Mitri *et al.*, 2013). Such mixed systems may then originate emergent behaviors observed in neither of the original systems (Halloy *et al.*, 2013). Establishing mixed societies takes the study of behavior from an individual-based level to the investigation of collective capabilities in animal groups. This approach is based on the identification of local interaction rules between individuals and the formulation of models that link these rules to emergent, self-organized behavior patterns at the collective level, e.g., via positive feedback mechanisms (Deneubourg and Goss, 1989). Subsequent integration of robotic nodes, which comply with these rules, into animal groups then allows to change these interaction parameters locally, and thus to observe their effect on global behavior patterns at the group level (Mondada *et al.*, 2013).

Despite keen interest in this area, research projects that have established a mixed society in a strict sense are still not very numerous. The most seminal contribution is a study by Halloy *et al.* (2007), who were able to socially integrate mobile robots into groups of cockroaches and change the preference of these insects for dark shelters in favor of lighter ones. This was achieved by biasing the robots behavioral algorithm in favor of the lighter shelters. A major challenge when trying to socially integrate robots into animal groups is to design a functional robot that is accepted as a conspecific individual by members of the group, and must, therefore, incorporate the critical stimuli for social behavior (Mondada *et al.*, 2013). In the cockroach-experiment, this social acceptance was mediated by olfactory cues and did not presuppose any visual resemblance of the robots to a cockroach (Halloy *et al.*, 2007). Other studies have relied on different mechanisms and sensory systems for social integration of robots into animal groups. Gribovskiy *et al.* (2010) exploited the innate filial imprinting mechanism of chickens (*Gallus gallus domesticus*) to integrate a mobile robot, which could subsequently influence the animal's behavior. Landgraf *et al.* (2012b) designed a robot that imitated the waggle dance of honey bees (*Apis mellifera*) to investigate how bees decode foraging information from fellow workers. Other studies have highlighted the importance of visual cues, such as realistic eyes for social attraction in guppies (Landgraf *et al.*, 2016), and it was shown that a mobile rover could infiltrate a group of penguins (*Aptenodytes patagonicus*) when it was disguised as a penguin chick (Le Maho *et al.*, 2014). Most recently, Bonnet *et al.* (2018) established a mixed society with live zebrafish using simple fishing baits to attract the animals and manipulate their behavior.

These studies on animal–robot interaction have shown that exact biomimetic replication is not necessarily a prerequisite for a robotic agent to be accepted as a conspecific by live animals. Instead, many species appear to rely on key stimuli, which can be identified in hypothesis-driven stimulus-response experiments and can subsequently be exploited in closed-loop experimental setups. The following two chapters test the assumption that mormyrid weakly electric fish might be particularly well suited as model organisms for the formation of a mixed society, because of their ability to interact and communicate electrically. Electrical signals can be used to trigger social responses (chapters 4 and 5) and may thus serve as the key stimuli that enable a mobile dummy to be accepted as a conspecific by live fish. In chapter 8, the design of a biomimetic weakly electric *Mormyrus rume* will be presented to test this hypothesis. The robot mimics live individuals in size, shape, motility, and electric signal generation. By combining motility cues with electric signaling displays, it will be shown experimentally that the latter are the more important attraction feature of the robot, both for single individuals and small groups of live *M. rume*.

Based on these results, the mobile robot will be systematically reduced to a moving playback source in chapter 9. By experimentally excluding stimuli from vision, the mechanosensory lateral line system, and eventually active electrolocation, it will be shown that *M. rume* can rely exclusively on its passive electrosensory system, most likely mediated by the knollenorgan pathway (see section 1.3), to track a mobile source of electrical communication signals. Thus, social interactions among mormyrids can be induced and mediated by passive reception of electrical communication signals without the need for direct perception of the location of the signal source through other senses. Electrical playback of the mormyrid EOD is, therefore, a powerful tool to socially integrate robotic dummy fish into groups of live mormyrids. This makes mormyrid weakly electric fish a unique model for the study of social dynamics in mixed societies of live and artificial fish.

8. Project 3: Investigation of Collective Behavior and Electrocommunication in the Weakly Electric Fish, *Mormyrus rume*, through a Biomimetic Robotic Dummy Fish

This chapter is based on my contribution to:

Donati, E., Worm, M., Mintchev, S., van der Wiel, M., Benelli, G., von der Emde, G. and Stefanini, C. (2016). Investigation of collective behaviour and electrocommunication in the weakly electric fish, *Mormyrus rume*, through a biomimetic robotic dummy fish. *Bioinspiration & Biomimetics* 11: 066009.

8.1 Introduction

Interactive robots are increasingly used to investigate animal behavior in experimental biology (Frohnwieser *et al.*, 2016; Krause *et al.*, 2011). In the classical studies that originated ethology as a research discipline, animal models were systematically reduced to simple stimuli to identify the key features that trigger stereotyped behavior. The observation that aggressive behavior in male sticklebacks (*Gasterosteus aculeatus*) is primarily triggered by the perception of the red coloration on the ventral side of a supposed opponent describes one of the most widely regarded examples of a so-called social releaser (Tinbergen, 1948). Incorporating such stimuli into robotic devices allows to systematically investigate the significance of different stimulus features in elaborated and standardized behavioral protocols (Klein *et al.*, 2012), as well as closing the feedback loop between stimulus presentation and behavioral reaction (Mondada *et al.*, 2013). Fish are popular model organisms in studies on animal–robot interaction because they display a large variety of stereotyped behavior patterns, and the tendency of many fish species to form shoals serves as a good indicator for a dummy's effectiveness in mimicking the relevant traits of a conspecific individual (Bartolini *et al.*, 2016; Butail *et al.*, 2013; Faria *et al.*, 2010; Kruusmaa *et al.*, 2016; Landgraf *et al.*, 2016; Polverino *et al.*, 2012; Romano *et al.*, 2017; Ward *et al.*, 2012). Robotic fish dummies are thus well suited to initiate and manipulate behavioral patterns in real animals and to unravel the key elements determining the expression of such behavior patterns in fish.

Stereotyped behavior in fish can be elicited by physical appearance, dynamics of movement patterns, and the generation of transient communication signals by a conspecific. Size, shape, coloration, and motility of fish dummies have been the subject of many studies that aimed to disclose the features that induce social behaviors and cause biomimetic robot fish to be attractive to live fish from a variety of species (Abaid *et al.*, 2012; Bonnet *et al.*, 2016; Kopman *et al.*, 2013; Polverino *et al.*, 2012; Polverino *et al.*, 2013; Polverino and Porfiri, 2013b). Biomimetic motility and locomotion were shown to be determinants of fish preference in several studies (Cazenille *et al.*, 2018; Landgraf *et al.*, 2016; Marras and Porfiri, 2012). These studies demonstrated that to successfully introduce a robot into a group of animals with the intention to manipulate natural behavior patterns, it is crucial to design the robot around cues that can cause its acceptance as a conspecific. Likewise, it must be designed to contain the stimuli that will trigger the behavioral patterns of interest (Mondada *et al.*, 2013). While most research, where dummies were used to investigate fish behavior, focused on visual or hydrodynamic cues, the current study exploits electrical signals as a communication strategy to trigger social responses in the mormyrid weakly electric fish *Mormyrus rume*.

Mormyrids communicate using electric signals, which can easily be brought under an experimenter's control through electrical playback protocols (Kramer, 1979) (see chapters 4 and 5). These fish produce short, pulse-type electric organ discharges (EOD), which are separated by inter-discharge intervals (IDI) of highly variable duration, resulting in temporal discharge patterns, which are associated with the animal's current behavior. Electric signals in mormyrids serve two purposes: they are used for active electrolocation (von der Emde, 1999), and they provide identity information about the sender (Hopkins, 1999) as well as behavioral information during electrocommunication (Gebhardt *et al.*, 2012a). The active space for electrocommunication thereby exceeds the range for active electrolocation due to the higher sensitivity of the respective receptor organs involved in communication (Bennett, 1971c). Many studies have demonstrated that electrical playback experiments are useful tools to study electrocommunication with respect to, e.g., territorial behavior (Hanika and Kramer, 2005), mate choice (Feulner *et al.*, 2009a), and the general attractiveness of specific signaling features (Kramer, 1979; Teyssedre and Serrier, 1986). In many fishes, shoaling behavior is mediated by vision and the lateral line system (Partridge and Pitcher, 1980), but mormyrids were shown to also rely on their electrosensory capabilities for group formation and coherence (Moller, 1976). The imitation of electrical signaling through playback experiments can, therefore, enable the acceptance of a dummy fish within a group of real fish, and makes mormyrids a novel model for the investigation of shoaling and group-communication (Worm *et al.*, 2014) (see chapter 11).

This chapter describes the development of an artificial dummy fish that mimics the weakly electric fish *M. rume* in shape, size, motility, and electric signal generation, and subsequently tests the acceptance of the dummy by live individuals in a set of behavioral experiments. The chapter is arranged into a robotic and a biological part. The main challenge from the roboticists' perspective was to integrate a tail-beat mechanism and the capability for electric signal generation and reception into a fish robot with the same morphology and dimensions as a live animal. The influence of tail movements and electric signal generation on the attractiveness of the dummy fish for single individuals and small groups of weakly electric fish is the subject of the second section. The results show that electric signal generation had a strong influence on the attractiveness of the moving dummy fish towards individuals and small groups of *M. rume*, but this effect did not depend on a particular tail-beat movement. This suggests that electrical signaling may be the most important stimulus triggering social interactions among mormyrids.

8.2 Design and implementation of the robotic dummy fish

From a biological point of view, the main objective of building a robotic dummy fish was to create a tool that allowed to disentangle the effects of morphology, naturalistic motion patterns, and electrical signaling on the release of social behaviors during interactive experiments with weakly electric fish. The robotic fish was designed in cooperation with the Biorobotics Institute of the Sant'Anna School of Advanced Studies in Pisa, Italy, where it was developed and manufactured at the Department of Creative Engineering Design. Technical specifications and validations are detailed in Donati *et al.* (2016). The dummy fish was designed around three central cues to (i) match the physical appearance of *M. rume* in size, shape, and color, (ii) exhibit forward movement and a tail-beat mechanism, and (iii) possess an artificial electric sense. The robot (Figure 8.1A) was 152 x 32 x 16 mm in size and was based on the morphology of a similarly sized specimen of *Mormyrus rume proboscirostris* (Figure 8.1B). It was composed of a rigid head, hosting the mechanism for tail oscillation (Figure 8.1D), and an actuated caudal section made of soft silicone rubber. The head of the dummy was connected through a rod to a linear stage that provided a forward motion to the fish during the behavioral experiments (Figure 8.1C). The physical appearance of the dummy was not systematically varied, and therefore its effect on the behavior of *M. rume* was not explicitly tested in this study. The tail-beat mechanism was designed to achieve oscillation frequencies of up to 3 Hz and deflection amplitudes up to 40 mm from the midline, enabling a systematic variation of combinations. The electric sense was implemented through a pair of electrodes, by which the dummy generated playback of electrical signaling sequences in the form of electric fields around its body. In addition, it was capable of recording electric signals in its surrounding in a bioinspired way via pairs of electrodes, which were strategically distributed along the body. The following sections describe the design of the dummy, focusing on the tail-beat mechanism and the integration of the electric sense.

8.2.1 Tail-beat mechanism and forward locomotion

Biomimetic tail-beat mechanisms have been designed with the aim to achieve propulsion efficiency and biomimetic locomotion and resulted in elaborate systems requiring a high degree of control for operation (Barrett *et al.*, 1999; Kumph, 2000). Here, forward locomotion was accomplished by tethering the dummy to a moving slide outside the tank (Figure 8.1C). The tail-fin movement was therefore not designed to achieve propulsion, but to generate a naturalistic motion pattern. The robotic fish was composed of a soft caudal section, actuated by a single DC motor (El Daou *et al.*, 2012) to mimic carangiform swimming locomotion. This mechanism resulted in a dummy fish with a simple design, with no joints, intrinsically waterproof, and easy to manufacture through silicone casting and 3D printing.

The tail-beat mechanism comprised two cables (Dyneema® fiber with a diameter of 0.26 mm) in an antagonistic configuration (i.e., one for each side of the body), which were molded inside the flexible body (Dragon Skin®, Smooth-On©, Pennsylvania, USA). The two cables were connected to a distal rib and routed through three intermediate ribs toward a pulley that was actuated by a DC motor (DC motor 210-002 from Precision Microdrives™, London, UK), which was located in the head of the dummy. The oscillation of the pulley led to the alternated pull and release of the two antagonistic cables, thus driving the oscillation of the caudal fin.

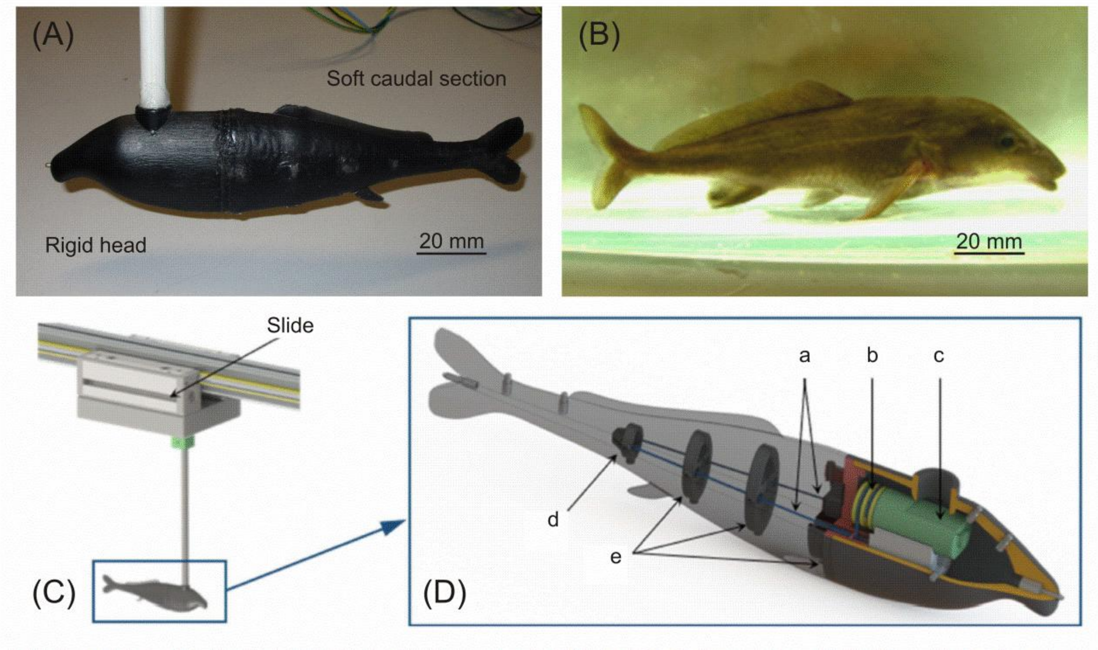

Figure 8.1: Design features of the mobile dummy fish. (A) Final design of the fully assembled dummy fish composed of a rigid head and a flexible caudal section, connected to a plastic rod to enable forward motion along a linear guide. (B) Live specimen of *Mormyrus rume proboscirostris*. (C) Schematic of the dummy fish connected to the linear guide. (D) Partial section of the main components of the tail-beat mechanism inside the dummy; a: antagonistic cables; b: pulley; c: motor; d: distal rib; e: intermediate ribs.

Externally to the dummy fish, a custom-built electronic board controlled the initiation of the tail-fin movement as well as the settings for tail-beat frequency and amplitude. Amplitude values were measured from the midline of the body to the maximum deflection of the tail. Two switches on the board sent input corresponding to the selected oscillation frequency and deflection amplitude to an Arduino ATmEGA microcontroller board, which sent an appropriate signal to the driver of the DC motor (L293 driver STMicroelectronics). The Arduino received a signal from the digital output of a CED (Power 1401, Cambridge

Electronic Design, Cambridge, UK), which was controlled by a computer running Spike2 (version 5.21, Cambridge Electronic Design, Cambridge, UK). Only when the trigger signal was on, the Arduino sent the commands to the driver and the motor was activated. This mechanism allowed synchronizing the movement of the dummy with electric playback generation within the experimental protocol. The wires of the motor were routed to the electronics outside the tank through a non-conductive rod connected to the head of the robot. The rod also connected the dummy fish to a linear slide (Schlitten LRF 8 D10 120 × 160, Item Industrietechnik GmbH, Solingen, Germany) that controlled the forward movement of the robot during the experiments (Figure 8.1C). The slide could be moved by powering a geared motor (Modelcraft RB350050-2273R, 12 V/50:1, not illustrated in Figure 8.1), to which it was connected via a cable linkage and a set of pulleys, and which was also controlled via the CED 1401.

8.2.2 Integration of an electric sense

The electroreceptive capabilities of weakly electric fishes are increasingly well understood (Caputi, 2017) and several research projects have been dedicated to the transfer of such perception abilities to robotic devices for underwater navigation (Boyer *et al.*, 2015; Boyer *et al.*, 2013; Lebastard *et al.*, 2010; Mintchev *et al.*, 2012), object detection and localization (Bai *et al.*, 2016; Bai *et al.*, 2015; Lebastard *et al.*, 2016; Lebastard *et al.*, 2012; Solberg *et al.*, 2008), as well as communication (Mintchev *et al.*, 2014; von der Emde *et al.*, 2012; Wang *et al.*, 2017). Here, an artificial electric sense was implemented into the dummy for the sole purpose of generating and receiving EODs.

As shown in Figure 8.2, the dummy was equipped with two playback electrodes (emitting electrodes) and eight sensing electrodes (receiving electrodes). These electrodes were made of stainless steel with a diameter of 1.5 mm. Conductive wires were soldered to the electrodes and routed from the body of the dummy to outside the tank through the plastic connection tube. The output of electrical playback signals occurred via electrodes S1 and S2 (Figure 8.2) and allowed approximating the dipole-shaped electric field along the longitudinal axis of the fish (Figure 8.4). Active electrodes S1 and S2 for the generation of electrical playback signals were integrated at the tip of the snout and at the very caudal end of the tail of the dummy to mimic the spatial properties of the electric field surrounding a weakly electric fish as closely as possible (Figure 8.4). Electrodes R1 to R6 were connected to a differential amplifier (Brownlee Precision Model 440, Palo Alto, CA, USA) and were designed to record electric signals in the surrounding of the dummy.

Inspired by a model by Hopkins (2005), four pairs of differentially recording electrodes were positioned in an orthogonal configuration as illustrated in Figure 8.3A. According to this model, weakly electric fish find a dipole source by aligning their body axis along the local electric field vector, which can be accomplished by turning into the direction of stronger stimulation and moving forward once receptors on both sides receive stimulation of the same magnitude (Figure 8.3B). The dummy would then find the signaling fish along the electric field vector by moving in order to minimize measured amplitude difference between electrode pairs R6L–R3L and R6R–R3R, i.e., by turning in the direction of the electrode pair that reports the higher value (Figure 8.3A). For as long as swimming backward is not an option, the dummy fish will approach the source when electrodes R1–R2 report a higher value than electrodes R4–R5. Recording electrodes should be deactivated during signaling via the playback electrodes. In live mormyrids, this is achieved by a corollary discharge mechanism in the brain (Baker *et al.*, 2013a). Figure 8.3B shows exemplary recordings by the left (L) and right (R) electrode pairs of a continuous sine wave stimulus emitted by the dipole source while continuously moving the dummy fish in parallel alignment to the stimulus electrodes on an orthogonal line connecting the positions drawn in Figure 8.3A. The dotted line shows the location of the dummy at the central position of Figure 8.3A. These results indicate that detection of and movement towards an active dipole source, e.g., the EOD of an electric fish, would be possible for a robotic dummy fish.

The EOD waveform of a specimen of *M. rume* was sampled as described in Gebhardt (2012) and assembled to an IDI-sequence pre-recorded from an animal that was foraging in a small group (Gebhardt *et al.*, 2012a). The resulting playback was transferred via a D/A-converter (CED Power 1401, Cambridge Electronic Design, Cambridge, UK) and an analog stimulus isolation unit (Model 2200, A-M Systems, Carlsborg, WA, USA) to the pair of electrodes integrated at the head and the tail of the dummy. To characterize the spatial extension of the dummy's electric field during playback emission, the dummy was placed at the center of a 60 cm x 30 cm tank. EODs were recorded with a spot electrode covering a 2 cm x 2 cm grid of the whole area in the central plane where the playback electrodes were located. Signal strength at a given location was calculated from the peak-to-peak voltage of the EOD at this position with positive values representing head-positive voltages. For comparison, the same measurements were conducted with a live specimen of *M. rume*, measuring 14.0 cm in standard length. The live fish was confined to a porous clay tube at the same position as the dummy during the measurements. Results normalized to the highest peak-to-peak value from each measurement are shown in Figure 8.4. Results were color-coded for the spatial distribution of signal amplitudes for the dummy (A) and the fish (B). Local amplitudes were more evenly distributed around the fish due to the resistive nature of its skin. In contrast, field amplitudes decreased more rapidly near the dummy, whose electric field essentially consisted of a dipole field emanating from the pair of stainless steel electrodes. The effect of this difference was less apparent with increasing distances from the signal source. Reference values of peak-to-peak voltages are reported at distances of 10 cm from the snout positions, demonstrating that the electric field generated around the dummy had about twice the strength of that of the living fish (Figure 8.4).

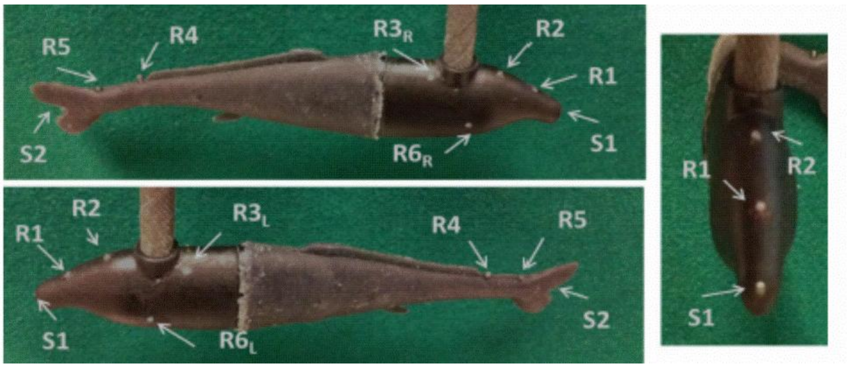

Figure 8.2: Electrode configuration of the mobile dummy fish. Arrows indicate the distribution of active playback electrodes (S) and recording electrodes (R).

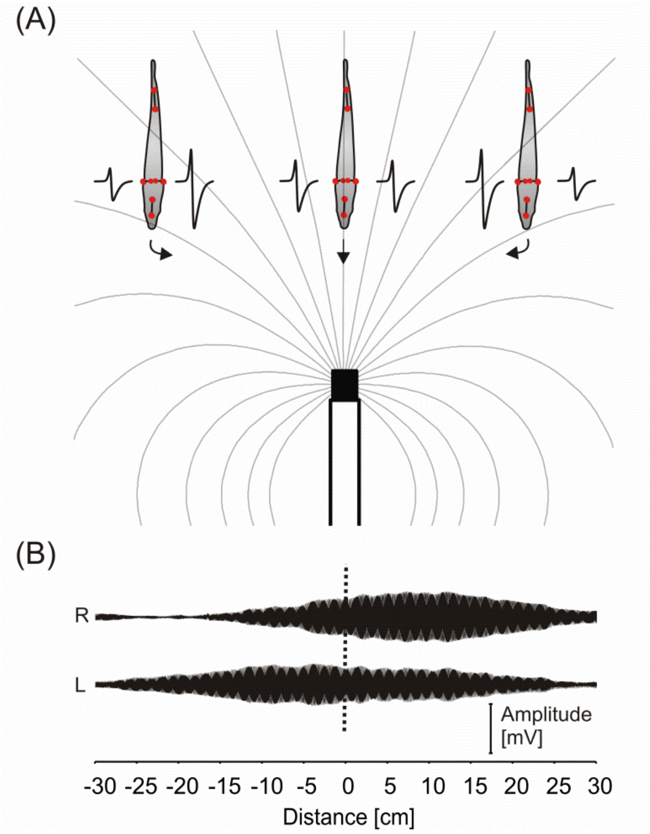

Figure 8.3: Schematic illustration of the proposed orientation mechanism towards an electrical dipole source. (A) The dummy would approach the dipole along the electric field lines by turning towards the lateral electrode pair (red) reporting higher signal amplitudes. (B) Recordings of the left (L) and right (R) electrode pairs of the dummy while moving it on a straight line orthogonal to the stimulus source, which was emitting a continuous sine-wave signal.

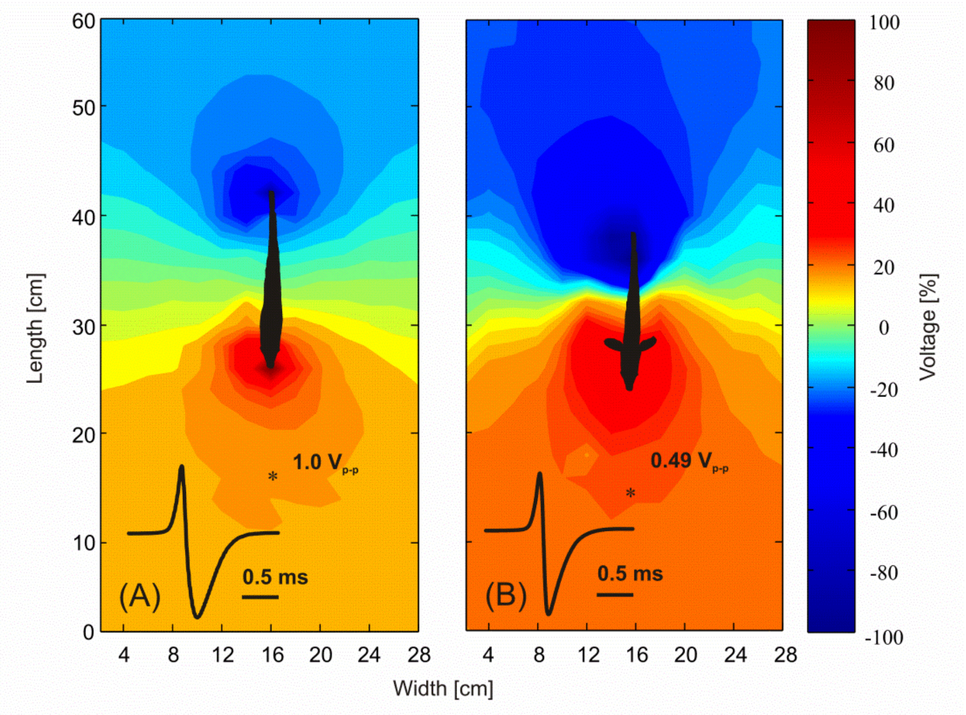

Figure 8.4: Electric field characteristics of the dummy and *M. rume*. Electric potentials measured in a horizontal plane around the dummy (A) and a similarly sized specimen of *M. rume* (B). Insets show the respective waveforms of real and artificial EODs. Asterisks mark reference values of peak-to-peak voltages measured at a distance of 10 cm in front of fish and dummy.

8.3 Experimental validation by behavioral experiments

8.3.1 Materials and methods

Here, the ability of the dummy to influence the behavior of single individuals and small groups of live *M. rume* was investigated using two types of stimuli: tail oscillations and electrical signaling. Behavioral experiments were performed with a total of 44 individuals of *M. rume* ranging from 6.4 cm to 17.6 cm in standard length. Fish were maintained at a light/dark cycle of 12/12 h with a water temperature around 25°C, and fed on a daily basis with defrosted *chironomid* larvae. The experimental tank had a base area of 200 cm x 50 cm with the water level at 20 cm. As illustrated in Figure 4.1, it was divided into a testing area and a living area, which were connected by a small gate. The living area was subdivided into a hiding area with shelters and an open area, which had to be crossed by the fish to reach the testing area. Animals were transferred to the experimental tank and acclimatized for at least 1 h before testing. The water temperature was kept at 25.0 ± 1.0°C and the water conductivity at 100 ± 5 µS cm^{-1} during all experiments. Experiments were triggered once a pair of electrodes within the open area registered the EOD of an animal. The dummy then started moving on a linear trajectory at an average speed of

0.11 m s^{-1} from its starting position at the gate through the entire testing area. All experiments were performed under visible light, which was provided indirectly by a pair of LED-Illuminators. Lights were regulated to yield an illuminance of approximately 10 lux directly above the center of the testing area (Light ProbeMeter™, 403 125, Extech Instruments), which was well suited for visual pattern recognition in the mormyrid *Gnathonemus petersii* (Schuster and Amtsfeld, 2002). Additionally, the testing area was illuminated with a pair of IR-illuminators (850 nm) to be monitored from above with an infrared-sensitive video camera (DBK 21AF04 FireWire Camera with Vari Focal T4Z2813CS-IR CCTV Lens, The Imaging Source, Bremen, Germany). Thus, it could be determined whether the dummy fish was effective in recruiting animals from the living area into the testing area. A virtual target line was defined at the level of the rear end of the dummy at its farthest position in the testing area (Figure 4.1). Animals crossing that line within 15 s after the onset of an experimental trial were counted as following the dummy.

Two sets of behavioral experiments were performed to discern the attractiveness of the various features of the dummy fish. In the first set, animals were divided among four experimental conditions that differed with respect to the dummy's tail-beat frequency and amplitude. These were: (i) 0 Hz and 0 mm (control), (ii) 0.5 Hz and 30 mm, (iii) 1.5 Hz and 10 mm, and (iv) 3 Hz and 5 mm. Each condition was performed with $n = 11$ individual fish. Given the constant forward movement of 0.11 m s^{-1}, these values resulted in Strouhal numbers of 0.27 for conditions (ii)–(iv), which is well within the range for efficient swimming movements as shown for a variety of fishes (Triantafyllou and Triantafyllou, 1995). Each animal was confronted with the respective condition ten times with and without the additional presentation of electrical playback. The presentation of different experimental conditions alternated in a pseudo-randomized order that allowed no more than three consecutive repetitions of the same condition within an experimental session. The succession of experimental sessions was randomized, and animals were assigned to the four conditions assuring equal size distribution. For the second set of experiments, the same animals were divided into $n = 11$ groups, each containing four similarly sized individuals. Each group was presented with ten repetitions of four different combinations of tail-fin movement and electrical playback emitted by the dummy fish: (i) electrical playback and tail fin movement with 1.5 Hz and 10 mm, (ii) electrical playback only, (iii) tail fin movement with 1.5 Hz and 10 mm without electrical playback, and (iv) no playbacks or tail fin movements as a control. Again, all stimuli were presented in pseudo-randomized order. Experimental trials in which at least one animal reached the following-criterion were defined to be successful. Inter-trial intervals of at least 10 min were maintained between all trials during all experimental sessions.

Data were analyzed in SPSS (version 22.0, IBM Corp., Armonk, NY, USA) using the non-parametric Kruskal-Wallis test for non-normally distributed data to compare the effect of tail-beat movement on recruitment efficiency for the playback condition and the electrically silent control condition. Wilcoxon signed-rank comparisons between these conditions were subsequently performed for each tail-beat configuration. The number of successful experiments during the group experiments was assessed with a non-parametric Friedman test followed by Bonferroni-corrected pairwise comparisons between results of the different experimental conditions. Statistical significance was accepted at the $\alpha = 0.05$ level.

8.3.2 Results

The tested combinations of cues evoked different attraction responses in individuals of *M. rume*. The attraction score of individual fish was measured for each as the proportion of trials during which the fish followed the dummy within 15 seconds. This measure was significantly affected by the presence or absence of the electrical playback signal during all tail-beat conditions, whereas the different tail-beat parameters during either the playback or the control condition had no significant effect. Single *M. rume* were mainly attracted when the dummy generated electrical playback signals, independently of its tail-beat movement (Figure 8.5). Concerning the experiments with groups of *M. rume*, the different combinations of cues generated by the dummy evoked significantly different attraction responses ($\chi^2_{(3)} = 23.19$; $p < 0.001$). Comparing the relative amount of successful trials showed that fish were more attracted by the dummy when it generated electrical playback compared with the electrically silent control conditions (Figure 8.6). The condition involving electrical playback and tail-fin movement was significantly more attractive than the electrically silent conditions with ($p = 0.002$) and without ($p = 0.006$) tail-fin movement.

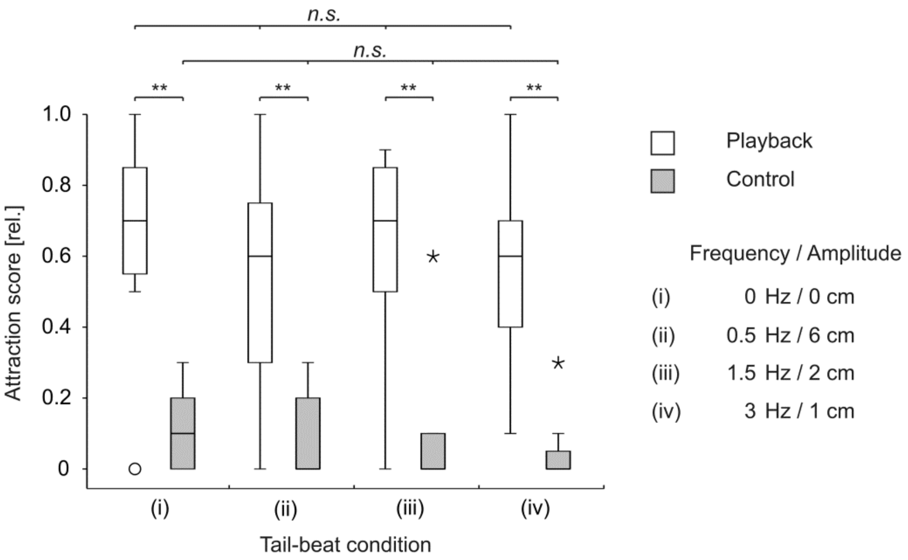

Figure 8.5: Attractiveness of the mobile dummy fish to single *M. rume* elicited by different combinations of visual and motility cues. Attraction scores of $n = 11$ individuals per tail-beat condition were always significantly higher in the presence of electrical playback compared to electrically silent controls based on related-samples Wilcoxon signed-rank tests for (i): $Z = -2.72$; $p = 0.007$; (ii): $Z = -2.68$; $p = 0.007$; (iii): $Z = -2.68$; $p = 0.007$; (iv): $Z = -2.94$; $p = 0.003$. Tail-beat movement influenced attraction scores neither during playback presentation ($\chi^2_{(3)} = 1.14$; $p = 0.77$) nor during electrically silent controls ($\chi^2_{(3)} = 1.75$; $p = 0.63$.

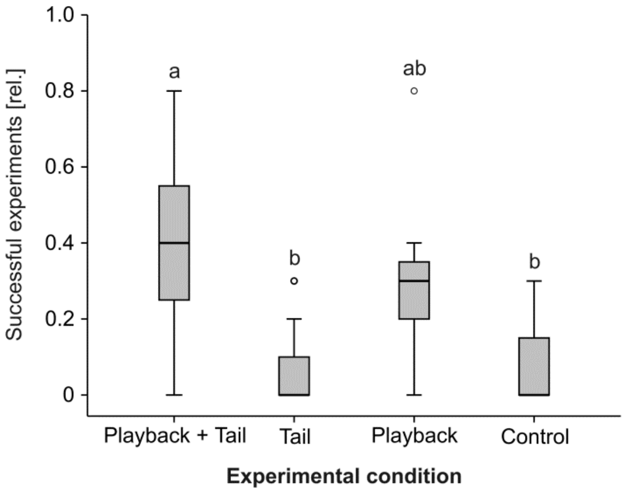

Figure 8.6: Attractiveness of the dummy fish to groups of four *M. rume* elicited by different combinations of cues. Box plots show the relative number of successful trials performed with $n = 11$ groups of four fish based on four experimental conditions featuring different combinations of electrical playback presentation and tail-fin movement by the dummy. Categories not sharing a common superscript letter differ significantly based on Bonferroni-corrected p-values.

8.4 Discussion

One of the most evident advantages of using robotic devices in behavioral biology is the possibility to test complex behavioral patterns in an automated and repeatable process. Appearance and behavior of a dummy can thus be brought under the researcher's control, which allows the systematic investigation of cues that determine the dummy's attractiveness for live animals and provide experimental validation of the significance of these cues also for animal-animal interactions. Weakly electric fish offer a unique opportunity to investigate the contributions of sensory cues from different modalities on social behavior, because the spatial and temporal aspects of electrical signaling are relatively easy to reproduce experimentally, and behaviorally highly relevant for orientation and intra-specific communication.

The aim of this work was to develop a robotic fish that can be used in behavioral studies on weakly electric fish, with the long-term goal of establishing a mixed society (Mondada *et al.*, 2013) of real and artificial *M. rume*. The resulting dummy fish mimicked the characteristics of *M. rume* on several levels. It was designed to resemble a live specimen as closely as possible in shape, size, and coloration. Although the effects of these properties on the robots attractiveness towards *M. rume* were not systematically investigated, the feasibility of integrating the components necessary for tail-fin actuation into a robot within the size-range of the tested fish could be demonstrated. Robots of considerably larger size than the tested fish have been used in behavioral experiments with several fish species (Kopman *et al.*, 2013; Marras and Porfiri, 2012; Polverino and Porfiri, 2013a) and it was argued, that aspect ratio may be more important as a parameter defining attractiveness than actual size (Abaid *et al.*, 2012). Nevertheless, zebrafish replicas of similar size appear to be more attractive to live conspecifics than larger ones (Bartolini *et al.*, 2016). Coloration and particular body features were shown in several studies to positively influence attraction of fish towards a replica in a variety of species (Abaid *et al.*, 2012; Landgraf *et al.*, 2016; Phamduy *et al.*, 2014; Polverino *et al.*, 2013). Other cues, such as hydrodynamic stimuli, were considered to be more important than visual cues by Marras and Porfiri (2012). Visual cues might be suspected to be of minor importance in *M. rume* since these animals are inconspicuous with respect to coloration and body features. In addition, their nocturnal lifestyle, as well as a grouped retina, leading to low spatial resolution in mormyrid vision (Landsberger *et al.*, 2008), suggest a more prominent role of electrical signaling for intra-specific interactions. This notion is supported by the fact that variations in EOD-waveform play an important role in the speciation of mormyrids (Feulner *et al.*, 2009b). However, electrically silenced *G. petersii* are, although to a lesser extent, attacked by conspecifics

(Kramer, 1976a). It seemed therefore reasonable to keep the visual appearance of the dummy fish as realistic as possible.

A large emphasis was set on the generation of naturalistic movement patterns, resulting in a robotic system capable of mimicking carangiform swimming movements in a wide range of tail-beat frequencies and amplitudes. The components and the mechanical design of the robot were optimized in a minimalist way to ensure reliability and ease of use during the experiments. The robot was composed of a flexible caudal fin and a rigid head housing the actuation unit. A DC motor controlled the oscillation of the tail through two cables in an antagonistic configuration. The robot was, however, not self-propelled and the power supplies for playback generation, tail actuation, and forward motion were situated outside the tank. The robot thus remained tethered and confined to a linear trajectory. The integration of receiving electrodes into the dummy potentially enables closing the feedback loop between live and artificial fish on two levels. Orthogonal arrangement of electrode pairs may allow spatial interactions between signaling fish and a completely mobile dummy by using a bioinspired approach algorithm (Hopkins, 2005) instead of visual feedback. It also opens the possibility to generate interactive playback patterns, which enables closed-loop feedback experiments on electrocommunication (compare chapters 5 and 11).

The behavioral experiments that were conducted using the dummy fish demonstrated that the robot could recruit single individuals and small groups of *M. rume* from a shelter into an exposed area. In the first set of experiments, the influence of different motion patterns on the attractiveness of the dummy towards single *M. rume* was investigated. By keeping swimming speed constant, tail-beat frequencies and amplitudes were systematically varied, all resulting in Strouhal numbers of 0.27, except for controls. This value was well within the range of numbers calculated from the motility parameters observed in a variety of fish species, where Strouhal numbers ranging from 0.25 to 0.35 were associated with high swimming efficiency (Triantafyllou and Triantafyllou, 1995). When implemented in the dummy fish, these combinations led to fairly natural movement patterns for frequencies of 1.5 Hz and 3 Hz, whereas the pattern at 0.5 Hz appeared exaggerated. While the presence of electrical playback signals had a highly significant and crucial effect on following-behavior, there was no significant effect of motion pattern on the attractiveness of the dummy (Figure 8.5). In contrast, Polverino *et al.* (2013) identified an optimum for tail-beat frequency on the attractiveness of a golden shiner (*Notemigonus crysoleucas*) replica. Studies on golden shiners were performed using stationary fish dummies in constant water flow (Marras and Porfiri, 2012; Polverino *et al.*, 2013) and attraction may have in part been due to hydrodynamic returns for swimming efficiency. This is unlikely as an explanation for the spontaneous behavior observed in the placid water of the experiments with *M. rume*.

The second set of behavioral experiments was performed to disentangle the influences of motion pattern and electrical signaling on small groups of *M. rume*. Again, it did not make a difference whether the dummy fish performed a natural movement pattern, while electrical signaling significantly improved recruitment efficiency (Figure 8.6). While the effect of swimming speed was not investigated in the current experiments, previous studies demonstrated that swimming speed (Butail *et al.*, 2013) and dynamics (Landgraf *et al.*, 2016) were determinants of a replicas attractiveness towards, zebrafish (*Danio rerio*) and guppies (*Poecilia reticulata*), respectively.

Playback experiments with weakly electric fish have traditionally been performed using stationary electrodes for signal generation (Feulner *et al.*, 2009a; Hanika and Kramer, 2005; Kramer, 1979). Incorporation of playback electrodes into a mobile fish dummy allowed to additionally investigate spatial aspects of interactions during electrocommunication in mormyrids (Worm *et al.*, 2014) (see chapters 4, 5, 9 and 11). By constructing a realistic model of *M. rume*, a complex set of cues was generated, and the influence of different motion patterns and electrical signaling on following-behavior of live fish could be systematically tested in behavioral experiments. Selective activation of caudal-fin oscillation and electrical signaling suggested that the latter played a more prominent role in inducing the observed behavior. The dominant effect of electrical signaling suggests that the robot could be reduced in a way analogous to Tinbergen's (1948) identification of social releasing mechanisms (see chapter 9). Developing an artificial dummy fish may thus help to understand fundamental aspects of collective behavior in weakly electric fishes, and the rules and properties necessary to initiate and sustain such behavior in closed-loop feedback experiments based on electrocommunication.

9. Project 4: Disembodying the Invisible: Electrocommunication and Social Interactions by Passive Reception of a Moving Playback Signal

This chapter is based on my contribution to:

Worm, M., Kirschbaum, F. and von der Emde, G. (2018). Disembodying the invisible: Electrocommunication by passive reception of a moving playback signal. *Journal of Experimental Biology* 221: jeb-172890.

9.1 Introduction

Mormyrid weakly electric fish have a multitude of sensory systems at their disposal, which they use to navigate their environment, detect predators and food, and mediate social interactions between individuals. Mormyrids share the ability to passively detect low-frequency electric signals via ampullary receptor organs with a variety of electroreceptive fishes (Engelmann *et al.*, 2010; Kalmijn, 1974). More prominent is their ability to probe their immediate environment using self-generated electric organ discharges (EOD) during active electrolocation (von der Emde, 1999). These signals are produced by an electric organ located within the caudal peduncle and generate an instant, three-dimensional dipole field around the fish (Bennett, 1971a). Animals detect their own discharges through mormyromast electroreceptor organs (Bell *et al.*, 1989), which are distributed over large areas of the body surface (Harder, 1968; Hollmann *et al.*, 2008). Object induced local modulations of EOD-amplitude and waveform, which are registered by mormyromasts, constitute an electric image that allows the fish to detect and differentiate objects based on their size and shape (von der Emde *et al.*, 2010), as well as material composition (von der Emde, 2006). Active electrolocation is thus used for finding food (Arnegard and Carlson, 2005; von der Emde, 1994; von der Emde and Bleckmann, 1998) and for orientation and navigation in the environment (Cain *et al.*, 1994; Cain and Malwal, 2002; Schumacher *et al.*, 2017b; Walton and Moller, 2010).

Electrocommunication relies on a third electrosensory system with its own electroreceptor organs and brain pathways. Like mormyromasts, knollenorgans are electroreceptors that respond to the high frequencies contained in an EOD, but their input to the central nervous system is inhibited by a centrally evoked corollary discharge each time an animal discharges its electric organ (Bell and Grant, 1989). Instead, the knollenorgans relay information about the timing of EODs emitted by other electric fish to the brain. Through their knollenorgan pathway, fish can thus detect variations in waveform and inter-discharge interval (IDI) of the signals of nearby conspecifics (Baker *et al.*, 2013a). While the EOD waveform mainly conveys information about the identity and status of the sender (Bass and Hopkins, 1983; Carlson *et al.*, 2000; Graff and Kramer, 1992; Hanika and Kramer, 2005; Hopkins, 1980; Terleph and Moller, 2003), immediate changes in IDI-distribution enable mormyrids to communicate behavioral states and motivations. Instantaneous discharge frequencies are also linked to the current needs of active electrolocation in a given behavioral context, such as resting, swimming, or foraging (Bauer, 1974; Gebhardt *et al.*, 2012a), or the detection and analysis of novel stimuli in the environment (Post and von der Emde, 1999; Toerring and Moller, 1984; von der Emde, 1992). Systematic variations in IDI-duration additionally result in specific signaling patterns that can encode intentional information (Baier and Kramer, 2007; Bratton and Kramer, 1989; Carlson and Hopkins, 2004b; Kramer, 1976a), and interactive signaling can lead to synchronization of discharge activity between individuals, which is also likely to play a role during communication (Arnegard and Carlson, 2005; Gebhardt *et al.*, 2012a; Gebhardt *et al.*, 2012b).

Social communication consists not only of detection and decoding of the senders signal and its content by the receiver, but often aims at initiating physical interactions, which requires the receiver to also determine its spatial relationship to the signal source. Social behaviors in mormyrids are quite versatile and include overt aggression and territorial behavior as well as social interactions and shoaling depending on both context and species (Carlson, 2016; Gebhardt *et al.*, 2012b; Moller, 1976). A multitude of context-depending motor patterns and behavioral sequences during social encounters have been described, many of which could be associated with stereotypical displays of electric discharge activity (Bell *et al.*, 1974; Kramer and Bauer, 1976; Wong and Hopkins, 2007), or were shown to depend on the capability to produce EODs (Crockett, 1986; Moller, 1976).

Many fishes are highly vision-dominated animals, as apparent from the innumerable amount of visual displays of postures, markings, and coloration during agonistic encounters, mate-choice, or group integration (Bakker and Milinski, 1993; Denton and Rowe, 1998; Simpson, 1968). Due to their nocturnal lifestyle (Moller *et al.*, 1979), social interactions among mormyrids often cannot rely on their visual system, which is adapted to dim light and turbid water conditions (Kreysing *et al.*, 2012; Landsberger *et al.*, 2008). Although the pulse-type electric signals emitted by mormyrids are well suited to encode information into temporal sequences, they do not provide directional information about the shortest distance towards the position of the signal source, because they exist as electrostatic dipole fields (Hopkins, 2005). Weakly electric fish therefore approach an electric dipole source by describing a curvilinear trajectory along the electric field lines of the emitted signal (Schluger and Hopkins, 1987). However, little is known about the relative roles of active and passive electrolocation during close-range encounters between mormyrids. Can social interactions among weakly electric fish be mediated by passive reception of electric communication signals alone? Or do mormyrids require input from other sensory modalities as well, to sustain close-range interactions between individuals during electrocommunication? These questions can only be addressed by restricting the source of the signal to its 'disembodied' electric signaling properties from the perspective of an electric fish.

Weakly electric *Mormyrus rume* have been shown to follow a mobile dummy fish emitting electrical playback of natural IDI-sequences from a shelter into an open area, apparently without relying on visual or motility cues (Donati *et al.*, 2016). Under non-visual conditions, the spatial relationship between the moving dummy and the following fish was affected by the presence of electric playback signals (Worm *et al.*, 2017), raising the question of what sensory systems are actually involved when following another individual. In that study, it was hypothesized that mormyrids can rely on the spatial information contained in the electrostatic dipole-fields generated by the EODs of a conspecific, and thus are capable of spatially interacting with a moving signal source based on information processed via the knollenorgan pathway during electrocommunication. In the current study, a similar design as in Worm *et al.* (2017) (chapter 4) was used to confront single individuals of *M. rume* with a mobile dummy electrode emitting EODs to entice the fish to swim out of a shelter and into a testing area. All sensory cues from the signal source that could have been perceived by vision, the lateral line system, and eventually also active electrolocation, were experimentally excluded. This approach should render all physical properties of the signal source, except the actively generated electrostatic fields of the playback EODs, undetectable for *M. rume* and allowed concluding on the significance of passive electroreception via the knollenorgan pathway during interactive social behaviors. The signal source was thus 'disembodied,' and it was tested whether its characteristics still sufficed to induce normal social following behavior. The results show that the presentation of electric playback signals reliably attracted the tested fish and triggered the emission of stereotypical signaling sequences, which usually can be observed during electrocommunication. Even animals that were deprived of their ability to generate EODs were still able to locate the moving signal source, track its movement throughout the testing area, and orient themselves relative to its position during spatial interactions. Thus, passive perception of electrocommunication signals was sufficient to initiate following-behavior in *M. rume* and sustained interactions with an otherwise imperceptible signal source.

9.2 Materials and methods

9.2.1 Experimental animals

A total of 27 *Mormyrus rume proboscirostris* were used during the experiments, all of which were bred in captivity by F. Kirschbaum (Humboldt University of Berlin) by imitation of rainy season conditions following the method described by Schugardt and Kirschbaum (2004). Animals were kept under tropical conditions and a 12/12h light/dark cycle, with water temperatures around 26°C. Food was provided at least five times a week in the form of defrosted *chironomid* larvae. All experiments were carried out in accordance with the guidelines of German law and with the animal welfare regulations of the University of Bonn. All procedures and methods were approved by the LANUV NRW (Landesamt für Natur, Umwelt und Verbraucherschutz Nordrhein-Westfalen, reference number: 84-02.04.2015.A444).

In a first set of experiments, 24 *M. rume* with standard lengths between 6.4 and 11.4 cm were used to test the reaction of intact fish to a mobile dummy dipole that was basically reduced to the emitted playback signal (see below). These animals were approximately two years of age at the time of experimentation and of undetermined sex. They were kept as a group in a communal tank, from where they were individually transferred to the experimental tank at least one day prior to testing.

9.2.2 Setup

The experimental tank had a base area of 200 cm x 50 cm and was subdivided into three compartments (Figure 9.1A): The first compartment (closed area) measured approximately 60 cm in length. It was made inaccessible to the fish using a fly screen and contained the inlet and outlet of the water filter, a heater and an aeration device, all of which were switched off during the experiments. The second compartment measured around 50 cm in length and served as a hiding area, which was connected via a 10 cm wide gate to the testing area, which had a length of 90 cm. The floor of both the hiding and the testing areas was covered with gravel. Water level was maintained at approximately 20 cm. In the testing area, an electrically transparent agarose tube was embedded in the ground, which served as the track for the moving electric dipole source. During all experiments, water temperature and conductivity were kept at 26.0 ± 1°C and 100 ± 5 $\mu S\ cm^{-1}$, respectively.

The agarose tube had a length of 1000 mm and defined the trajectory of the mobile playback electrode inside the third compartment, the testing area. It had a cubic outer cross section of 55 x 50 mm and an inner diameter of 15 mm and was made using 20 $g\ l^{-1}$ Universal Agarose ("Seakem® LE", Axon Labortechnik, Kaiserslautern, Germany). Water with a conductivity of 100 $\mu S\ cm^{-1}$ was used to manufacture the tunnel to ensure electric transparency during the experiments (Heiligenberg, 1973). The tunnel was embedded in the gravel, running in parallel to the longitudinal axis of the tank, through the gate and centrally through the testing area. The upper surface of the tunnel was planar with a final layer of fine white sand (Sansibar S'now, JBL GmbH & Co. KG, Neuhofen, Germany) which provided good contrast for subsequent video tracking, but did not cover the surface of the tunnel.

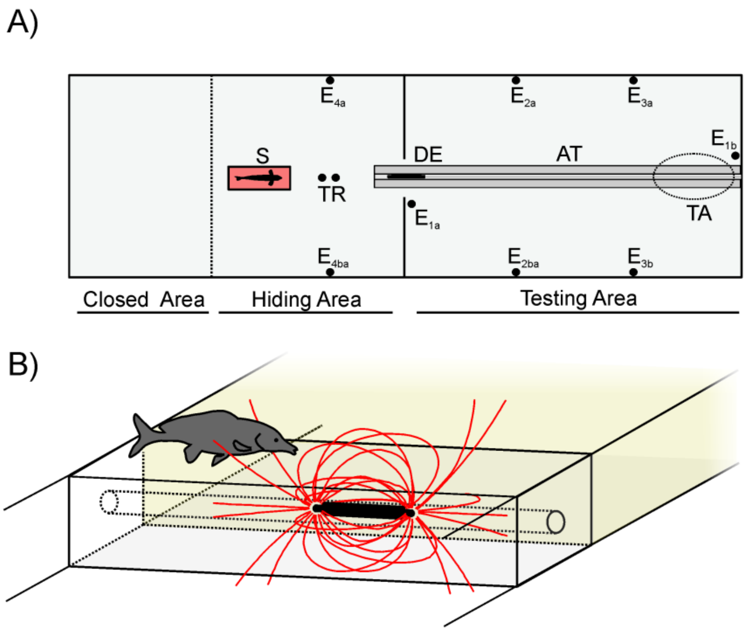

Figure 9.1: Experimental setup. A) Top view of the experimental setup (base area: 200 x 50 cm). Upon registration of electric activity by a pair of trigger electrodes (TR), the mobile dummy electrode (DE) crossed the testing area through an electrically transparent agarose tube (AT) while emitting playback of a natural IDI pattern. Fish that left the shelter (S) and reached the target area (TA) within 15s of onset of the experiment were defined as following the dummy electrode. Experiments were videotaped by an infrared-sensitive camera and electric activity was recorded by a mulitielectrode array (E) for further analysis. B) Schematic illustration of *M. rume* detecting the mobile dummy electrode emitting a dipole field within the elctrically transparent agarose tube at the bottom of the tank. Images are not drawn to scale.

The hiding area was provided with a single shelter made from a 20 cm x 5 cm red transparent plastic tube (Bioscape GmbH, Castrop-Rauxel, Germany), with the opening directed towards the gate at a distance of 30 cm. The front end of the shelter was endowed with a pair of trigger electrodes. Electric activity of the test fish was amplified differentially (Brownlee Precision Model 440, Palo Alto, CA) between these electrodes and was used to generate a TTL-pulse via a digital oscilloscope (Yokogawa DL1620, Yokogawa Electric Corp., Tokyo, Japan) once a certain threshold was passed. This threshold was determined for each fish prior to an experimental session. Its sensitivity was set to trigger the onset of an experiment once the fish stuck its snout out of the front end of the shelter.

9.2.3 Electrical playback and EOD recordings

A playback dipole was made from a 9 cm plastic rod with a diameter of 8 mm, which was fitted with a pair of carbon electrodes situated at the front and rear ends of the rod. This dummy electrode was placed inside the agarose tunnel (Figure 9.1B) and could be dragged at a speed of 0.11 m s^{-1} by a wire through the tunnel using a small DC motor (Modelcraft RB350050-2273R, 12 V/50:1) to which it was connected via a cable linkage and a set of pulleys. The wire also connected the dummy electrode to a stimulus isolator (model 2200, A-M Systems Inc., Carlsborg, WA) that also served as a power source for electrical playback generation.

An electrical playback sequence was generated in Matlab (version R2013b, The MathWorks Inc., Natick, MA) using a custom-written script to concatenate single EODs to a highly regular, but natural pulse-sequence with an average IDI duration of 59 ± 9 ms (*mean* ± *s.d.*) and a total duration of 14 seconds. This sequence had previously been recorded during a similar experiment. Template EODs were recorded head-to-tail (Brownlee Precision Model 440, high-pass: 1 Hz) from a *M. rume*, digitized at a sampling rate of 50 kHz (CED Power 1401, Cambridge Electronic Design, Cambridge, UK) and averaged from 50 signals using Spike2 (version 5.21, Cambridge Electronic Design, Cambridge, UK). The second positive phase of the EOD (Kramer, 2013) was omitted from the playback signal because it declines 'asymptotically' and arguably contains low-frequency signal components. The output of the assembled playback sequence occurred at 50 kHz via the Spike2 sequencer, a D/A-converter (CED Power 1401), a dB-attenuator (University of Regensburg, Germany), and the stimulus isolator to the playback electrode inside the agarose tube. The stimulus isolator was turned on also during trials without electrical playback to control for effects of any low-frequency offsets it may have caused. Signal strength was adjusted to match the EOD-amplitude of a living fish of similar size, resulting in a maximum signal strength of 118 mV cm^{-1} measured outside the agarose tube.

The EODs of the fish and the dipole were recorded differentially (Brownlee Precision Model 440) via a five-channel multi-electrode array (Figure 9.1A), which included the trigger electrodes to account for all signals irrespective of the test fish's position in the tank. Recording electrodes, which consisted of single-wires funneled through 5 mm plastic tubes with a short silver wire (AG-8W, Science Products GmbH, Hofheim, Germany) soldered to the tip, were placed closely above the gravel. Waveform data were digitized (CED Power 1401) and recorded to disk using Spike2 software. All experiments were performed under infrared illumination only (850 nm, IR Illuminator Model SA1-60-C-IR, Itakka, Wattens, Austria). They were recorded using Spike2 Video Recorder and monitored remotely via a pair of infrared-sensitive cameras (DBK 21AF04 FireWire Camera with Vari Focal T4Z2813CS-IR CCTV Lens, The Imaging Source, Bremen, Germany) mounted above the hiding compartment and the testing area to avoid the possibility of visible light influencing the behavior of the fish.

9.2.4 Experimental protocol

Single animals were taken from the communal tank and adapted to the experimental tank overnight. To establish basic parameters for random swimming, electric communication patterns, and communication distances, trials with the moving electrode were preceded by a set of baseline experiments, during which the playback electrode was placed stationary at the farthest position within the testing area. During these baseline experiments, movement patterns and electric activity of each fish were recorded ten times with and without playback presentation for 15 seconds after activation of the trigger by the test fish. The presentation order was pseudo-randomized, allowing no more than three consecutive repetitions of the same condition. For each trial, it was noted whether the fish entered the testing area and whether it subsequently reached the stationary dummy dipole, which was defined by a perimeter criterion (see below).

Following the baseline experiments after a short break, each fish was presented three times with the moving dummy electrode emitting the electric playback sequence and three times with the silent control condition without playback. The dummy dipole arrived at the target positions after seven seconds and remained there motionless while continuing to emit electrical playback for another seven seconds. Again, the presentation order was pseudo-randomized, allowing no more than two consecutive repetitions of the same condition. Half of the animals were confronted first with the control, and the other half with the playback sequence. Inter-trial intervals of at least five minutes were maintained in between trials. Again, all movement patterns and electrical activity were recorded to disk for 15 seconds after the fish had activated the trigger.

To investigate a possible influence of active electrolocation for detecting the moving playback electrode, three additional fish (standard length: 10.9–12.9 cm) were subjected to the same experimental protocol with the only difference that the number of trials with the moving electrode was increased to ten repetitions per condition. After this initial experimental session, these animals were then electrically silenced and tested again six to eight days later. As after silencing the animals were no longer capable of producing EODs, the start of each experimental trial had to be initiated manually by the experimenter once the fish stuck its snout out of the front end of the shelter.

Electric silencing was achieved by sectioning the spinal cord directly in front of the electric organ with a needle. Animals were anesthetized with 150 mg l^{-1} MS 222 (Acros Organics, Geel, Belgium) before the procedure. The success of the intervention was verified by an audio monitor (RadioShack® mini amplifier-speaker, Tandy Corp., Fort Worth, TX) after the operation, and directly before re-testing these animals in the behavioral experiments.

9.2.5 Data analysis

Two criteria were defined to quantify the effectiveness of moving and stationary playback presentations on following-behavior and to distinguish the effect of these treatments from random swimming. For each condition, the proportion of trials, in which the fish entered the testing area completely within 15 seconds of onset of the experiment, was determined. For the second criterion, fish had to cross an elliptic perimeter around the dummy dipole at its final position (Figure 9.1A) with any part of their body. Ellipses were drawn in ImageJ (version 1.46r, National Institutes of Health, USA) with a major axis of 190 mm and a minor axis of 110 mm, defining an area extending about 50 mm around the dipole. All videos were evaluated manually. The relative proportion of trials in which each fish fulfilled this perimeter criterion during all experimental conditions was determined seven and 15 seconds after onset of the experiment. Additionally, for both the experimental sessions with the stationary and the moving dummy electrode, fish that had fulfilled the perimeter criterion within 14 seconds of onset of the experiment at least once during playback presentation and the control condition were selected for further analysis. Of the 24 *M. rume* that were used in these experiments, this was the case in $n = 15$ animals for the stationary and $n = 13$ animals for the moving condition. For these animals, those trials in which they reached the criterion for the first time were used for more detailed analysis of electric signaling and swimming trajectories.

Spike2 waveform data from these experiments were converted into time series by marking the occurrence of each EOD in time. Signal sequences of playback and fish were then separated into individual time series from which IDIs were subsequently calculated. Histograms of the relative occurrence of IDI-distributions were calculated for each experimental condition by pooling IDIs into bins of 2 ms. IDI-sequences were then analyzed for communicative signaling patterns, in particular double-pulses and long cessations, as well as discharge synchronizations with the playback sequence.

Double-pulses are signaling sequences involving alternations of long and short IDIs. The minimal requirement for a double-pulse sequence was defined by setting a lower and an upper threshold at 50 and 60 ms, respectively. A double-pulse sequence had to consist of at least five pulses, where IDI1, IDI3, and IDI5 had durations of ≥ 60 ms, and IDI2 and IDI4 had durations of ≤ 50 ms. All signals in that sequence were then treated as double-pulses. This analysis was performed automatically using a custom-written Matlab-script.

Long cessations were characterized by the absence of electric signals and were defined as periods of at least 1000 ms without an EOD by the fish. Using ImageJ, the distance between the test fish and the dummy electrode was determined within that video frame which was recorded simultaneously with the last EOD before a cessation for each trial involving the stationary playback condition.

Discharge synchronizations were quantified by calculating adaptive cross-correlations between the IDI-sequences of playback and fish according to the procedure described in Gebhardt *et al.* (2012a) (see also materials and methods in chapters 4 and 5). Maximum correlation values occurring within a 100 ms response time between EODs of the playback sequence and the fish were averaged over the time course of a trial for the moving playback condition (n = 24) and the stationary playback condition (n = 13). As a control, randomly occurring correlations between the playback and IDI-sequences of *M. rume* recorded independently during the moving control condition (n = 23) were also calculated.

Detailed swimming trajectories of the fish were obtained by video tracking using Ctrax (Branson *et al.*, 2009) for all trials involving the moving dummy electrode. Video tracking was also performed for the three fish before and after silencing of their electric organs in the trials involving the moving dummy electrode and electrical playback.

All statistical tests were performed in SPSS (version 22.0, IBM Corp., Armonk, NY). Normality of data was assessed by a Shapiro-Wilk test, and parametric or non-parametric tests were used accordingly. Statistical significance was accepted at the $\alpha = 0.05$ level.

9.3 Results

9.3.1 Attraction of the dummy

Once they detected the electric playback signals, all 24 animals were highly attracted to the dummy dipole and responded with communicative signaling patterns and physical interactions. In a first set of trials featuring the stationary playback electrode, the active space for electrocommunication was determined, and a basic value for explorative behavior into the testing area, as well as for the detection of the dummy dipole based on a perimeter criterion, was established. For the experiments with the stationary dummy dipole, the average ratio of animals entering the testing area was 0.47 ± 0.03 (*mean* ± *s.e.m.*) during the silent controls compared with 0.50 ± 0.03 (*mean* ± *s.e.m.*) during electric playback presentations from the target area. This indicates that the intensity of the playback from the end position of the dipole was not sufficient to recruit animals from the hiding area into the testing area at a rate higher than what would be expected due to normal exploring behavior (paired-samples t-test, $t_{(23)} = -0.88$, $p = 0.39$; Figure 9.2A). Similarly, the fish were not attracted by the moving dummy dipole alone when it did not emit electric playback signals. Median values for the relative number of trials in which animals entered the testing area were 0.50 for the silent stationary and 0.33 for the silent moving control, respectively, and did not differ significantly from each other based on a paired-samples Wilcoxon signed-rank test ($Z = -0.80$, $p = 0.42$; Figure 9.2B).

Of the 24 *M. rume* participating in these trials, a total of $n = 15$ animals reached the perimeter criterion at least once both during the stationary playback experiments and during the stationary silent control tests. The latency for reaching the criterion did not differ significantly between the control condition (9.64 ± 0.84 seconds; *mean* ± *s.e.m.*) and electrical playback presentation (8.96 ± 1.00 seconds; *mean* ± *s.e.m.*; paired-samples *t*-test, $t_{(14)} = 0.47$, $p = 0.64$), indicating that the playback signal was not strong enough to recruit animals reliably from the hiding area to reach the perimeter criterion (Figure 9.2C). This was not the case for the moving conditions, during which $n = 13$ animals reached the perimeter criterion at least once both during playback presentation and the silent control condition. Here, the latency for reaching the criterion was significantly longer for the silent controls (8.58 ± 0.78 seconds; *mean* ± *s.e.m.*) compared with when electrical playback was emitted (5.96 ± 0.18 seconds; *mean* ± *s.e.m.;* paired-samples *t*-test, $t_{(12)} = 3.73$, $p = 0.003$). In this case, the fish reliably reached the criterion as soon as the dummy electrode stopped at its target position. This reliability is also indicated by the smaller error bars for the playback compared with the control (Figure 9.2D).

To determine the recruitment efficiency of the mobile dummy dipole, the relative number of trials where $n = 24$ fish had reached the perimeter criterion was determined seven and 15 seconds after onset of a trial for all experimental conditions (Figure 9.3). Most fish had already fulfilled the criterion after seven seconds in response to the moving playback (*median* = 1), whereas very few animals reached the criterion at that time during all other conditions (*median* values: 0.0–0.1; Friedman test for repeated measures, $X^2_{(3)} = 56.79$, $p < 0.001$; post hoc tests $p < 0.001$ for all comparisons with the moving playback condition; Figure 9.3A). After 15 seconds, *M. rume* had followed the mobile dipole almost without exception when it emitted playback (*median* = 1), still differing significantly from all other conditions (Friedman test for repeated measures $X^2_{(3)} = 52.49$, $p < 0.001$; post hoc tests $p \leq 0.001$ for all comparisons with the moving playback condition; Figure 9.3B). By that time, the stationary playback condition (*median* = 0.35) had, however, also attracted test fish at a significantly higher rate than the stationary control (*median* = 0.1; post hoc comparison $p = 0.044$). The playback dipole was therefore within the active space of the sheltered fish when placed at the starting position, but it was only detected at the target position if animals were already swimming in the testing area.

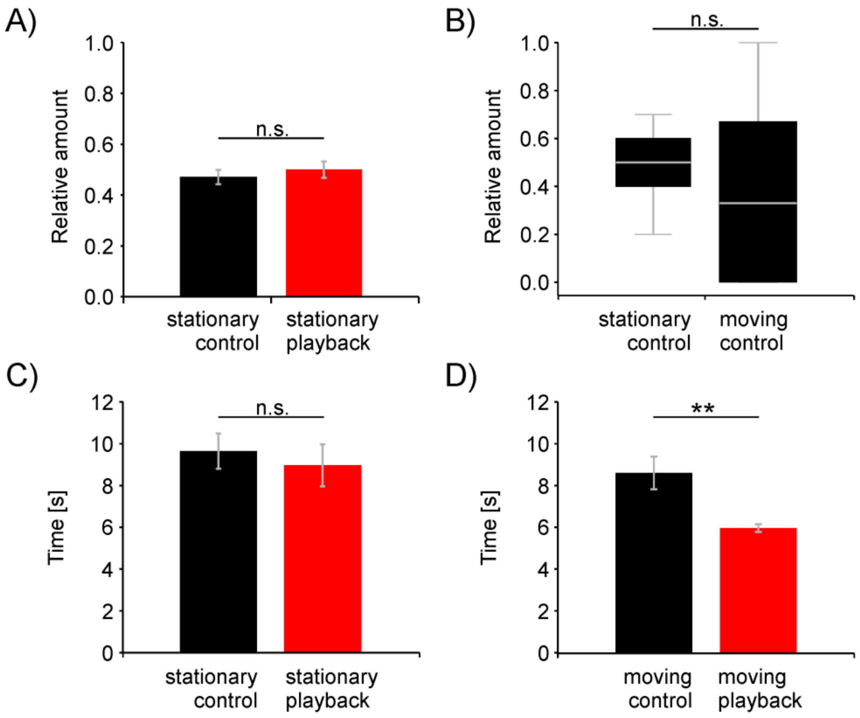

Figure 9.2: Influence of experimental condition on attraction. A) Relative number (*mean* ± *s.e.m.*) of trials for *n* = 24 *M. rume* where animals entered the testing area during presentation of stationary playback and stationary silent control. B) Relative proportion of trials where *n* = 24 fish entered the testing area during stationary and moving silent controls. C) Average time (*mean* ± *s.e.m.*) when *n* = 15 *M. rume* reached the perimeter criterion for the first time during stationary playback presentation and stationary silent control. D) Average time (*mean* ± *s.e.m.*) when *n* = 13 *M. rume* reached the perimeter criterion for the first time during moving playback presentation and moving silent control. ** $p \leq 0.01$; ns: not significant.

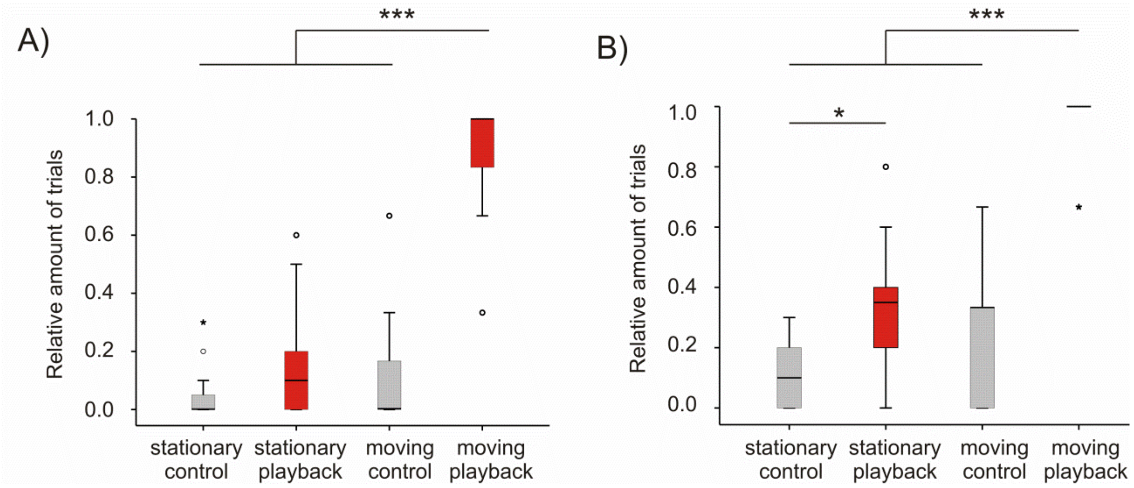

Figure 9.3: Recruitment efficiency. Relative number of trials for *n* = 24 *M. rume* where animals reached the perimeter criterion within seven (A) and 15 seconds (B) of onset of the experiment during the presentation of stationary and moving playbacks and controls. * $p \leq 0.05$; *** $p \leq 0.001$.

9.3.2 Electric signaling and locomotor behavior

Signaling sequences by *M. rume* in response to electrical playback were highly stereotypical. IDI-distributions during the silent control conditions were irregular, with high variability resulting in wide distributions and modes at relatively long IDIs between 71 (moving control) and 55 ms (stationary control; see upper panels of Figure 9.4A and B). In contrast, signaling sequences emitted in response to electrical playback were characterized by long cessations, double-pulse patterns and regularizations at higher frequencies (see lower panels of Figure 9.4A and B). Typical IDI-sequence of *M. rume* in response to the moving playback dipole (Figure 9.4B, lower panel) started with a cessation upon detection of the electric playback signal, continued with a short sequence of double pulses and ended with a regularized discharge sequence, synchronized at approximately the same frequency as the playback sequence. This pattern is reflected in the overall histogram on the right-hand side of the lower panel of Figure 9.4B, with a narrower IDI-distribution around the mode at 59 ms, and additional modes at 71 and 27 ms, representing the alternation of long and short intervals during double-pulse sequences, compared with the histogram of the moving control condition in the panel above.

Fundamentally different reactions based on whether the moving dummy dipole emitted electrical playback were also observed in the swimming trajectories of *M. rume* (Figure 9.5). During the electrically silent control condition (Figure 9.5A), swimming trajectories dispersed throughout the testing area with a tendency of the fish to swim along the walls of the tank, but without obvious relation to the trajectory of the moving dummy dipole. In contrast, fish stuck close to the dummy's trajectory in case of electric playback presentation (Figure 9.5B), showed multiple instances of circling and trajectory cut-off, and did not leave the dummy electrode after it reached its target destination, as they would do during the silent control condition. Instead, animals kept searching the surface of the agarose tube at the front end of the dummy dipole until the playback stopped. This difference in following-behavior is reflected in a highly significant difference in average distances between *M. rume* and the dummy dipole during the two conditions (paired samples *t*-test, $t_{(12)} = 8.49$, $p < 0.001$; Figure 9.6A). With an average distance of 250 ± 20 mm (*mean* ± *s.e.m.*), *M. rume* spent most of the time out of reach for active electrolocation during silent controls, whereas the average distance of 64 ± 3 mm (*mean* ± *s.e.m.*) during electric playback presentation was well within the range of active electrolocation (von der Emde, 1999).

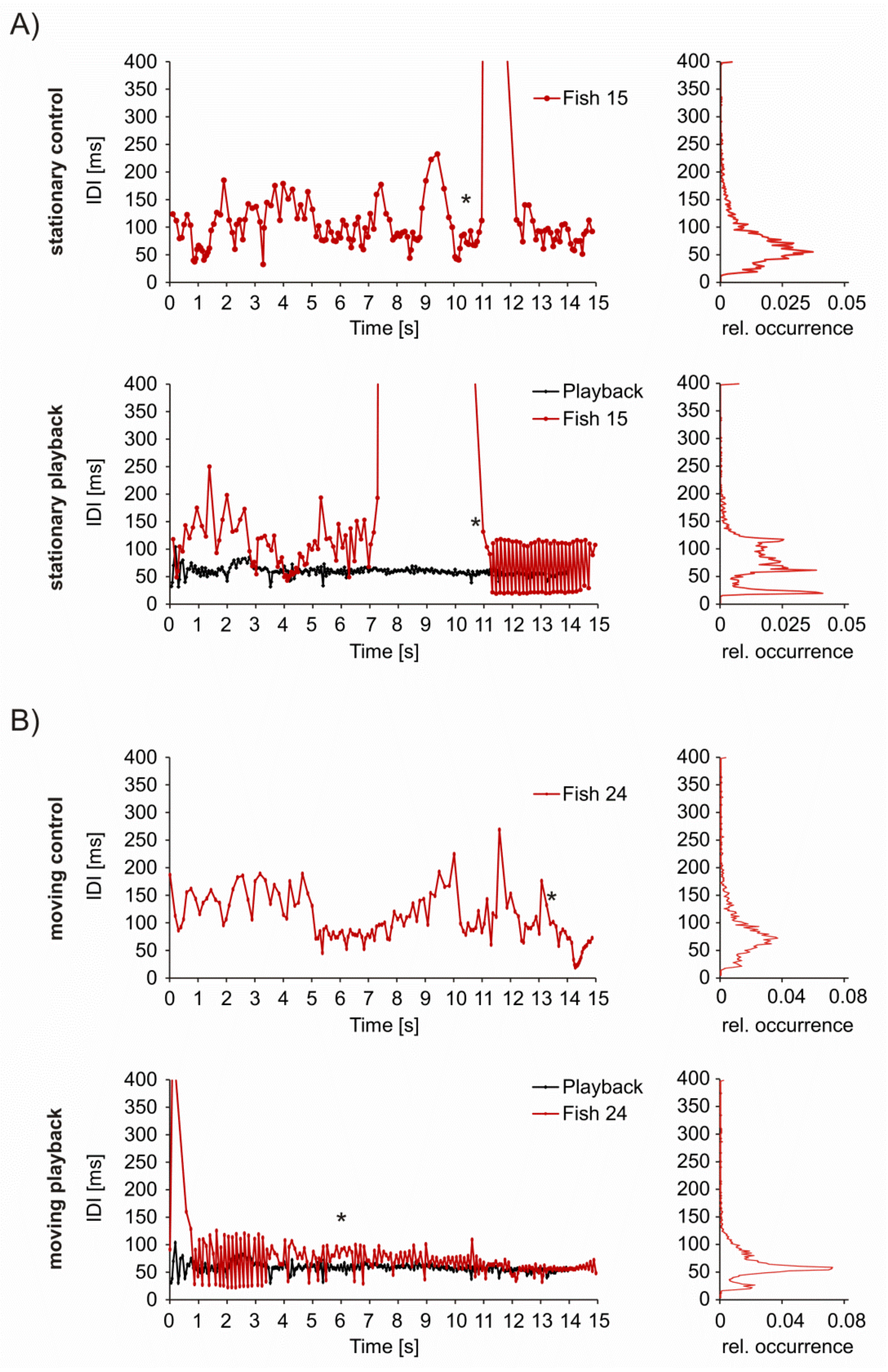

Figure 9.4: Electric signaling patterns. Exemplary IDI-sequences of individual *M. rume* reaching the target area within 15 seconds of onset of the experiment during presentation of electrical playback (lower panel) and the silent control (upper panel) for the stationary (A) and the moving (B) dummy electrode. Asterisks mark the points in time when the perimeter criterion was met. Histograms to the right represent pooled IDIs of *n* = 15 (A) and *n* = 13 (B) fish that reached the perimeter criterion during the four experimental conditions.

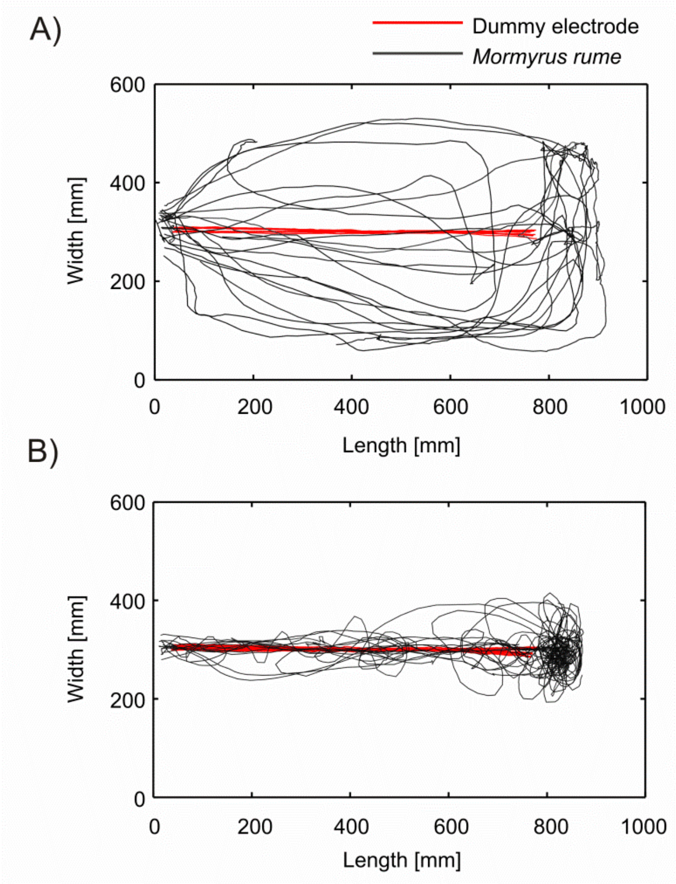

Figure 9.5: Swimming trajectories of intact fish. Swimming trajectories of *n* = 13 fish (black) that followed the mobile dummy electrode (red) emitting electrical playback (B) and during silent controls (A).

Typical IDI-sequences in response to the stationary playback condition (Figure 9.4A, lower panel) started with the same irregular discharge pattern as observed during the silent control condition (upper panel). Upon detection of the electric playback stimulus, *M. rume* usually responded with a long cessation of up to several seconds, directly followed by a highly regular double-pulse pattern, which is reflected by additional modes at 19 and 117 ms in the overall IDI-histogram. The onset of the double-pulse pattern usually occurred directly after the test fish had crossed the perimeter criterion (asterisk in the lower panel of Figure 9.4A). Long cessations occurred with 87.7% significantly more often during the playback trials compared with the control trials with only 20% (exact McNemar's test *p* = 0.002; Figure 9.6B). An evaluation of onset and offset times of long cessations during playback trials (Figure 9.6C) demonstrated that long cessations always occurred before the fish crossed the perimeter criterion (*median* = -2.02 seconds) and usually stopped shortly thereafter (*median* = 0.34 seconds). The median distance between *M. rume* and the dummy dipole, where *n* = 20 animals first responded to the detection of the playback signal with a long cessation, was 355 mm (Figure 9.6D). *M. rume* thus marked the outer limit of the playback's active space with the onset of a long cessation. It then approached the signal source silently and started to discharge double-pulse patterns when it was near it. Double pulses were only produced in response to electrical playback (Figure 9.7A and C). They were virtually absent during the control conditions (Figure 9.7B and D) and were not displayed prior to long cessations during stationary playback presentation (Figure 9.7C), suggesting that *M. rume* attempted to interact socially with the discharging dummy dipole.

Discharge synchronizations of *M. rume* to the electric playback sequence were frequently observed in response to the moving playback condition (Figure 9.8). In contrast, during the stationary playback condition, correlations did not exceed those calculated for an independently recorded IDI-sequence, suggesting that detection and initial approach of a stationary conspecific are not associated with interactive signaling in *M. rume*.

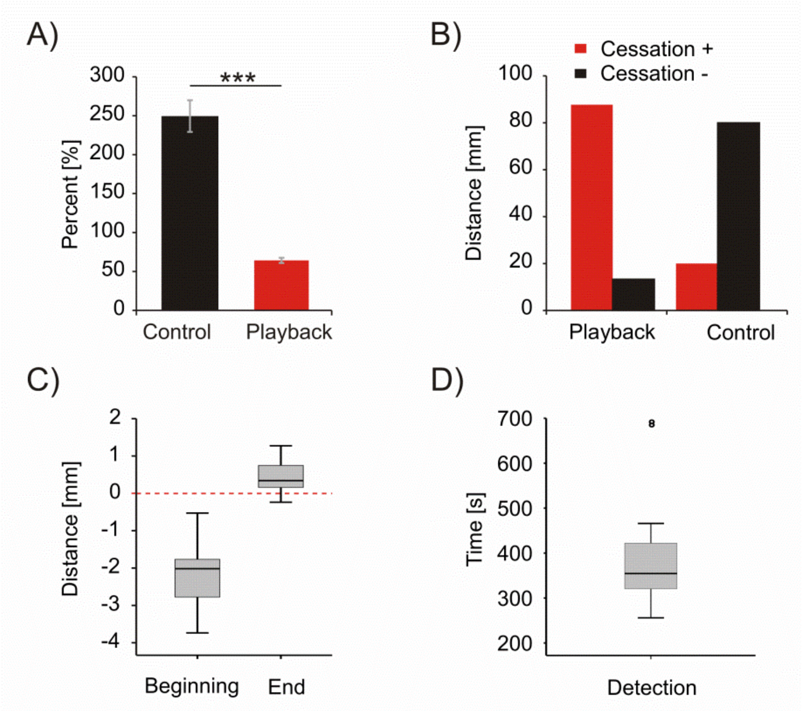

Figure 9.6: Spatial parameters and electric signaling activity. A) Average (*mean* ± *s.e.m.*) of the mean distance between test fish and dummy electrode for the trajectories depicted in Figure 9.5. B) Relative amount of IDI-sequences featuring at least one discharge cessation ≥ 1000 ms during experiments with the stationary dummy electrode; pairwise comparisons for $n = 15$ individuals that had reached the perimeter criterion during playback presentation and the control condition. C) Time differences for beginning and end of discharge cessations ≥ 1000 ms in relation to the time when the perimeter criterion was met (dotted red line) shown by $n = 20$ individuals in response to stationary playback presentation. D) Distance between test fish and dummy electrode at the time of the last EOD before the discharge cessations shown in (C).

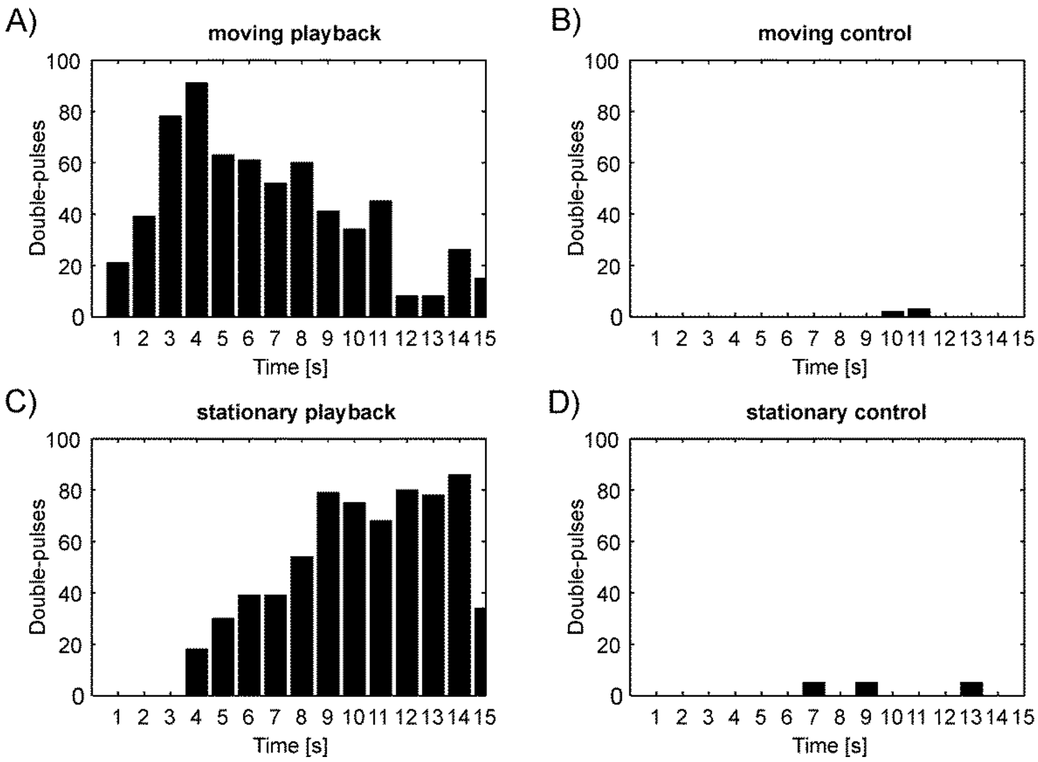

Figure 9.7: Double-pulse patterns. Number of double pulses displayed over the time course of the experiments by n = 13 individuals in response to the moving dummy electrode emitting electrical playback (A) and during silent controls (B), and for n = 15 individuals that reached the perimeter criterion during the trials with the stationary dummy electrode in response to electrical playback (C) and control (D).

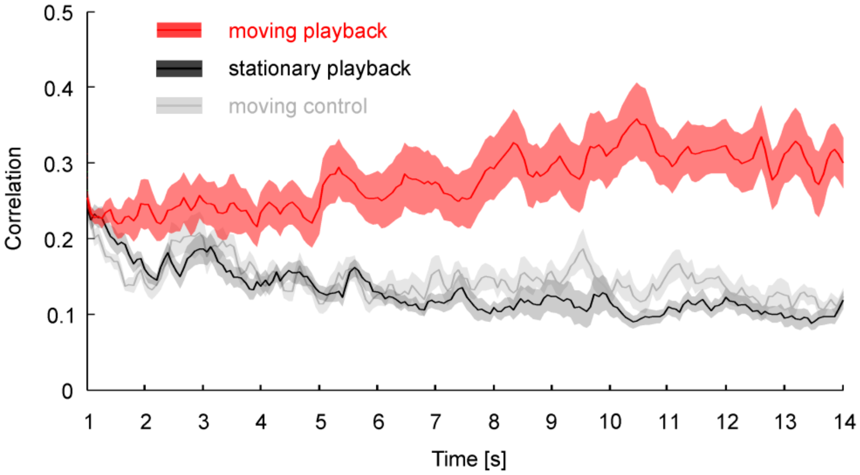

Figure 9.8: Signaling interactions of *M. rume* with the playback sequence. Averaged maximum cross-correlation coefficients for a 100 ms response time window over the time course of a trial. Correlations represent synchronization of the fish's signaling sequence to the electrical playback during the experiments with the moving playback (red), the stationary playback (black), and an independently recorded control using the signals emitted by the fish during the moving control condition (grey). Shaded areas represent the standard error of the mean.

9.3.3 Silenced fish

To determine the importance of active electrolocation during social interactions, three additional animals were tested before and after silencing of their electric organs. When the moving dummy dipole produced electrical playback, all animals reached the perimeter criterion in ten out of ten trials both before and after surgery. This was in all cases significantly more often than during the silent control condition (Fisher's exact test, $\alpha = 0.05$; Figure 9.9). Animals did not seem to behave differently during the post-surgery trials and were similarly active as before the intervention. While following, silenced *M. rume* remained close to the moving dummy electrode and displayed the same type of circling behavior previously observed in intact fish, even though they could not use their active electrolocation system. Swimming trajectories of the fish observed during the movement period of the dummy electrode are shown on the left-hand side of Figure 9.10. They demonstrate that the fish do not require active electrolocation to locate the physical source of a communication signal and to determine their position relative to the signaler. However, once the playback electrode stopped moving but continued emitting electrical playback, intact fish vigorously tried to reach the positive electrode of the dummy dipole (Figure 9.10, upper right panels), whereas swimming trajectories after silencing of the electric organ described a symmetrical, curvilinear path (Figure 9.10, lower right panels), which would be expected if fish followed the field lines along the dipole source. This distinction in searching strategies was observed in fish #3 and #6, whereas fish #8 employed the latter strategy both before and after surgery (Figure 9.10C), suggesting that knollenorgans play the major role in spatial interrelations between socially interacting mormyrids.

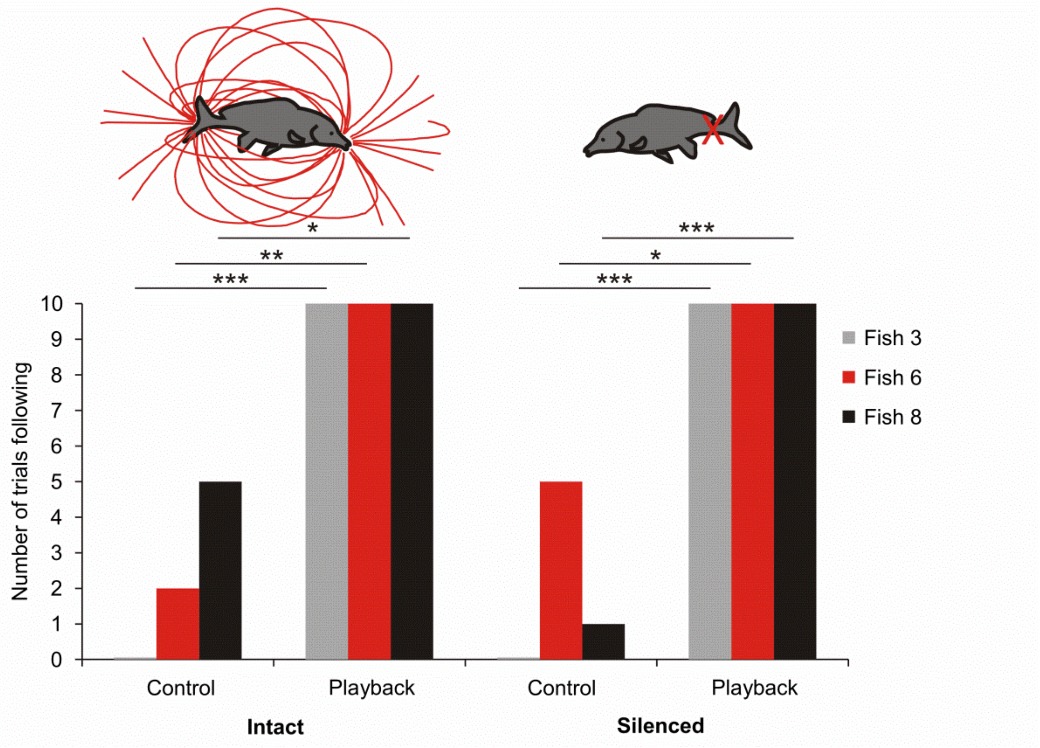

Figure 9.9: Comparison of following behavior between intact and electric silenced fish. Number of trials out of ten for three fish reaching the target area during playback presentation and controls both before and after silencing of their electric organs. Fish followed significantly more often during playback presentation, independent of the functionality of their active electrolocation system. Scores differ significantly based on Fisher's exact test ($\alpha = 0.05$). * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

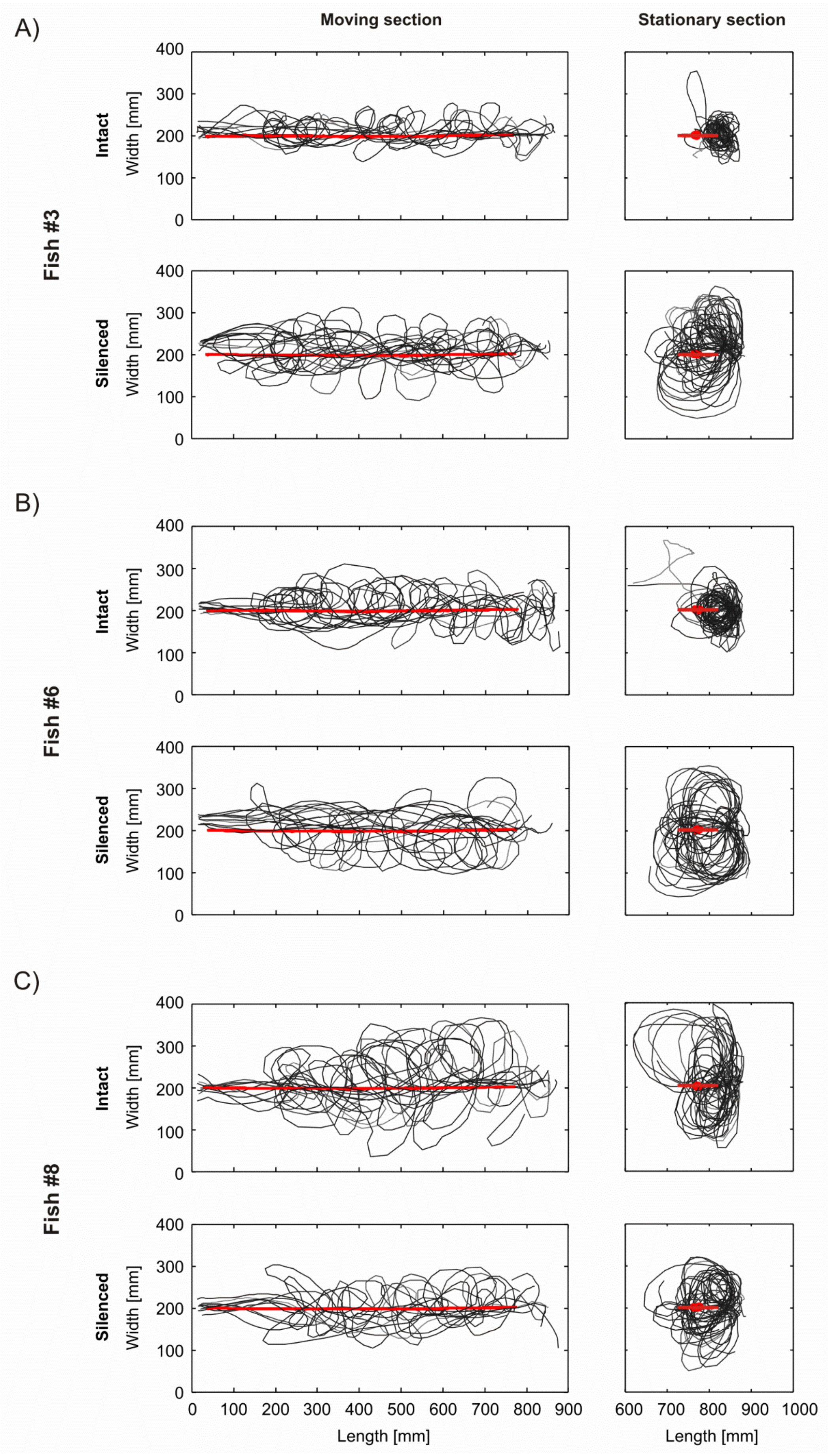
A)
B)
C)
Moving section
Stationary section
Fish #3
Fish #6
Fish #8
Intact
Silenced
Width [mm]
400
300
200
100
0
0
100
200
300
400
500
600
700
800
900
600
700
800
900
1000
Length [mm]

Figure 9.10 (previous page): Swimming trajectories before and after electric silencing. Exemplary depiction of ten swimming trajectories (black) of fish #3 (A), fish #6 (B) and fish #8 (C) in response to the moving dummy electrode emitting electrical playback. Tests were performed before (upper panels) and after (lower panels) silencing the electric organs of the fish. Trajectories are split between the first half of the trial, where the dummy electrode was moving, and the second half, where it remained motionless in the target area. Dummy trajectories are shown in red. On the right-hand side, the size of the dummy electrode is indicated by a red bar.

9.4 Discussion

Like most animals, mormyrids use multiple sensory systems synergistically to perceive their environment, and the loss of a particular modality may be compensated for by a different sense to accomplish a specific goal (Rojas and Moller, 2002; Schumacher *et al.*, 2016a; Schumacher *et al.*, 2017a; von der Emde and Bleckmann, 1998). In the current experiments, the behavior of *M. rume*, when following the disembodied dummy electrode moving along the ground, closely resembled the behavior of single *M. rume* following an EOD-emitting dummy fish moving through the water (Worm *et al.*, 2017) (chapter 4). In both cases, the following fish moved at comparable distances from the dummy and communicated with it electrically by producing double-pulses and by synchronizing their EODs to the playback sequence. By experimentally excluding all sensory cues mediated through vision, the lateral line system, and active electrolocation, it was demonstrated that only passive electroreception of EODs, probably mediated through the knollenorgan pathway, is sufficient to initiate and perpetuate following-behavior and evoke electrical signaling typical for electrocommunication in *M. rume.*

Based on electrical playback of natural EOD-sequences, fish followed a mobile dummy dipole from a shelter into an open area and to a final position, which was outside the communication distance from that shelter under non-visual conditions. The active space of the playback signal was pre-determined in control trials with a stationary playback source, using the onset of long discharge cessations as a means to define the outer limit where fish reacted to the signal. Animals then silently approached the dummy electrode and subsequently resumed discharge activity by displaying a double-pulse pattern that was only observed in response to electrical playback. These behavioral sequences were very similar to those observed by Moller *et al.* (1989) in *Brienomyrus niger*. Taken together, the combination of discharge cessations and double-pulse patterns clearly demonstrates that live fish attempted to interact socially with the electrically active dummy dipole and engaged in electrocommunication.

Visual guidance during following was excluded by performing all trials under infrared illumination only, which is invisible to the fish (Ciali *et al.*, 1997). Under these non-visual conditions, the fish could, in principle, use either active electrolocation or the mechanosensory lateral line system (Walton and Moller, 2010) to follow a moving object. In the present study, lateral line stimuli were ruled out by moving the playback dipole inside an electrically transparent agarose tube. Active electrolocation is a close-range sensory system that allows weakly electric fish to detect objects up to a distance of approximately one body length, but discrimination performance rapidly drops at distances greater than 4 cm in *G. petersii* (Fechler and von der Emde, 2013; von der Emde *et al.*, 2010) and probably at similar distances in *M. rume*. Given the distance of 30 cm between the shelter and the gate, it is unlikely that *M. rume* detected the moving electrode by active electrolocation from within the shelter. The following-distance under the electric playback condition was, however, well within that detection range. Active electrolocation could, therefore, have been used during close-range interactions in all trials with intact fish. The significance of active electrolocation for interactive behaviors in *M. rume* was controlled for by comparing three animals before and after surgically silencing their electric organs, making it impossible for them to use active electrolocation. The silenced fish showed the same tendency to follow the mobile dummy dipole as before the intervention. They also showed the same motivation to interact with the moving dummy by circling it, a behavior never observed in response to the silent controls. Although active electrolocation might, in principle, be used synergistically with passive electric sensing during close-range encounters between mormyrids, this result demonstrates that active electrolocation is not required to track and interact with a signal source mimicking a moving conspecific. Hence, fish only need the information contained in the electric fields generated by a conspecific's EODs to position themselves with respect to the signaler during close-range interactions.

A functional electric organ was also no precondition for the searching behavior that the fish displayed once the moving electrode had come to a stop but continued producing electrical playback. However, two out of three animals changed their searching strategy after silencing (the third animal had used this strategy already before silencing). Instead of probing for the positive pole of the dipole, which represents the rostral part of an *M. rume* producing an EOD, they circled the dummy dipole along curvilinear trajectories. This should be expected if animals orient themselves along the current lines of the electric dipole field established during EOD emission. The application this strategy for approaching a signaling conspecific from outside the range of active electrolocation was demonstrated by Schluger and Hopkins (1987) for the mormyrid *Brienomyrus brachyistius*. This behavioral response was proposed to be mediated through a directional sensitivity of knollenorgan electroreceptors by analogy to similar behaviors of the gymnotiform weakly electric fish *Hypopomus*, which possesses functionally similar time coding tuberous electroreceptor organs with directional sensitivity characteristics (Yager and Hopkins, 1993).

As other senses could not have mediated recruitment from the shelter, and active electrolocation was apparently not essential for following the moving dipole, it is proposed that it was passive electroreception of the electric signals of the dummy dipole, mediated through the knollenorgan system, which effectuated these behaviors. However, also ampullary receptor organs can be used for passive electroreception in weakly electric fish (Engelmann *et al.*, 2010). Due to the experimental design, a significant contribution of the low-frequency ampullary receptor system to the recruitment of the fish from the hiding area is not very likely, as animals did not follow in the absence of high-frequency EOD-signals. However, during following-behavior, a combined use of ampullary receptor organs and knollenorgans cannot be completely ruled out. Ampullary receptors are tuned to low frequencies of < 10 Hz (Engelmann *et al.*, 2010), whereas knollenorgans are broadly tuned to the higher frequencies contained in the species-specific EODs (Hopkins, 1981b) that were used for playback in this study. It was shown by Bell and Russell (1978) that in *G. petersii* ampullary receptor organs do not respond to EOD-like biphasic positive-negative signals with phases of equal amplitudes. Thus, if ampullary receptors are involved, they would have to respond to the low-frequency component of the playback EODs used in the experiments, which had, however, a significantly lower amplitude than the high-frequency components. An involvement of mormyromast electroreceptor organs in mediating the observed behavior cannot be ruled out completely but is also rather unlikely. The sensory threshold of mormyromasts is approximately one order of magnitude higher compared with that of the knollenorgans (Bennett, 1971c). This probably makes the mormyromasts not sensitive enough for the perception of the dummy's playback EODs. Afferent electro-sensory input from mormyromasts is enhanced by a corollary discharge at the level of the electrosensory lateral line lobe in the hindbrain, which makes the system most sensitive only when the fish produce their EODs during active electrolocation (Bell and Maler, 2005).

The conditions in this experiment were highly artificial and may have resulted in mismatched sensory information that would not usually be encountered in a natural situation. Due to their nocturnal lifestyle and reduced neuronal correlates in the visual system, vision was considered to be of minor importance for mormyrids (Lázár *et al.*, 1984). Nevertheless, they are able to perform visual tasks (Schumacher *et al.*, 2016a; Schumacher *et al.*, 2017a; Schuster and Amtsfeld, 2002). The loss of vision under dim light conditions reduced group cohesion in *G. petersii* (Moller *et al.*, 1982), indicating a contribution of vision to social behaviors. However, imitation of visual appearance and motility patterns by a mobile dummy had no noteworthy effect recruiting individual *M. rume* from a sheltered area, when compared with electrical playback (Donati *et al.*, 2016). This supports the notion that electroreception is the dominant modality during social interactions. In a previous study (Worm *et al.*, 2017), *M. rume* also followed a mobile dummy, which did not emit any electrical playback, even in complete darkness. Swimming trajectories in these experiments suggested an involvement of the lateral line in following-behavior, as the fish closely reproduced the swimming path of the dummy. Path-following behavior based on lateral line information has previously been described in piscivorous catfish tracking their prey (Pohlmann *et al.*, 2001). In *M. rume,* however, such path-following behavior was mainly observed in the absence of electrical signals, whereas following-behavior in response to electric playback caused a shift of swimming trajectories to a more lateral position in relation to the dummy (Worm *et al.*, 2017). This supports the hypothesis that passive electroreception by knollenorgans constitutes a major determinant of social spacing in *M. rume*.

The importance of active electrolocation for detection and characterization of animate and inanimate objects is well established. Whether mormyrids make use of active electrolocation during intra-specific interactions has rarely been addressed. Evidence that mormyrids might be able to recognize conspecifics by active electrolocation comes from the observations by Moller *et al.* (1982), who found blind *G. petersii* to be still attracted by electrically silent conspecifics. Terleph (2004) suggested that regularized discharge patterns during parallel lineups could have been ritualized into a communication signal from mutual size estimation during sequential assessment between opponents (Enquist and Leimar, 1983). Insights from modeling active and passive electric images in the gymnotid *Gymnotus omarorum* suggest that both active and passive electroreception could be used during assessment of an opponent, but passive information is more likely to trigger aggressive interactions (Pedraja *et al.*, 2016). The experiments with *M. rume* support the hypothesis that information from passive electroreception serves in triggering following-behavior and social interactions in mormyrids.

In conclusion, it is suggested that passive location via the knollenorgan pathway serves in mediating communication not by detecting EOD-waveform differences and variations in IDI-sequences. The evidence presented in this study supports the hypothesis that this sensory pathway is also used to mediate spatial interactions between individuals, where it is sufficient, although not necessarily exclusively employed, during close-range encounters between mormyrid weakly electric fish.

10. Discussion: Ethorobotics

The collaboration between roboticists and biologists holds great potential for joint research projects that aim to implement specialized animal capabilities, like sensory performances or locomotion strategies, into innovative technical devices and applications. From a scientific perspective, robotic animal models provide a methodic approach to test hypotheses regarding the internal control of behavior (Webb, 2008). For the behavioral biologist, ethorobotics is increasingly well positioned to provide the answer to Tinbergen's (1963) question: "how does one make an experimental animal which lacks just one behaviour pattern and is otherwise normal?" While simple mockups, dummies, and decoys have long been used to identify the key stimuli that trigger social responses and innate behavior patterns (Tinbergen, 1948), robots offer the possibility to test multimodal and cross-modal contributions to such behavior patterns in controlled and repeatable experimental conditions, mainly because all aspects of the robot's behavior are under the experimenter's control at all times (da Silva Guerra *et al.*, 2010; Krause *et al.*, 2011). Additionally, many behavior patterns may rather be considered to be chains of stereotyped behaviors, which require a certain amount of interactivity for complete expression (Lissmann, 1932). The possibility to equip robot models with sensors that monitor animal reactions in real-time enables researchers to close the feedback loop between live animals and robotic dummies (Caprari *et al.*, 2005; Gribovskiy *et al.*, 2010). This approach allows the investigation of more complex behaviors in elaborate experimental designs, in which a dummy can respond to the reactions it induces (Landgraf *et al.*, 2013).

The use of interactive robots can also elevate behavioral studies from the level of individuals to group-level interactions by building mixed societies of live and artificial animals (Halloy *et al.*, 2013; Schmickl *et al.*, 2013). This methodology requires that researchers succeed in building robots that are accepted by animals as conspecifics (Mondada *et al.*, 2013). This acceptance, however, does not presuppose identical replication of all animal features. Instead, only the relevant components that trigger social behavior have to be identified and implemented to successfully generate collective behavior (Halloy *et al.*, 2007). One way to identify such features is by constructing a replica and then systematically reduce it to determine the relevant cues in behavioral experiments.

The potential of mormyrid weakly electric fish as model organisms for establishing a mixed society, in which the acceptance problem can be solved by using playback of electrical communication signals, was systematically explored in the preceding two chapters of this thesis. Since the discovery of the active electric sense (Lissmann, 1951; Lissmann and Machin, 1958), it has become increasingly evident that communication is a vital aspect of electrical signaling in mormyrids and that both the waveform and temporal sequence of their EODs contain behaviorally relevant information (see section 3.5). Pioneering studies have shown that playbacks of electric signals can differentially evoke behavior in mormyrids based on EOD-duration (Hopkins and Bass, 1981) and IDI-sequence (Kramer, 1979). It is therefore not surprising that electric signals are also important during communication in groups, where they may promote coherence among group members (Arnegard and Carlson, 2005; Gebhardt *et al.*, 2012a; Gebhardt *et al.*, 2012b; Khait *et al.*, 2009; Moller, 1976), communicate identity, dominance, motivation, and current activity (see section 3.5), but also the location of an individual (Hopkins, 2005; Schluger and Hopkins, 1987). Here, the focus was set on the sensory contributions to the behaviors observed in chapters 4 and 5 rather than on the specific 'meaning' of a particular signaling pattern. Electrical playback sequences were, therefore, not systematically modified. The objective was to isolate the general influence of electrical signaling on behavioral responses of *Mormyrus rume proboscirostris* and contrast their relevance for induced social behavior with cues from other sensory modalities of the fish. This was done in experiments with a biomimetic dummy fish (chapter 8) (Donati *et al.*, 2016), which were contrasted with experiments in which the stimulus was reduced entirely to the electric signals (chapter 9) (Worm *et al.*, 2018).

10.1 Experiments with the biomimetic dummy fish

In chapter 8, a biomimetic dummy fish was designed that resembled a live specimen of *M. rume* morphologically and had realistic size dimensions compared with a real fish. The robot contained an actuation mechanism to generate tail-fin oscillations while moving forward and could additionally produce playback sequences of pre-recorded EODs. By systematically combining motility cues with and without electrical signaling displays, the contributions of the robot's features to the attraction of fish were investigated in a behavioral paradigm based on the quantification of following-responses, similar to the experiments in chapter 4. The results were unequivocal and showed that electrical signaling was the main attraction feature, whereas the tail-fin oscillations of the dummy had no significant effect on fish behavior (Figure 8.5). There was also no convincing evidence for multimodal synergy effects. The combination of motility cues and electrical playback was not significantly more attractive than electrical signaling alone (Figure 8.6). However, the assessment of social responses of *M. rume* in chapter 8 was solely based on quantification of following-behavior. Electric signaling responses by the fish were not analyzed.

Compared with the following-responses that were observed with comparable experimental setups in chapters 4 (Figure 4.1) and 9 (Figure 9.1, see section 10.2 below), using a biomimetic robot had no advantage over a simple fishing bait with electrodes, or even a 'disembodied' dipole electrode, which the fish could only perceive by passive electric sensing. In fact, following-scores were considerably lower in chapter 8, which might have been due to some intrinsic properties of the biomimetic robot fish, or caused by general differences in test conditions and the experimental protocol. In theory, a potential positive effect of motility cues could have been counteracted by an aversive effect of the acoustic noise that was caused by the internal actuation mechanism of the robot. Activation of the tail-beat movement caused sound emission at frequencies of 3 kHz and higher (see Figures C.1 and C.2 in Appendix C for a more detailed characterization). Mormyrids are 'hearing specialists' (see section 3.3.2), and it has been shown that *Gnathonemus petersii* can hear frequencies up to approximately 2500 Hz (McCormick and Popper, 1984). Since there are no hearing curves for *M. rume* in the literature, it cannot be excluded that the animals could have heard the acoustic noise generated by the biomimetic dummy and that this noise might have had a negative effect on attraction. In this respect, the robot may have failed to restrict sensory stimuli to distinct and defined sensory perception channels of the fish.

Nevertheless, the experiment was designed to contrast sensory stimuli that live fish detect via different sensory channels, including vision. In a visual discrimination task, *G. petersii* was most successful at an illuminance of 10 lux (Schuster and Amtsfeld, 2002). Accordingly, this illumination level was chosen for the current experiments to allow visual detection and discrimination of the stimuli generated by the dummy. However, most mormyrids are nocturnal (Moller *et al.*, 1979) and even low light intensities cause immediate shelter seeking. Consequently, attraction scores obtained during the experiments in chapters 4 and 9 might have been higher because of the absence of visual stimuli, and illumination may have made *M. rume* more reluctant to leave the sheltered area during the experiments in chapter 8. Finally, the short acclimatization time of only 1 h may have caused higher anxiety levels and, therefore, a decreased willingness to explore the open area of the experimental tank compared with the studies in chapters 4 and 9, during which the fish were acclimatized overnight and tested under infrared illumination.

10.2 Experiments with the reduced dummy fish

In a second behavioral study (chapter 9) (Worm *et al.*, 2018), all features of the robot were controlled to create a dummy that was reduced to only the electrical signaling stimuli from the perspective of the fish. This was accomplished by moving a playback electrode inside an electrically transparent agarose tube to exclude hydrodynamic stimuli, and by performing experiments under non-visual conditions using infrared illumination. Additionally, some fish were electrically silenced to control for active electrolocation. Electrical signaling could thereby be identified as a key stimulus that is sufficient to induce social behavior in *M. rume*. Moreover, it was established that electrical signals could also sustain social interactions and guide them spatially, most likely via the knollenorgan pathway (see section 1.3). These results emphasize the importance of this sensory channel for electrocommunication.

Even though the sensory information the fish had about the position of the dummy dipole was highly restricted, attraction scores during this experiment were highest compared with all other studies. Fish almost always followed the moving dummy dipole, given it emitted electrical playback (Figure 9.3). The main improvement to the setup was the provision of only a single shelter, in which the fish had to assume a defined position within the active space of the playback signal before a trial could be triggered (Figure 9.1A). Additionally, swimming trajectories and electric signaling responses of the fish were analyzed in detail to confirm the social nature of the responses evoked by the electrically active dummy dipole. The occurrence of social signaling patterns, in particular double pulses (Figure 9.7), long cessations (Figure 9.6B), and discharge synchronizations with the electrical playback sequence (Figure 9.8) allow the conclusion that the observed behavior was indeed of social nature and not a mere effect of curiosity. Similar behaviors have previously been observed in socially interacting mormyrids (Gebhardt, 2012; Gebhardt *et al.*, 2012a; Gebhardt *et al.*, 2012b; Moller *et al.*, 1989), and could not be observed in isolated individuals or control trials with electrically silent dummy fish (chapter 4) (Kersten, 2017a; Worm *et al.*, 2017).

10.3 Sensory contributions to the observed behavior

Mormyrids have multiple sensory systems to perceive their environment (Moller, 2002) and for communication (Schuster, 2006) (see sections 3.3 and 3.5). The objective of the preceding two chapters was to test the hypothesis that electrical communication signals can mediate the acceptance of a mobile dummy as a conspecific by mormyrid weakly electric fish and assess the contributions of multiple sensory modalities to social behavior in live *M. rume*. For this purpose, sensory inputs to all but the passive electrosensory modalities of the fish were experimentally controlled, which allowed addressing the question whether the fish ultimately need to directly perceive the morphological body features of another individual to communicate.

Behavioral responses to the visual properties of the various dummy fish that were used throughout this thesis were not systematically investigated. However, the visual system in mormyrids is highly specialized and very sensitive in dim light conditions (Kreysing *et al.*, 2012; Landsberger *et al.*, 2008). It must, therefore, be assumed that the visual appearances of the fish dummies used in chapters 5, 8 and 11 were perceived by the fish, and may have influenced their behavior, because these studies were performed using visible light. However, it appeared that animals did not pay much attention to either the morphological features of the dummies or to the visible aspects of the motility cues generated by tail-fin oscillations in chapter 8. In fact, following-behavior (Figure 4.3) and motor interactions (Figure 4.20) were also observed under infrared illumination, which cannot be perceived by the fish (Ciali *et al.*, 1997).

The use of mobile fish dummies under visual conditions did not allow to differentiate between the potential effects of visual and hydrodynamic stimuli. The question whether the visual perception of morphology and motility features influences social responses in mormyrids, could, for instance, be investigated by using computer animated fish models (Gierszewski *et al.*, 2017). However, *M. rume proboscirostris* are by default nocturnal animals that even spawn at night (Schugardt and Kirschbaum, 2004). Visual information was no precondition for following-behavior, and the social interactions with moving dummies in chapters 4 and 9 were observed under infrared illumination. It is therefore suggested that vision should be generally excluded from mixed live-artificial experiments with mormyrids (with the possible exception of those members of the subfamily Petrocephaline that specialized in visual communication (Stevens *et al.*, 2013)), because it introduces a confounding variable that is at best unnecessary with regard to the desired behavioral observations, and possibly disturbs the natural behavior of nocturnal fish.

The active tail-fin oscillations of the biomimetic dummy fish in chapter 8 did provide not only visual cues but also generated hydrodynamic effects. Objects that move in relation to water generate vortices that fish can perceive via their mechanosensory lateral line system (Bleckmann and Zelick, 2009). Consequently, hydrodynamic information may have influenced fish behavior in all experiments involving mobile dummies, except the one in chapter 9. Here, movement of the dummy dipole was confined to an agarose tube, which was transparent for electric signals, but not for water movements that could have stimulated the lateral line neuromasts of the fish. Even though electroreceptor organs have their evolutionary origin in the lateral line system (Szabo, 1965), the mechanosensory component of the lateral line has hardly been investigated in mormyrids. In *G. petersii*, both the number of superficial neuromasts and the morphology of the head-canal system are reduced, possibly in favor of the electrosensory system (Schumacher, 2017). This is likely to cause a reduced functionality in comparison with the mechanosensory lateral line of other teleosts However, in chapter 4 (Worm *et al.*, 2017), *M. rume* were able to follow an electrically silent dummy under non-visual conditions. Since the animals followed the trajectories of the electrically silent dummy very closely (Figure 4.22), this behavior could have been mediated by the lateral line, analogous to the wake-following behavior of predatory catfish (Pohlmann *et al.*, 2001). Based on the observation that fish followed in a more lateral position when electrical playback was presented (Figure 4.21B and Figure 4.22), and the fact that lateral line information was no precondition for following-behavior in chapter 9, it is suggested that the electrosensory systems provide mormyrids with more reliable information during social interactions than the lateral line. In analogy to the concept of electrosensory capture proposed by Schumacher *et al.* (2017a), electrosensory guidance seems to dominate over lateral line information during following-behavior in the presence of electrocommunication signals.

Within a range of approximately one body length, active electrolocation provides weakly electric fish with behaviorally relevant information about objects in their environment (see section 1.3). Thus, animals could have used actively acquired electrosensory information to follow the mobile fish dummies. The shift in following-behavior that was observed in chapter 4 can only be explained by passive electroreception, because the fish were at all times able to rely on active electrolocation in these experiments. While vision might theoretically fool mormyrids into mistaking a biomimetic fish dummy for a conspecific individual based on morphological features, this is highly unlikely for the active electric sense. During active electrolocation, living organisms are easily discriminated from inanimate objects based on their capacitive nature, which provides fundamentally different electrical information compared with the purely resistive properties of nonconductive objects (Gottwald *et al.*, 2017a). In behavioral experiments, active electrolocation can be controlled for by surgically silencing the electric organ of test fish (Moller *et al.*, 1982; Schumacher *et al.*, 2016a). This treatment neither impaired the motivation, nor the ability of *M. rume* to follow the mobile dipole electrode in chapter 9, thus proving the importance of passive sensing for social behavior. However, trajectories of silenced animals differed from those obtained from intact fish in chapter 4 (fish #3 in Figure 4.22B was the same individual as fish #6 in Figure 9.10B). Trajectories obtained from fish that could not rely on hydrodynamic stimuli and active electrolocation were way more curvaceous compared with those of fish that followed an 'embodied' version of the dummy, which was physically represented by a fishing bait of similar size as the test fish. These results are consistent with what would be expected if animals relied on passive sensing and navigated along the electric field lines of the playback EODs to follow the source of the signal (Hopkins, 2005). These findings also expand the results by Schluger and Hopkins (1987), who showed that *Brienomyrus brachyistius* approached a stationary fish along the field lines of its EOD.

Here, it was shown for *M. rume* that also the position of a swimming conspecific may be tracked by passive detection of the information contained in the spatial properties of actively generated electric fields. While this behavior could, in theory, be mediated by either mormyromasts, ampullary receptor organs, knollenorgans, or a combination of the three, it is argued that knollenorgans are most likely involved. Knollenorgans are more sensitive to foreign signals than mormyromasts (Bennett, 1971c) and respond to the high-frequency signal components contained in the self-generated EOD, whereas ampullary receptor organs are sensitive to low-frequency electric signal components (Engelmann *et al.*, 2010).

The fact that there was no necessity for any sensory cues other than the high-frequency electric playback EOD to induce following-behavior and social interactions does not imply that fish ignore them during normal, unrestrained behavior in their natural habitat. The lateral line may not usually be involved in following-behavior among mormyrids, but it most certainly plays a role during lateral displays between competing fish (see section 6.2). Also, there is no reason to classify the tracking of the electrically silent dummy by *M. rume* in chapter 4 as social behavior. The 'embodied' version of the dummy in chapter 4 was followed on more efficient trajectories than the 'disembodied' dummy dipole used in chapter 9. This suggests that at close range, passive sensing might have rather served as a backup for active electrolocation than to represent the default sensory strategy for social interactions. It is, therefore, reasonable to conclude that mormyrids make the most of all their sensory systems during social interactions in their natural habitat.

For the design of interactive, biomimetic dummy fish, and the possibility of their integration into mixed societies of live and artificial weakly electric fish, the results obtained so far have some useful implications. A major challenge when constructing biomimetic robots for interactive behavioral experiments with live animals is to determine the right set of cues that will be perceived by the animals (Mondada *et al.*, 2013). Restricting stimuli to as few as possible and as many as necessary should be the adequate approach to guarantee the expression of the desired behavior by research animals, and enable researchers to draw meaningful conclusions from experiments. In chapter 8, it was demonstrated that the design of a biomimetic robotic fish that mimics live *M. rume* in size, morphology, and motility is possible, but not necessary for the investigation of mormyrid social behavior. Comparing the results with those from chapters 4 and 9 allowed the conclusion that, if possible, visual stimuli should be avoided altogether. In chapter 9, it was shown that passive reception of electric communication signals is sufficient to induce social behavior, but animals without a functional electric organ switched to a different searching strategy, which apparently did not represent their normal behavior. An embodied representation of the artificial agent as a dummy fish, which cannot only be perceived through passive electric sensing by live fish, is, therefore, an advantage not only from a methodical perspective.

An elaborate mechanism to generate biomimetic movement patterns does not appear to be significant, and may even introduce disturbances, such as acoustic noise. The hydrodynamic effects of artificial tail-fin oscillations in chapter 8 were not quantified and could therefore not be compared to the vortices generated by swimming *M. rume* (see for instance Polverino *et al.* (2013)). However, the results of chapters 4 and 5 have demonstrated that commercial fishing baits, which are designed to generate body movements and vortices when dragged through water, are sufficiently suited to represent the body of an artificial mormyrid (also compare Cazenille *et al.* (2018) and Bonnet *et al.* (2018)). In conclusion, the essential feature of a dummy fish representing an artificial mormyrid is the capability to generate playback of electrical communication signals, i.e., sequences of artificial EODs. Live *M. rume* displayed both motor and electromotor social behaviors towards electrically active mobile dummies, indicating that the presence of electrical communication signals is behaviorally more relevant than the presumed ability of mormyrids to distinguish a real fish from a plastic replica by using active electrolocation. Thus, it could be shown that electrically active dummy fish were 'accepted' by live mormyrids according to the propositions by Halloy *et al.* (2013). From a sensory perspective, and with regard to the behavioral relevance of the information contained in artificial stimulus EODs, the hypothesis that the imitation of electrical communication signals can be used to systematically induce and manipulate social behavior in *M. rume*, could be confirmed. The third and last part of this thesis will investigate and evaluate the potential of social group-behavior in *M. rume* as a prerequisite for establishing mixed live-artificial experiments that are interactive at the levels of motor behavior and electrical signaling.

IV. Part Three: Collective Behavior in Groups

11. Project 5: Influence of an Interactive Mobile Dummy Fish on Small Groups of the Weakly Electric Fish *Mormyrus rume proboscirostris*

In this final chapter, the influence of a mobile dummy on single fish and small groups of two, three, and four *M. rume* will be investigated and discussed in the context of current knowledge about mormyrid group behavior. The experiments in this chapter represent an extension of those presented in chapter 5. They allowed the experimenter to modify the swimming trajectories of the dummy based on real-time video recordings, and the dummy was programmed to generate artificial echo responses to EODs of nearby fish. Interactive behavior between dummy and test fish could, therefore, be investigated on both the motor and the electromotor level.

The chapter starts with a summary of the general cost and benefits of group-living with an emphasis on shoaling behavior in fish. The potential of mobile dummies for investigating and influencing group behavior in fish in general, and mormyrids in particular, are then summarized and discussed in the context of the results of the experiments.

11.1 Introduction

Many animals are social and live in groups of different sizes and different degrees of complexity. Animal groups can be small with rigid hierarchies (Cafazzo *et al.*, 2016), form complex and dynamic fission–fusion societies (Couzin, 2006), or constitute large aggregations like flocks of birds, herds of ungulates, and shoals of fish. Compared to a solitary existence, group-living provides several adaptive advantages to social animals, but may also incur costs that are context-dependent and have to be traded off by individuals (Krause and Ruxton, 2002). In particular, large schools of marine pelagic fish are fascinating phenomena that have captivated both researchers and layman and are also of great commercial and ecological importance. However, animals benefit from group formation also at smaller scales. Consequently, it has been estimated that approximately 50% of fish species exhibit shoaling tendencies at least during some stages of their lives (Shaw, 1978). Collective behavior in fishes has been investigated on many levels ranging from global scale migration patterns (Berdahl *et al.*, 2016; Makris *et al.*, 2009) to the local interactions between individuals that are prerequisite for the organization of larger groups (Hunter, 1969; Katz *et al.*, 2011; Partridge and Pitcher, 1980; Pitcher, 1979; Pitcher *et al.*, 1976). According to Pitcher and Parrish (1993), *shoals* are groups of fish that stay together due to social attraction, whereas *schools* are characterized by synchronous swimming activity and a high degree of polarization, i.e., a similar spatial orientation of group members. This means that *schooling* is a particular form of *shoaling*.

11.1.1 Costs and benefits of shoal formation

Avoiding predation is the most important reason for shoaling in fishes (Pitcher and Parrish, 1993) and there are several ways in which group formation can reduce an individual's predation risk. According to the 'selfish herd' concept proposed by Hamilton (1971), gregarious behavior can be explained as a selfish strategy that minimizes an individual's risk of getting caught by predators with a tendency to capture the nearest individual they encounter. Group formation could thus have evolved as a form of cover-seeking that is particularly beneficial in featureless open-water habitats, which do not provide any other kind of shelter (Parrish, 1992). The combination of this *dilution effect*, which minimizes individual predation risk at the cost of everybody else, and a general *predator avoidance effect*, which rests on the assumption that predators are more likely to encounter dispersed individuals than a locally aggregated group of animals, has been proposed to be an adaptive strategy that causes *attack abatement* (Turner and Pitcher, 1986).

In addition to individual risk reduction, gregarious behavior may be adaptive because group members benefit from effects that cannot be produced by solitary animals and emerge only at the group level. The *confusion effect* describes the fact that predators have a decreased attack success when trying to capture prey from larger aggregations (Landeau and Terborgh, 1986). This reduced success is attributed to a higher difficulty in neural mapping that leads to a decreased attack rate and an increased spatial targeting error during attacks (Ioannou *et al.*, 2007). Fish in larger schools can also perform coordinated escape maneuvers to evade predation (Nøttestad and Axelsen, 1999; Pitcher and Wyche, 1983) (Figure 11.1).

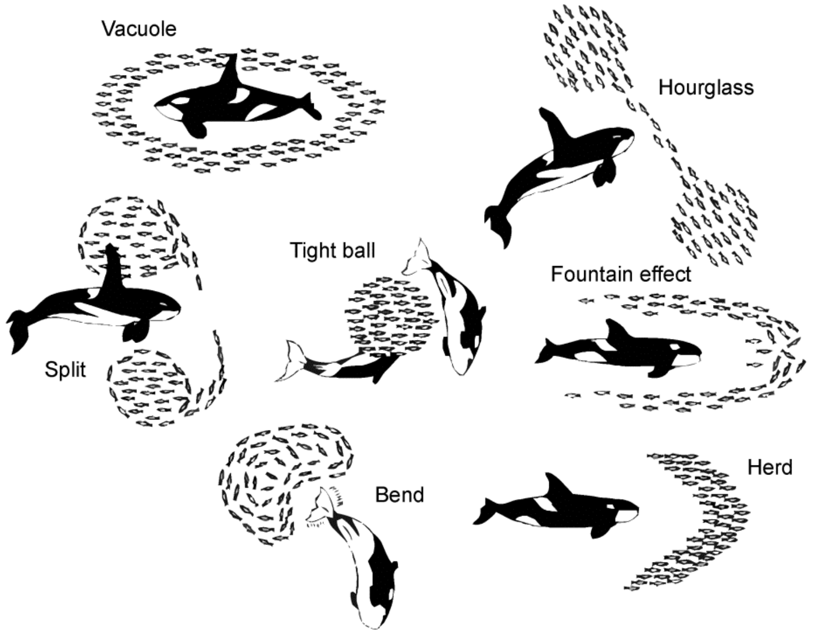

Figure 11.1: Escape maneuvers of schooling prey. Schematic representation of group-level behavioral strategies of herring in response to a killer whale attack. Modified after Vabø and Nøttestad (1997).

Animals in groups can further benefit from increased information, which can manifest on several levels. In the immediate environment, the *many-eyes effect*, which is afforded by higher corporate vigilance in groups of larger sizes in combination with socially mediated flight responses among shoal members (Godin *et al.*, 1988), can contribute to the reduction of predation risk. Consequently, individuals in groups can allocate more time to foraging instead of monitoring for predators (Magurran *et al.*, 1985). Individuals also benefit from shared information when it comes to finding food and the development of new foraging sites. For example, fish in groups have been shown to find a food source faster than solitary individuals (Böhme, 2011; Pitcher *et al.*, 1982).

On a larger scale, shared information can be an advantage during navigation and migration. Many fishes perform seasonal long-distance migrations that are related to their respective live cycles. Prominent examples are herring and salmon, who repeatedly visit traditional spawning areas (McQuinn, 1997) or return to their natal freshwater habitats for reproduction (Quinn, 2005). Irrespective of the sensory information that is acquired and used by individual fish during migration (Døving and Stabell, 2003; Hawryshyn, 2010; Quinn, 2005), collective movement can increase the precision of homing if school members adopt a common mean direction and individual errors are canceled out (Larkin and Walton, 1969). Additionally, the combination of taxis and schooling behavior can enable large groups to track small gradients, especially in noisy environments where such gradients cannot be perceived by individual animals (Grünbaum, 1998). Under laboratory conditions, the ability of golden shiners (*Notemigonus crysoleucas*) to orient to a faint light gradient increased with group size and was mediated by social cohesion rather than by individual responses to the environment (Berdahl *et al.*, 2013). Collective navigation has therefore been suggested to be a strategy for homing in anadromous salmon (Berdahl *et al.*, 2016) and a relatively small proportion of experienced individuals could be sufficient to influence the direction of movement in schools of herring (Huse *et al.*, 2002). In the context of migratory behavior, the synchronization of reproduction is also an essential function of group formation (Makris *et al.*, 2009).

Group living can also reduce the energetic costs of locomotion (Krause and Ruxton, 2002). Based on the assumption that fish in groups can reduce the energetic costs of swimming by exploiting vortices generated by other fish, Weihs (1973) proposed that schooling fish should adopt a diamond-shaped spatial array to yield the highest possible hydromechanical advantage. While expression of this particular group structure could not be confirmed for real schools (Pitcher and Parrish, 1993), more recent experiments demonstrated energetic benefits of schooling that do not depend on a precise geometric alignment of group members (Marras *et al.*, 2015).

Group living does, however, also involve disadvantages for individual group members. The benefits of predator avoidance and enhanced food detection abilities may come at the cost of increased competition for resources, which can cause aggressive behavior among conspecifics, and large groups can be vulnerable to parasites and even attract predators (Krause and Ruxton, 2002). This means that there are tradeoffs for individuals, who have to outweigh the costs and benefits of joining a group against solitary existence. However, this also implies that costs and benefits vary as a function of group size, and with respect to an individual's position within a given group (Parrish, 1992). Individual predation risk is highest at the front and in the marginal regions of a group, but so is the chance to encounter food (Krause, 1993). Individuals may, therefore, adjust their position within the shoal according to their current motivation, to find a compromise between hunger levels and perceived predation risk (Krause, 1993). But sorting in shoals also occurs with respect to phenotypic traits (Pitcher *et al.*, 1985; Ranta *et al.*, 1994), especially under acute predation risk (Krause, 1994). Sorting counteracts the *oddity effect*, which may enable predators to focus on individuals that are easy to distinguish from the rest of the group (Landeau and Terborgh, 1986). The specific positions that individuals occupy in a group are thus non-random, and different individuals may benefit differentially from grouping based on size and composition of the shoal. Additionally, there are asymmetries between the fitness costs for established group members that are joined by additional animals, and the fitness benefits for solitary individuals joining the shoal. This conflict may cause naturally occurring groups to be larger than optimal (Krause and Ruxton, 2002). Consequently, the elective group size an individual prefers to shoal with varies according to current motivation, environmental conditions, and the life history traits of its species (Pitcher and Parrish, 1993).

Especially large schools of marine fish can attract large numbers of predators like birds, cetaceans, larger fish, and fishing vessels (Parrish, 1992; Pitcher and Parrish, 1993). Baleen humpback whales (*Megaptera novaeangliae*) exploit the schooling tendencies of their prey during bubble-net feeding by enclosing and capturing large amounts of fish between self-generated walls of air bubbles (Wiley *et al.*, 2011). In this respect, group hunting provides an advantage for gregarious predators because it constitutes a strategy to counteract the shoaling defense of grouping prey. Cooperatively hunting bottlenose dolphins (*Tursiops truncatus*) were shown to divide labor between group members to catch schooling fishes (Gazda *et al.*, 2005), but coordinated attacks on schooling prey have also been observed in piscivorous fish (Handegard *et al.*, 2012; Herbert-Read *et al.*, 2016; Schmitt and Strand, 1982). Predator attack strategies must, therefore, be taken into consideration when discussing the evolution of schooling behavior as an adaptation against predation (Parrish, 1992).

11.1.2 Rules and mechanisms underlying collective behavior

The apparent synchrony and organization of large fish schools are fascinating phenomena that are, however, not readily accessible to the experimenter who is interested in the physiological and behavioral basis of these larger-scale patterns. Detailed studies, in which the dynamic interactions between individuals in large schools were analyzed with high temporal resolution over extended periods of time, are challenging and therefore rare (Partridge, 1981). Still, several methodological approaches can shed light on the rules and mechanisms underlying collective animal behavior. With increasing shoal size, it becomes increasingly unlikely that individuals have information concerning the global pattern of the group, and how their current position relates to the group structure. Instead, natural selection likely acts on individual interaction rules that allow positioning within the group through self-organized sorting, without requiring any knowledge of the global group pattern (Couzin and Krause, 2003).

In self-organized systems, global patterns emerge as a consequence of local interactions among individuals, which act according to local information and without any reference to the global structure of the system as a whole (Camazine *et al.*, 2001). Some of the global patterns in which animal groups are organized can also be observed in inorganic, particle-based systems, and may, therefore, represent emergent epiphenomena rather than adaptations (Parrish and Edelstein-Keshet, 1999; Parrish *et al.*, 2002). But goal-directed movements and coordinated escape responses require that individuals comply with behavioral rules to cause global-scale effects. Behavior at the global level must, however, not be explicitly encoded to generate these effects. Instead, it emerges as a result of local interactions between individual group members and is based on recursive, nonlinear feedback-loops between individual interactions and the collective behavior of the group. Context-dependent modifications of these local interaction rules as a result of changes in environmental conditions can then lead to adaptive responses at the group level (Couzin and Krause, 2003). In guppies (*Poecilia reticulata*), predation pressure leads to larger and more cohesive shoals because it modifies the decision rules of individuals for social attraction and repulsion, and changes the dynamics of acceleration and deceleration responses (Herbert-Read *et al.*, 2017). Similarly, in Berdahl *et al.* (2013), a tendency of individual golden shiners to slightly increase swimming speed in response to brighter light levels caused the entire shoal to turn towards more shaded areas due to social cohesion.

Decoding such rules requires studying the sensory performance of individuals as well as their interactions with the environment and with conspecifics in different contexts. Shoaling behavior in fishes is mainly mediated by vision and the mechanosensory lateral line system (Partridge and Pitcher, 1980; Shaw, 1978). By temporally blindfolding individual saithe (*Pollachius virens*), Pitcher *et al.* (1976) demonstrated that these fish were able to school with conspecifics unless the researchers additionally deprived the animals of hydrodynamic information by sectioning the lateral line. Based on a set of follow-up experiments, Partridge and Pitcher (1980) concluded on a synergistic use of the two sensory systems, with vision being of greater importance for maintaining positions and angular orientation in relation to other individuals, and the lateral line for monitoring swimming speed and direction of their neighbors in the school.

But knowledge of the sensory requirements for shoaling does not sufficiently explain the interaction rules that are necessary to achieve behavioral synchrony during collective movements. On the group level, video analysis has been used to quantify response latencies and information transfer in fish schools during directional changes (Shaw, 1978). Similarly, social reactions have been measured in individual fish. By selectively startling individual jack mackerel (*Trachurus symmetricus*), Hunter (1969) found that nearby individuals reacted with response latencies of 0.15 to 0.25 s to the startled fish, depending on its angular position. While sensory contributions and behavioral response latencies can be studied in individual fish or by analyzing dyadic interactions, extrapolating such results to explain the behavior of larger groups is problematic. This is because shoaling behavior is qualitatively different from dyadic interactions and does not depend linearly on the number of individuals in a group (Partridge, 1980).

Theoretical approaches to formulate the principles underlying shoal formation while circumventing such experimental difficulties have been made using individual-based computer simulations (Couzin *et al.*, 2002; Huth and Wissel, 1992; Vabø and Nøttestad, 1997). To specify the mechanisms that generate synchronized behavior in fish schools, Huth and Wissel (1992) designed a model in which they assigned behavioral response rules to simulated individuals, who were influenced only by the position and orientation of their respective nearest neighbors. Each was programmed to have a concentric zone of repulsion to avoid collisions, followed by a zone of alignment, in which individuals assumed parallel orientation, and a zone of attraction to approach other individuals (Figure 11.2). By averaging the influence of at least four nearest neighbors per individual, Huth and Wissel (1994) were able to simulate schools resembling the behavior of real fish schools with respect to group cohesion, polarization, and internal dynamics. In a refined simulation model, Couzin *et al.* (2002) were able to induce behavioral transitions of shoaling behavior at the group level by changing the range of the zone of parallel orientation. In a simulation of flocking behavior in starlings, using the topological distance to a fixed number of 6–7 nearest neighbors, independently of their absolute distance, produced better results for flock coherence when compared with the use of metric distance, which only accounts for interactions within a spatially defined zone (Ballerini *et al.*, 2008). Computer models have also been employed to investigate how large groups of animals can make collective decisions, and it has been demonstrated that in theory, only a small subset of individuals needs information to influence goal-directed movements of a group (Couzin *et al.*, 2005; Huse *et al.*, 2002). These predictions are in line with field observations of herring school formation and spawning that were obtained through large-scale acoustic imaging (Makris *et al.*, 2009).

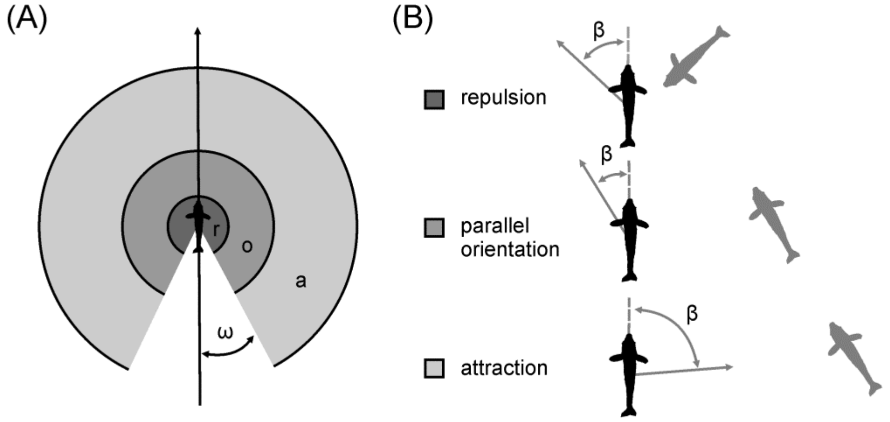

Figure 11.2: Interaction rules for an individual-based model of shoaling behavior. (A) Representation of an individual fish surrounded by a zone of repulsion (r), a zone of parallel orientation (o), and a zone of attraction. Other individuals are not detected within the dead angle (ω). (B) Behavioral reactions of the focal fish (black) upon encounter of a conspecific (grey) depending on the interaction zones specified in (A). The animal changes its direction of movement (hatched line) by the angle β to obtain a new heading (arrow). Modified from Huth and Wissel (1994).

The fact that simple rules in computer simulations can cause behavioral patterns resembling those of living systems does not necessarily mean that living systems follow these exact rules (Parrish and Edelstein-Keshet, 1999). Models can not demonstrate whether real animal groups are organized according to their assumptions, nor whether their assumed behavioral rules are hard-wired into an animal's behavioral physiology (Herbert-Read *et al.*, 2011). By studying schooling dynamics in golden shiners, Katz *et al.* (2011) found no evidence that fish average the headings of their nearest neighbors to adjust their own swimming direction. Instead, alignment resulted from attraction-repulsion dynamics and the adjustment of swimming speeds. It has therefore been suggested that models of shoaling behavior should be developed in a bottom-up approach relying on experimental data from individual interactions rather than by specifying parameters that result in qualitatively similar behavior patterns at the group level (Lopez *et al.*, 2012).

11.1.3 Robotic fish for the investigation of group behavior

Manipulating the behavior of individuals in groups is notoriously difficult, which makes it challenging to conduct controlled experiments on social interaction rules with live animals. One way to test the effect of specific behavioral rules on shoal formation is by introducing robotic fish dummies into groups of live fish and make them 'behave' according to rules that are specified by an experimenter (Landgraf *et al.*, 2013; Landgraf *et al.*, 2014; Swain *et al.*, 2012). The general features determining attraction of biomimetic robots, and possibly their recognition as conspecific individuals in behavioral studies on communication and social interactions, have been discussed in chapters 8 and 9. If live fish accept such robotic dummy fish, this approach makes it possible to test hypotheses regarding the rules of local interactions on behavior at the group level and to study how these mechanisms are used to achieve collective movement and decisions (Swain *et al.*, 2012).

By using a remote-controlled fish replica, Faria *et al.* (2010) investigated recruitment and leadership in small groups of sticklebacks (*Gasterosteus aculeatus*) and provided evidence that topological rather than metric distance is likely to be important during directional changes of the shoal. To investigate individual differences in shoaling behavior of sticklebacks, Pearish *et al.* (2017) replaced an entire shoal of sticklebacks with robotic conspecifics. Collective decisions in groups of sticklebacks were shown to comply with relatively simple quorum rules (Sumpter *et al.*, 2008). With increasing group size, additional artificial dummy fish were necessary to lead the shoal towards or away from a food source (Ward *et al.*, 2012). If the number of replica fish exceeded the quorum, even maladaptive decisions, like collectively moving into an area with increased predation risk, could be triggered experimentally in live fish (Ward *et al.*, 2008). By integrating a robotic fish replica into small shoals of guppies (*Poecilia reticulata*), Landgraf *et al.* (2016) were able to demonstrate the influence of realistic eyes and swimming movements on the attraction of live fish. They also provided a framework for closed-loop interactive experiments in which the robot reacts to the behavior of the fish. Most recently, Bonnet *et al.* (2018) integrated small robotic fish dummies into groups of zebrafish (*Danio rerio*) in a closed-loop interactive experimental design, and showed that self-organized decisions in these mixed societies could be modulated by biasing the robot's behavior.

11.1.4 Electrocommunication and group dynamics in mormyrids

Due to their electroreceptive capabilities (see section 1.3) and versatile social behavior, mormyrids are interesting model organisms for the study of collective behavior. The active production of electric organ discharges (EOD) generates transient three-dimensional electric fields around these fish, which they use to probe their immediate environment. At the same time, mormyrids can exchange identity information based on the waveform of their EOD (see section 3.5.1), as well as contextual information through modification of inter-discharge intervals (IDI; see section 3.5.2) during electrocommunication. Additionally, electrical signaling provides information that allows individuals to localize and approach a conspecific (Hopkins, 2005; Schluger and Hopkins, 1987) and to track a mobile source of electrocommunication signals (Worm *et al.*, 2018) (see chapter 9). It has been shown experimentally, that mormyrids can rely on sensory modalities other than vision and the lateral line system for group coherence and shoal formation. Evidently, electric signal generation and perception play important roles in these situations (Khait *et al.*, 2009; Moller *et al.*, 1982). By silencing the electric organ of *Marcusenius cyprinoides*, Moller (1976) demonstrated that animals that were deprived of their active electric sense no longer engaged in 'single file swimming' and 'parallel lineups.'

Social behavior in mormyrids is highly complex and ranges from territorial and aggressive interactions in *Brienomyrus* (Friedman and Hopkins, 1996) to nocturnal hunting associations in *Mormyrops anguilloides* (Arnegard and Carlson, 2005), and the formation of large diurnal shoals in *Petrocephalus* (Carlson, 2016; Kramer, 1990). The analysis of electric signaling in grouping mormyrids revealed that these animals frequently engage in episodes of temporal EOD-synchronizations. These episodes are mediated by mutual echo responses to each other's EODs and have been interpreted as a communicative strategy to promote group coherence (Arnegard and Carlson, 2005; Gebhardt *et al.*, 2012a). Echo responses and discharge synchronizations have been introduced earlier (see sections 1.4, 3.5.2, 4.1, and 5.1) and were investigated in dyadic interactions between *M. rume* and a mobile dummy that generated electrical playback sequences in chapters 4 and 5. In groups of mormyrids, these synchronization episodes can switch rapidly between individuals, who frequently change their synchronization partner (Gebhardt *et al.*, 2012b) (Figure 11.3). Similar observations were also made during interactions in small groups of *M. rume*, in which a single group member was replaced with a mobile dummy that generated playback of electrical communication signals (Pannhausen, 2017; Toma, 2014a).

The electrical playback signals used in these experiments were static, i.e., their temporal pattern was fixed and did not depend on the signaling activity of the fish. The present study expands these findings. By imitating the species-specific echo response latency of *M. rume*, an interactive electrical playback was generated by a dummy that could be moved on arbitrary trajectories. This enabled both motor and electromotor interactions with the test fish. The effect of this dynamic echo playback on recruitment and following-behavior in single individuals and small groups of live *M. rume* was investigated and contrasted with the animal's responses to static random playback patterns. The effect on following behavior and shoal formation could thus be tested depending on group size, and social constellations—during which electrical discharge synchronizations occur in groups—were identified. The results corroborate the findings from chapter 5 by demonstrating that synchronizations are often initialized by individuals that approach a conspecific or the dummy. This observation supports the hypothesis that the echo response provides mormyrids with a mechanism to address a particular individual and selectively share social attention during electrocommunication in groups.

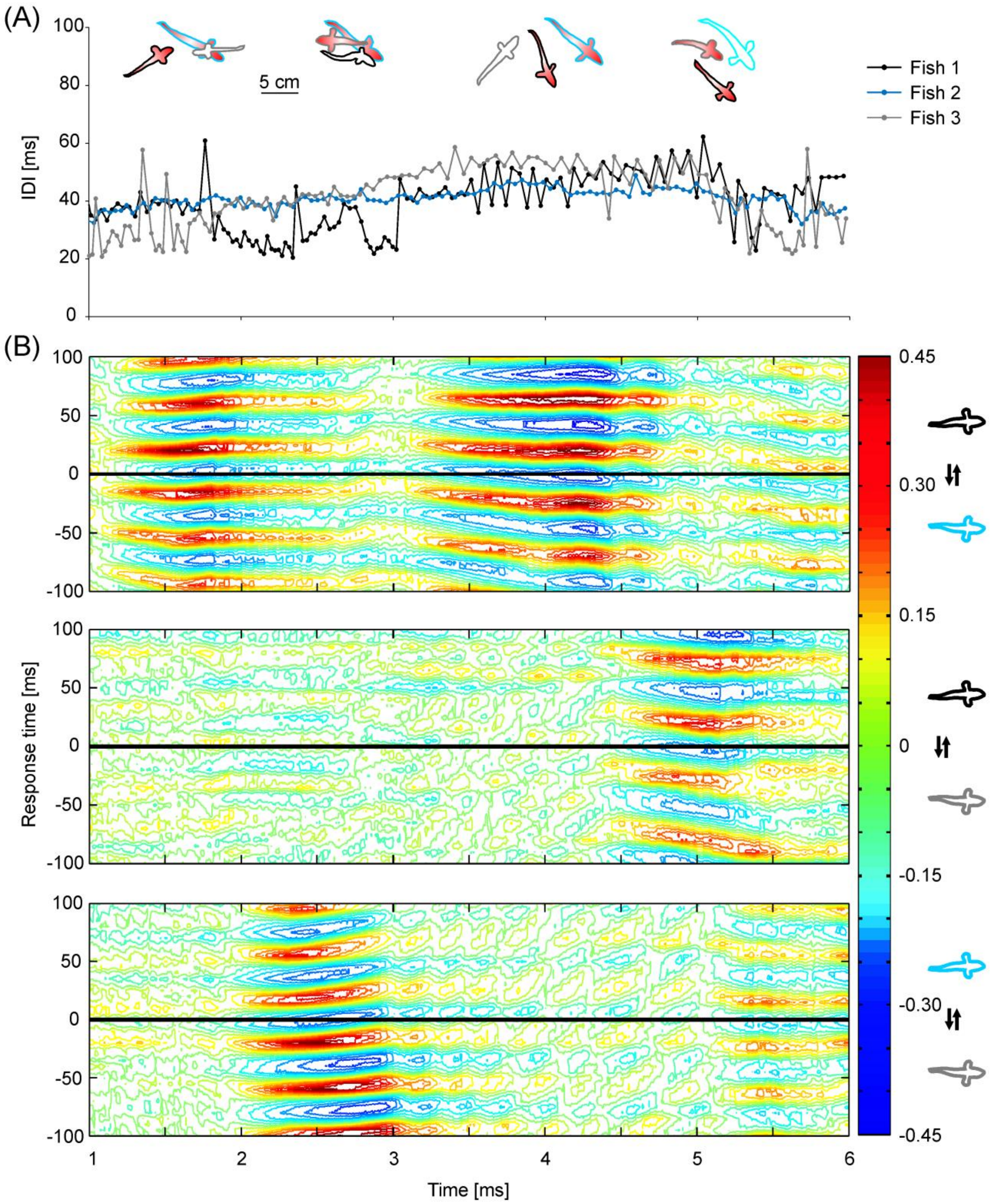

Figure 11.3: Switching of synchronization partners during electrocommunication in a group. Short behavioral interaction sequence of three *Mormyrus rume proboscirostris* during feeding. (A) Time series of the IDI-sequences of each fish. Line colors correspond to the outlines of the inset drawings, which represent behavioral snapshots from the interaction episode. (B) Cross-correlation analyses of all possible pairs of electric signaling interactions highlight the time course of synchronizations between individuals. Correlation coefficients are color-coded and represent reactions of the individuals indicated on the right-hand side of the diagrams to the respective other fish. Synchronizations were observed at the response time corresponding to the latency of the echo response in *M. rume*. Synchronizing individuals are marked in red in the inset drawings of (A).

11.2 Materials and methods

The experiments presented in this section were part of a larger research design that was introduced in chapter 5. The same 23 *Mormyrus rume proboscirostris* were used, and detailed descriptions of animal keeping and handling conditions can be found in sections 5.2.1 and 9.2.1. The exact design of the experimental setup (section 5.2.2) and the details of electrical playback assembly (section 5.2.3) and presentation (section 5.2.4) can also be obtained from there. In short, groups of different sizes were confronted with a mobile dummy fish (Figure 5.2B) that could be moved on arbitrary trajectories via magnetic coupling from underneath an experimental tank with a base area of 120 cm x 100 cm (Figure 5.1). The dummy emitted electrical playback with a natural EOD-waveform assembled either to static sequences of randomized natural IDI-durations, as an interactive sequence that responded to EODs of the fish with a latency characteristic of the echo response in *M. rume*, or remained electrically silent as a control. All groups were also tested without the dummy.

11.2.1 Group sizes and randomization

Tests were performed with single fish and small groups of two, three, and four individuals. For each group size, $n = 9$ groups were tested. Because all animals were also tested individually for the study presented in chapter 5, nine individuals were preselected to also go into the analysis of the current study. Since the number of available research animals was limited, animals had to be re-used in different group sizes. In groups of three, four animals had to be used twice also within that category, although never more than one per group. In groups of four, 13 animals were used twice also within that category, and no more than two animals were re-used per group. Groups were assembled using similarly sized animals, and experimental trials were arranged to assure that no animal was tested more than once per day. Similar to the randomization procedure described in section 5.2.4, trials were pseudo-randomized with respect to the order in which the static random playback, the dynamic echo playback, and the electrically silent control condition were presented within a session. Whether a given group was confronted with the dummy in the first or the second session was also randomized. To assure that groups were composed of the same individuals across experimental session, fish were made distinguishable by clipping small parts from their caudal and/or pectoral fins.

11.2.2 Data analysis

Cartesian coordinates and angular orientations were obtained for all fish and the dummy every three seconds to analyze spatial behavior patterns of *M. rume* in response to the different test conditions. This yielded 15 measurements per fish and trial for each analysis.

Also, the shortest distance between the snout of each fish and the closest wall of the tank was measured at the same time points. These measurements were performed manually using ImageJ (version 1.46r, National Institutes of Health, USA). Because fish could not be consistently identified across successive trials based on video recordings, values obtained from different individuals were not differentiated. Instead, the analysis was based either on mean values or minimum/maximum distances. Nearest neighbor distances (NND) between dummy and fish, as well as between the fish, were calculated from Cartesian coordinates using Matlab (Version R2013b, The MathWorks Inc. Natick, MA). Group coherence was quantified according to Huth and Wissel (1994) by calculating the average mean NND of all fish excluding the dummy for the 15 measures per trial.

Group polarization was quantified in a procedure inspired by Huth and Wissel (1992) by averaging the mean vectors of all individuals either with or without the dummy using the circ_stat toolbox for Matlab (Berens, 2009). Similar to the analysis by Huth and Wissel (1994), mean vectors of 0 represent groups with maximally randomized orientations, whereas a mean vector of 1 characterizes a group in which all individuals are perfectly aligned in parallel.

The difference between the orientation of the dummy and the mean orientation vector of the remaining group was calculated to quantify the effect of electrical playback presentation on group behavior. In addition, the number of the dummy's turns that were followed by at least one fish in response to the different experimental conditions was quantified manually from video recordings. File names were randomized to leave the experimenter blind to the playback condition during which the video had been recorded.

Statistical comparisons were performed using SPSS (version 22.0, IBM Corp., Armonk, NY). Within the different group sizes, experimental conditions were compared using repeated-measures designs. A repeated-measures ANOVA was performed if data were assumed to be normally distributed based on Shapiro-Wilk's test. Otherwise, the non-parametric Friedman test for non-normally distributed data was used. No inferential statistics were performed to compare the results between the different group sizes, because the re-use of individuals in groups of similarly sized individuals neither justified the use of repeated-measures testing procedures, nor the assumption of independent data across different group sizes.

All experiments with a group size of two that involved electrical playback were screened for episodes during which both fish showed instances of following the mobile dummy. Spike2-waveform data recorded during these episodes were converted to time series marking each EOD that was generated by either fish or playback during these sequences. After the identification of the playback signals, the remaining EODs of the test fish were assigned to the respective sender according to Gebhardt *et al.* (2012a). This assignment was accomplished by manually associating amplitudes and polarities of EODs that were recorded on multiple channels via the multi-electrode array, with the spatial positions the fish occupied in relation to the recording electrodes. Adaptive cross-correlations for a response time of ±100 ms were calculated over the time course of each episode for the IDI-sequences of the dummy and each fish, as well as for the two fish. This analysis was performed according to Gebhardt *et al.* (2012a). The maximum correlation coefficient for all possible pairings within the analyzed response time was extracted (see also sections 4.2.7 and 5.2.5). For correlations of the signals of either fish with the dummy, and with those of the other fish, these maximum correlation values were screened for episodes with a correlation coefficient of 0.3 or higher that lasted for at least 500 ms. Behavioral patterns displayed during such episodes were further characterized by manual inspection of the corresponding video recordings. In particular, spatial relationships between the two synchronizing partners in the video frame corresponding to the time when the 0.3 threshold was crossed were analyzed. This was done in ImageJ by determining the angle between the line connecting the centers of the communication partners and the orientation of the individual that synchronized or was synchronized to, respectively.

11.3 Results

Creating mixed groups by introducing a mobile dummy fish allowed to reliably influence the behavior of single individuals of *M. rume*, as well as small groups of two, three, and four fish. This influence mainly persisted when the dummy emitted electrical playback signals. Particularly single fish abandoned their preference for wall-following behavior in the presence of the dummy. But the animals, in general, were attracted to follow the dummy and interacted with it, both spatially and electrically, even in the presence of other live individuals. However, the influence of the playback emitting dummy on the behavior of groups was decreased compared with its effect on single fish. This is illustrated in Figure 11.4 through the visualization of fish positions recorded at fixed intervals during different experimental conditions. In the absence of the dummy, animals responded to the new environment with a strong preference for staying close to the tank walls, a behavior that persisted independently of group size. The presence of the electrically silent dummy could occasionally motivate the fish to explore further towards the center of the tank, but only when the dummy emitted playback of electrical signals was wall-following behavior abandoned almost entirely by single fish. With increasing group size, animals increasingly resumed their preference for the area close to the walls.

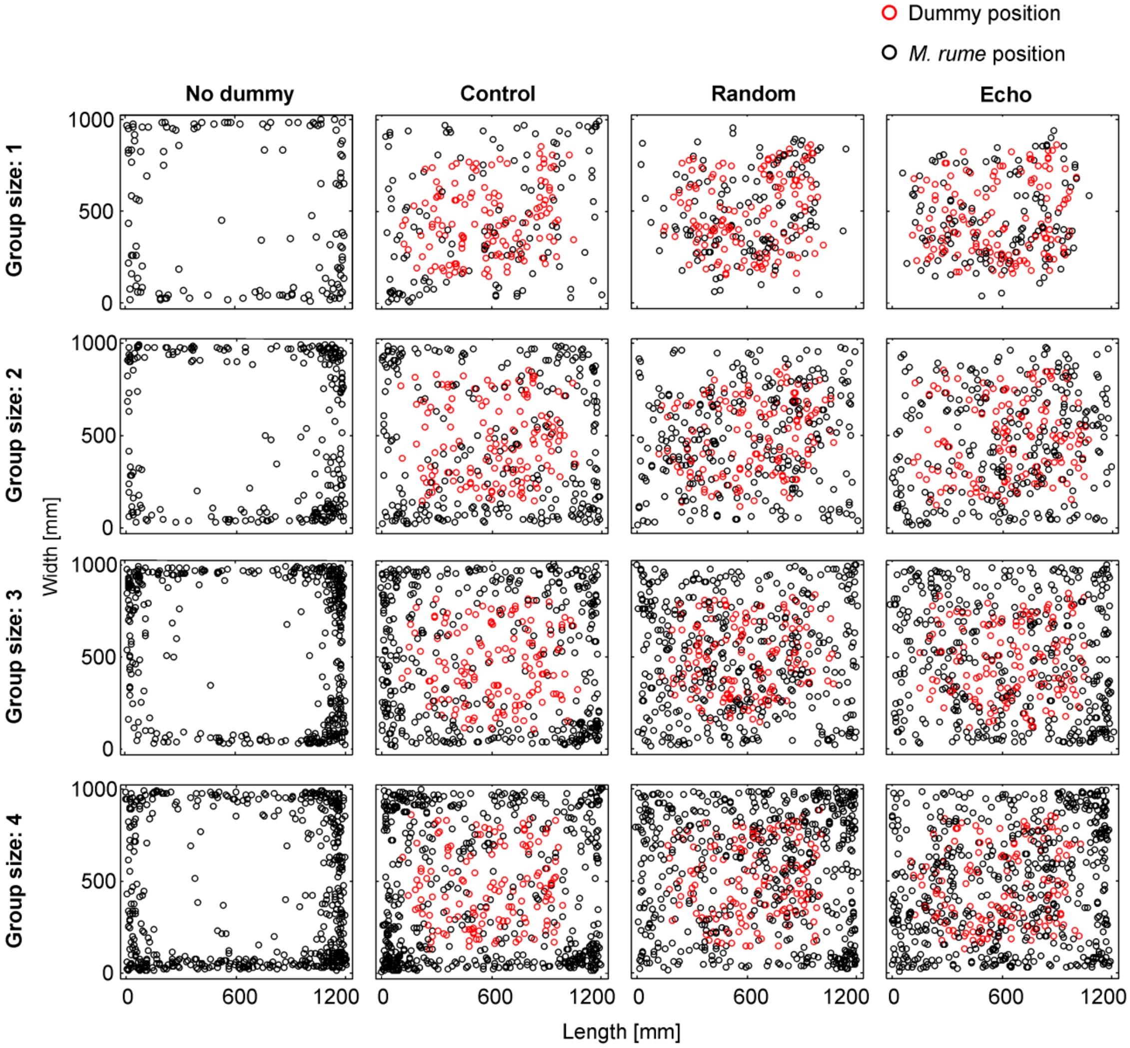

Figure 11.4: Influence of a mobile dummy on fish positions in groups of different sizes. The figure shows snapshots of Cartesian coordinates taken every three seconds during different experimental conditions to indicate the positions of fish (black) and dummy (red) in the tank. Each panel represents consolidated data from trials with *n* = 9 single individuals or groups, ranging in size from two to four fish. During the tests with no dummy present (first column), animals showed a strong preference for staying close to the tank walls. This preference was less pronounced in the presence of an electrically silent dummy (control) and was almost entirely abandoned by single fish during trials with the static random playback (third column) and the dynamic echo playback (fourth column). This influence diminished with increasing group size.

The influence of the mobile dummy on wall-following behavior was quantified and is illustrated in Figure 11.5. The distance between the fish and the tank wall was significantly influenced by the dummy in groups of all sizes (single fish: $\chi^2_{(3)} = 23.13$; $p < 0.001$; Figure 11.5A; groups of two: $\chi^2_{(3)} = 17.03$; $p < 0.001$; Figure 11.5B; groups of three: $\chi^2_{(3)} = 21.13$;

$p < 0.001$; Figure 11.5C; groups of four: $\chi^2_{(3)} = 18.47$; $p < 0.001$; Figure 11.5D). The overall pattern was the same for all group sizes. Animals stayed close to the tank walls during trials without the dummy (*median* distances: 55–96 mm) and increased that distance significantly in the presence of the dummy when it emitted either a static random playback or a dynamic echo playback (*median* distances: 209–274 mm). Based on Bonferroni corrected *p*-values, the two playback conditions never differed significantly. During control trials with the electrically silent dummy, recorded distances (*median* values: 119–188 mm) were always intermediate to the condition with no dummy and the two playback conditions. Only the fish furthest away from the tank wall was incorporated into this analysis for trials with more than one live individual.

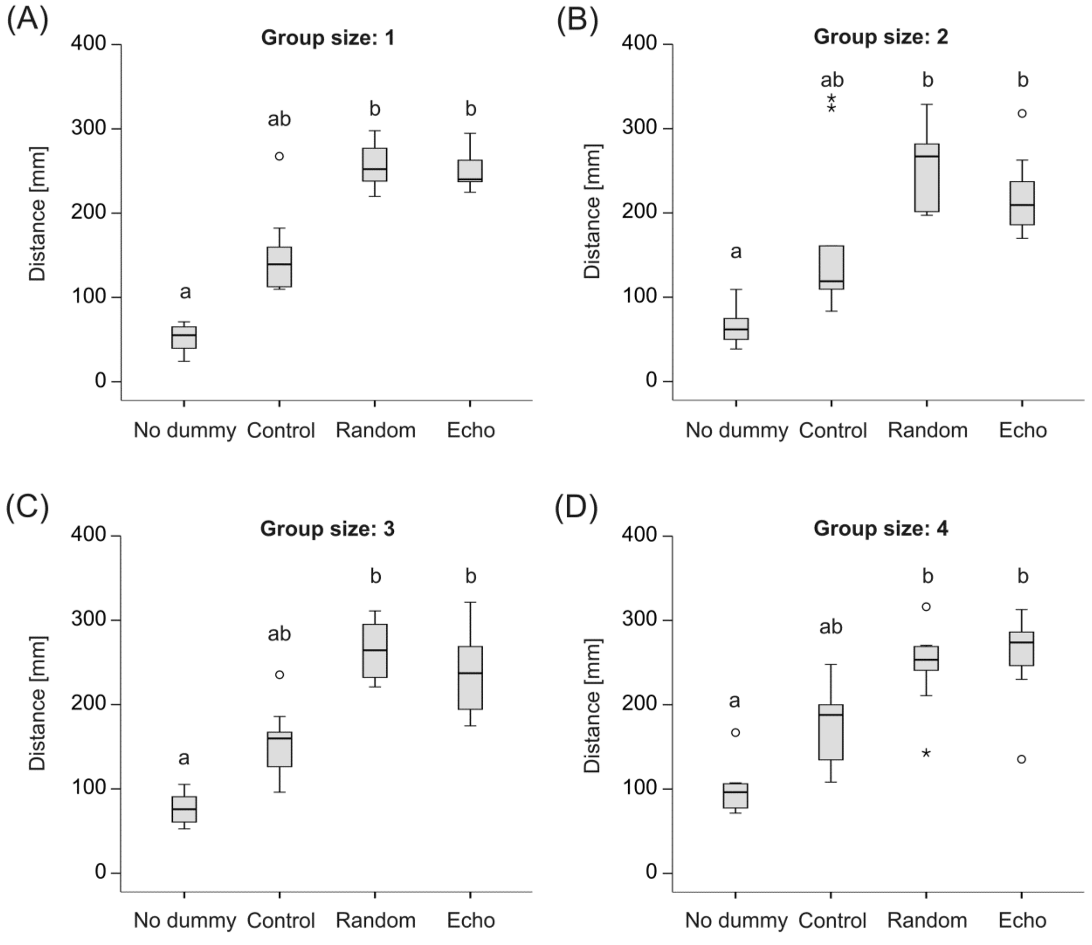

Figure 11.5: Influence of a mobile dummy on wall-following behavior in groups of different sizes. Box plots indicate the distance to the tank wall for single individuals (A) and the maximum distance, i.e., the distance of the fish closest to the center of the tank, for groups of two (B), three (C), and four (D) fish during experimental conditions with no dummy present, the electrically silent control condition, the static random playback, and the dynamic echo playback. Results from conditions not sharing a common superscript letter differ significantly based on Bonferroni corrected *p*-values.

The attractiveness of the dummy during the different experimental conditions was assessed by calculating nearest neighbor distances (NND). Figure 11.6 contrasts the results for all group sizes and confirms a similar general pattern that could be observed in groups of all sizes. The experimental condition had a significant influence on the dummy's NND in all group sizes (single fish: $F_{(2,16)} = 51.62$; $p < 0.001$; Figure 11.6A; groups of two: $F_{(2,16)} = 16.17$; $p < 0.001$; Figure 11.6B; groups of three: $F_{(2,16)} = 33.55$; $p < 0.001$; Figure 11.6C; groups of four: $F_{(2,16)} = 19.03$; $p < 0.001$; Figure 11.6D). In all cases, distances were significantly longer during the electrically silent control conditions compared with the two playback conditions, which never differed statistically. The difference between the influence of the two playback conditions and the control treatment was, however, most pronounced during the trials with single individuals and diminished slightly in the presence of other live fish.

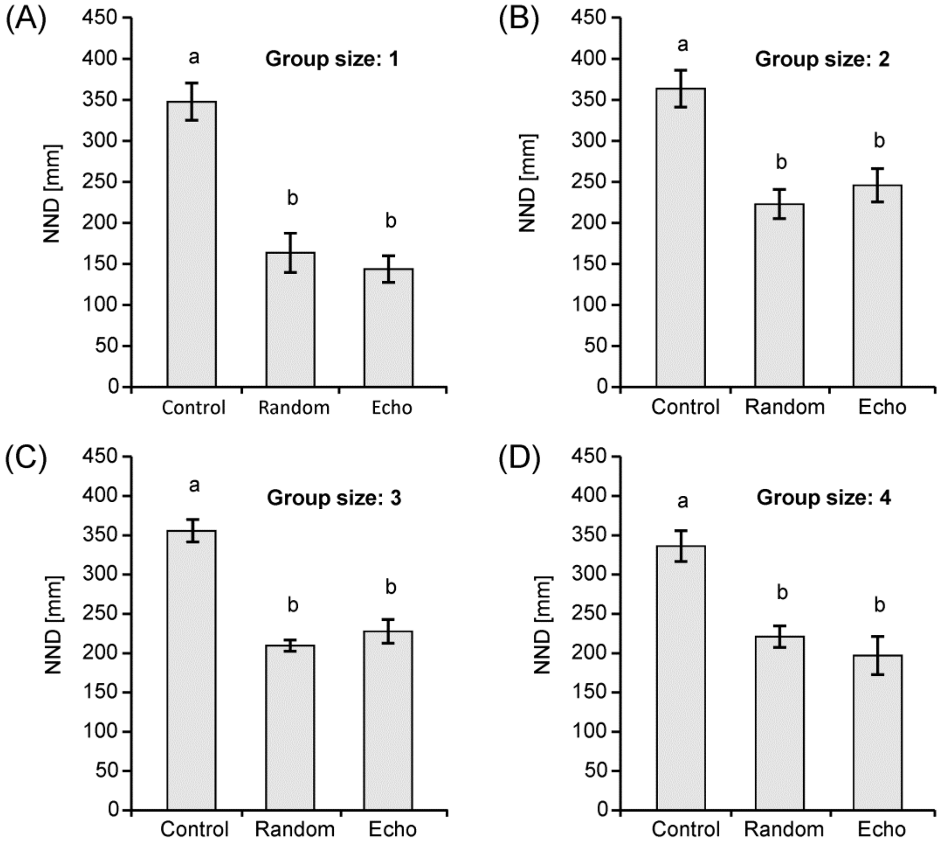

Figure 11.6: Influence of playback condition on the dummy's NND. Average distance between the mobile dummy and the test fish (A), and the distance to the dummy's nearest neighbor, respectively, for groups of two (B), three (C), and four (D) fish. Comparisons were made for each group size between the electrically silent control condition, the static random playback condition, and the dynamic echo playback condition. Categories with different superscript letters differ significantly based on Bonferroni corrected *p*-values.

Shoals of fish can be characterized by the coherence of individuals within the group. Group coherence was quantified by averaging the nearest neighbor distances of all fish excluding the dummy (Figure 11.7). The resulting parameter describes how close the animals tended to stay together during the trials and was not affected by different experimental conditions (groups of two: $\chi^2_{(3)} = 7.13$; $p = 0.07$; Figure 11.7A; groups of three: $\chi^2_{(3)} = 4.33$; $p = 0.23$; Figure 11.7B; $\chi^2_{(3)} = 1.40$; $p = 0.71$; Figure 11.7C). Median values for average group cohesion ranged from 123 to 214 mm, corresponding to 1.4 to 2.5 body lengths of the test fish based on their median standard length of 85 mm.

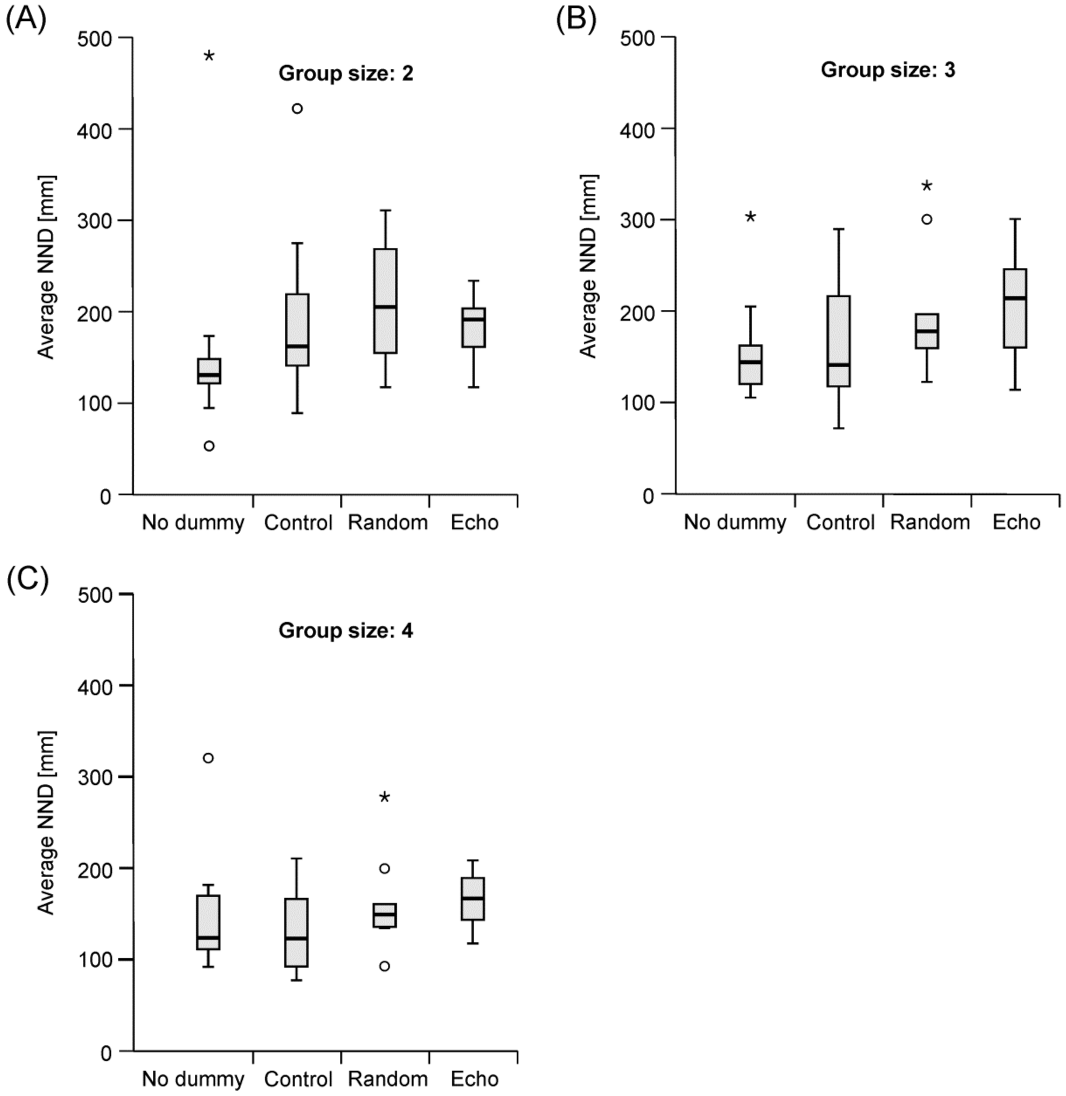

Figure 11.7: Group coherence. Box plots indicate the average NND of all fish in $n = 9$ groups of two (A), three (B), and four (C) individuals during experimental conditions with no dummy, the electrically silent control, the static random playback, and the dynamic echo playback. The dummy was not counted as the nearest neighbor. An extreme outlier at 776 mm was omitted in (A) in the static random playback condition. Results from different experimental conditions did not differ significantly.

The coherence of the fish with the mobile dummy was analyzed by comparing the nearest neighbor distance of the dummy with the distance to the corresponding individuals' nearest neighbor among the remaining fish (Figure 11.8). During the electrically silent control condition, this comparison resulted mostly in positive values, indicating that the dummy's nearest neighbor generally stayed closer to the other fish than to the dummy during these trials. Confidence intervals for each group size exclude the zero line, at which the two distances would be precisely the same (group of two: 95% CI, 57 to 277; group of three: 95% CI, 65 to 264; group of four: 95% CI, 80 to 250). This indicates a statistically significant difference between the distances. This relationship was not that unambiguous for the two conditions involving electrical playback. Although the inter-quartile range always included the zero line during the two playback conditions, a definite statement whether the playback-emitting dummy induced a similar coherence as observed among live fish cannot be made due to the non-normal distribution of the data. However, median values in Figure 11.8 show a stronger coherence during playback presentation compared with the electrically silent control condition.

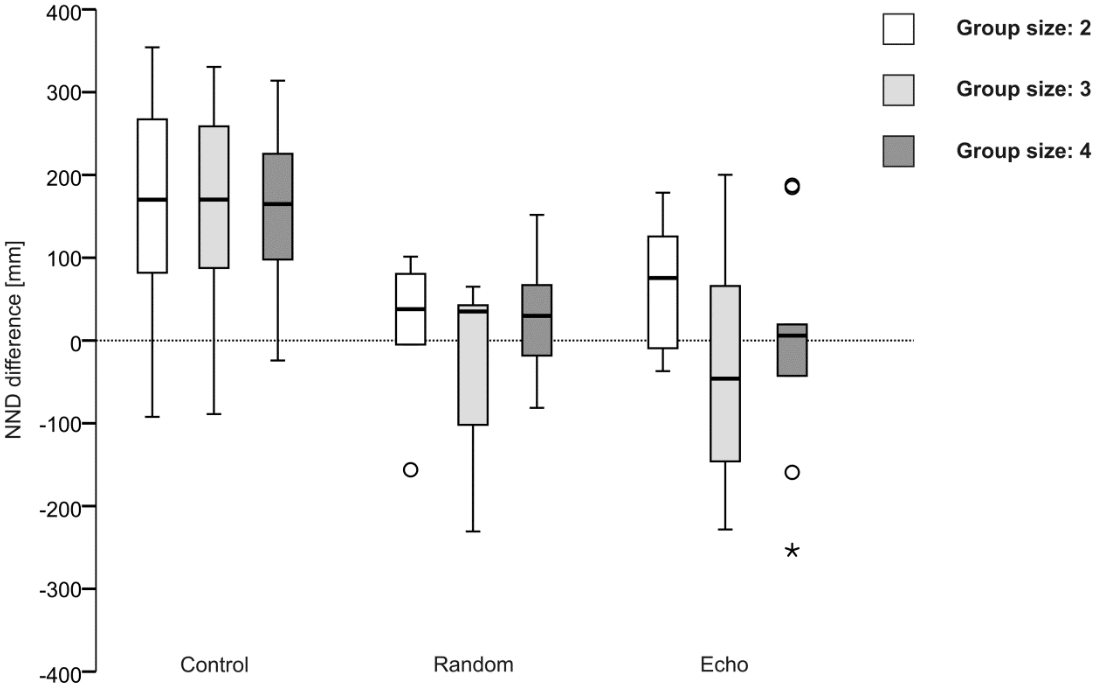

Figure 11.8: Group coherence between test fish and dummy. Box plots showing the difference in distance between the fish closest to the dummy and the dummy, and the distance to the nearest neighbor of that individual among the remaining fish in the group. This analysis was performed for groups of two (white), three (light grey) and four (dark grey) individuals for the electrically silent control condition, the static random playback condition, and the dynamic echo playback condition. Positive values indicate that the distance to the dummy is larger than the distance to the other fish. An extreme outlier at −599 mm was omitted in the group of two of the static random playback condition.

A second parameter for the characterization of shoaling fish is group polarization. Groups with low polarization consist of individuals that are randomly orientated on the horizontal plane, whereas individuals in groups with high polarization tend to be aligned in parallel to one another. Mean unit vectors of the orientation of dummy and fish were used to rank polarization of differently sized groups from zero (random orientation) to one (parallel orientation, Figure 11.9). Relatively high polarization values and consistent differences of the control condition to both playback conditions were only observed during the response of single fish to the mobile dummy ($F_{(2,16)} = 17.98$; $p < 0.001$; Figure 11.9A). Significant differences in group polarization were also recorded for groups of two ($F_{(2,16)} = 5.79$; $p = 0.013$; Figure 11.9B) and groups of four ($F_{(2,16)} = 4.63$; $p = 0.03$; Figure 11.9D). In either case, only one playback condition differed significantly from the electrically silent control condition after Bonferroni adjustments for multiple comparisons, and results obtained during the static random playback never differed statistically from those obtained during the dynamic echo playback. In groups of three, no statistically significant differences in group polarization were recorded at all ($F_{(2,16)} = 1.07$; $p = 0.36$; Figure 11.9C).

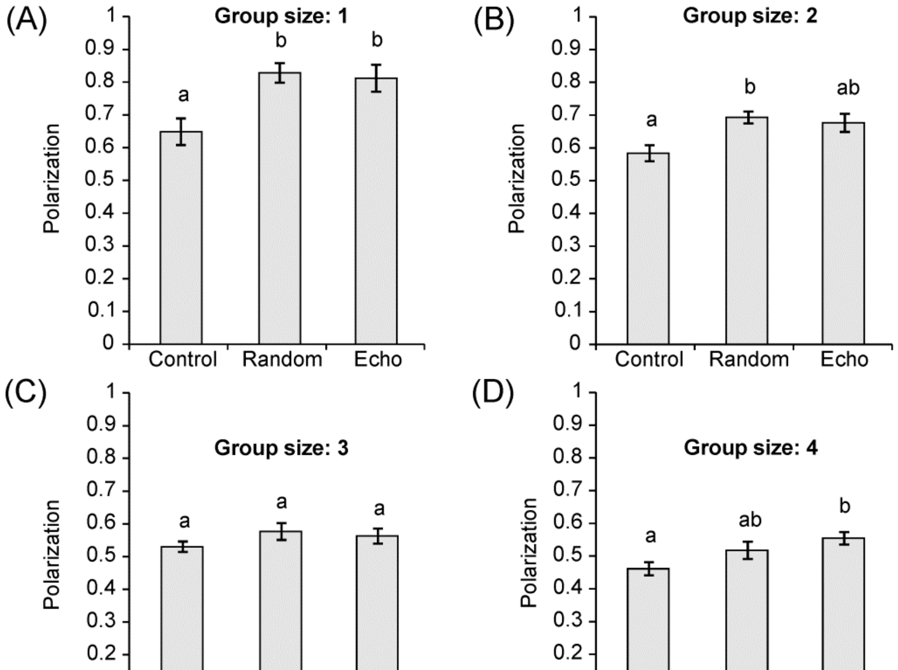

Figure 11.9: Group polarization in mixed groups. Polarization was quantified considering the dummy's orientation for single fish (A) and groups of two (B), three (C), and four (D) fish. A value of 0 indicates random orientations, whereas a value of 1 indicates parallel alignment of all group members. Results were compared between the electrically silent control condition, the static random playback, and the dynamic echo playback. Categories without a common superscript letter differ significantly based on Bonferroni corrected *p*-values.

When the dummy's orientation was excluded from the analysis (Figure 11.10), no statistically significant differences in polarization existed between the different experimental conditions at any group size (groups of two: $F_{(3,24)} = 1.71$; $p = 0.19$; groups of three: $F_{(3,24)} = 0.29$; $p = 0.84$; groups of four: $F_{(3,24)} = 2.24$; $p = 0.11$). Polarization was not particularly high in general, indicating that *M. rume* did not spend much time in polarized formations in the group sizes that were investigated in this study.

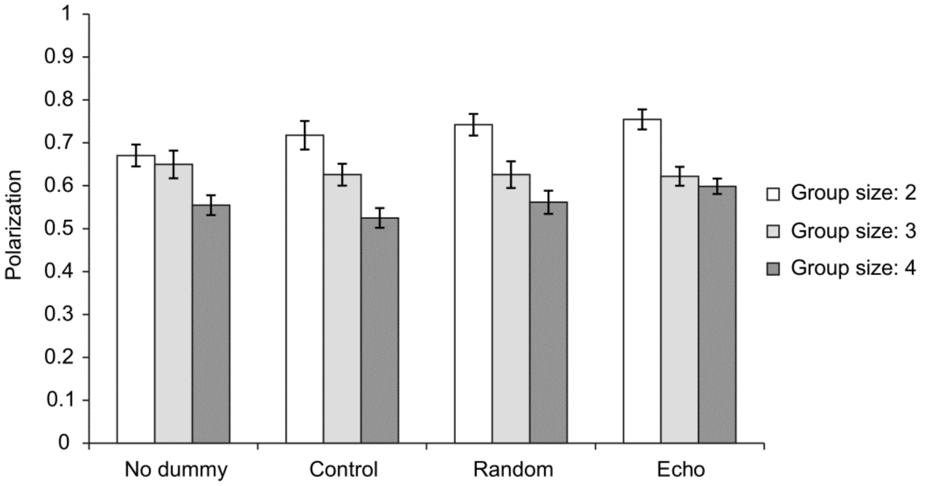

Figure 11.10: Group polarization excluding the dummy's orientation. Polarization was quantified among the test fish by omitting the dummy's orientation for groups of two (white), three (light grey), and four (dark grey) individuals. A polarization value of 0 indicates completely random orientations within the group, whereas a value of 1 indicates parallel alignment of all group members. Results were compared between the experimental condition with no dummy present, the electrically silent control condition, the static random playback, and the dynamic echo playback. No statistically significant differences existed between the results from different experimental conditions within a given group size.

Differences in orientation were also used to investigate whether groups of different sizes headed in the same direction as the dummy when they were attracted. Figure 11.11 shows the difference between the orientation of the dummy and the mean vector of the orientation of all fish. This angular difference decreased significantly in response to either of the two playback conditions for single fish ($F_{(2,16)} = 21.88$; $p < 0.001$; Figure 11.11A) and partly also in groups of two ($F_{(2,16)} = 7.68$; $p = 0.005$; Figure 11.11B), but not in groups of three ($F_{(2,16)} = 3.62$; $p = 0.050$; Figure 11.11C) and four, where Bonferroni corrected *p*-values did not confirm any differences between the categories, even though the overall model was significant ($F_{(2,16)} = 3.88$; $p = 0.042$; Figure 11.11D).

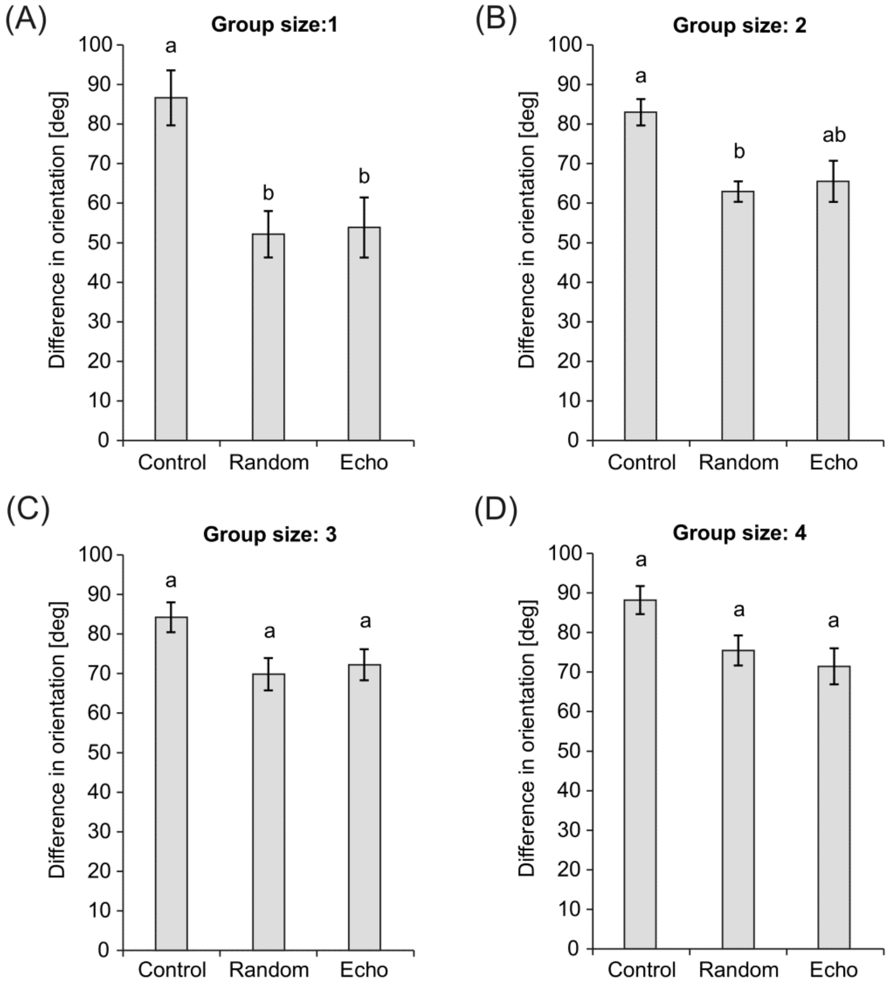

Figure 11.11: Difference in the orientations of the dummy and the group of fish. Angular difference between the orientations of the dummy and the test fish (A), or the mean vector of the orientations of two (B), three (C), and four (D) test fish, respectively. Results were compared between the electrically silent control condition, the static random playback, and the dynamic echo playback. Categories not sharing a common superscript letter differ significantly based on Bonferroni corrected *p*-values.

The relative amount of the dummy's turns, which were followed by at least one fish, was calculated for all group sizes to determine whether the fish specifically followed the dummy (Figure 11.12). In all cases, electrical playback presentation led to a significant increase in the relative amount of turns that were followed by at least one fish (single individuals: $\chi^2_{(2)} = 14.11$; $p = 0.001$; Figure 11.12A; groups of two: $\chi^2_{(2)} = 13.56$; $p = 0.001$; Figure 11.12B; groups of three: $\chi^2_{(2)} = 12.400$; $p = 0.002$; Figure 11.12C; groups of four: $\chi^2_{(2)} = 9.77$; $p = 0.008$; Figure 11.12D). The electrically silent control condition induced relatively few followed turns in fish (*median* values: 0–0.13), which was, except for group size three, always significantly different from the two playback conditions. In groups of three, only the static random playback induced significantly more turns compared with the electrically silent control, but the general pattern remained the same. The dummy always elicited more turns in the following fish when it emitted either the static random playback (*median* values: 0.29–0.77) or the dynamic echo playback (*median* values: 0.36–0.71), but the two playbacks never elicited statistically different responses in *M. rume*. Single fish followed most turns in response to playback, but following-behavior declined in larger groups.

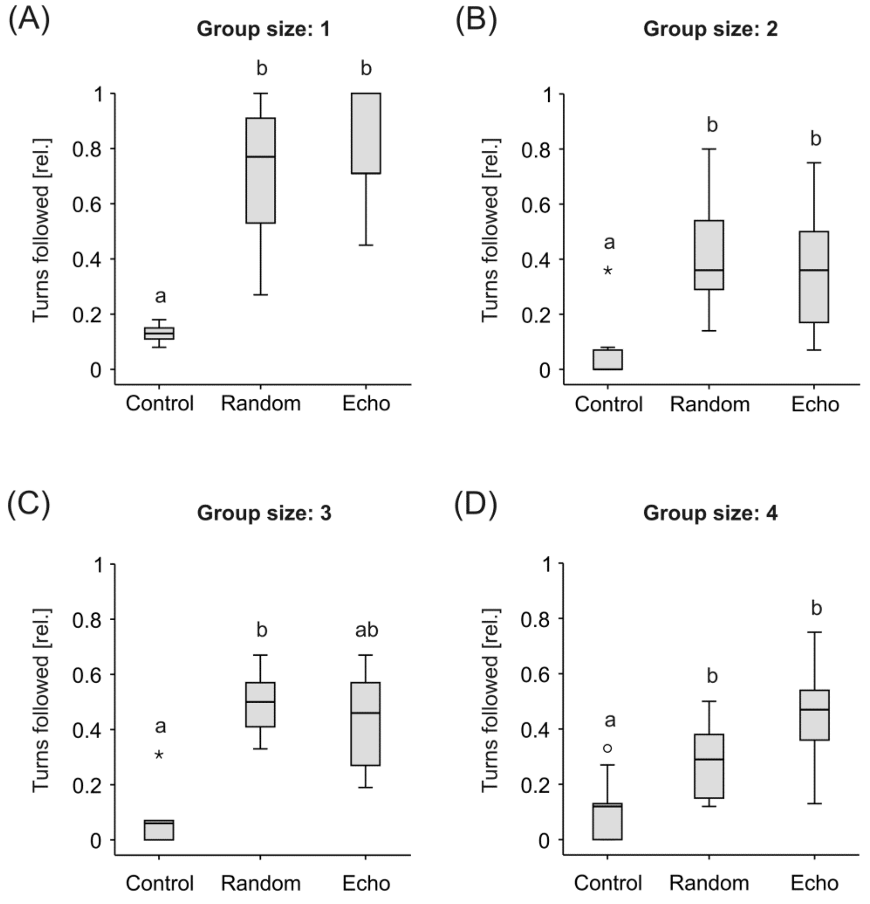

Figure 11.12: Following-behavior in response to electrical playback. Box plots show the relative number of the dummy's turns that were followed by at least one fish during the electrically silent control condition, the static random playback condition, and the dynamic echo playback condition in tests with single individuals (A) and groups of two (B), three (C), and four (D) fish. Results from categories not sharing a common superscript letter differ significantly based on Bonferroni corrected *p*-values.

Figure 11.13 summarizes the actual number of fish that followed the dummy's turns in differently sized groups, and in response to the different experimental conditions. It demonstrates that the dummy was also capable of inducing following-behavior in multiple fish, although following-behavior by single fish dominated in groups of three (Figure 11.13B) and groups of four (Figure 11.13C). Following-behavior by larger groups of fish hardly ever occurred in response to the electrically silent control condition.

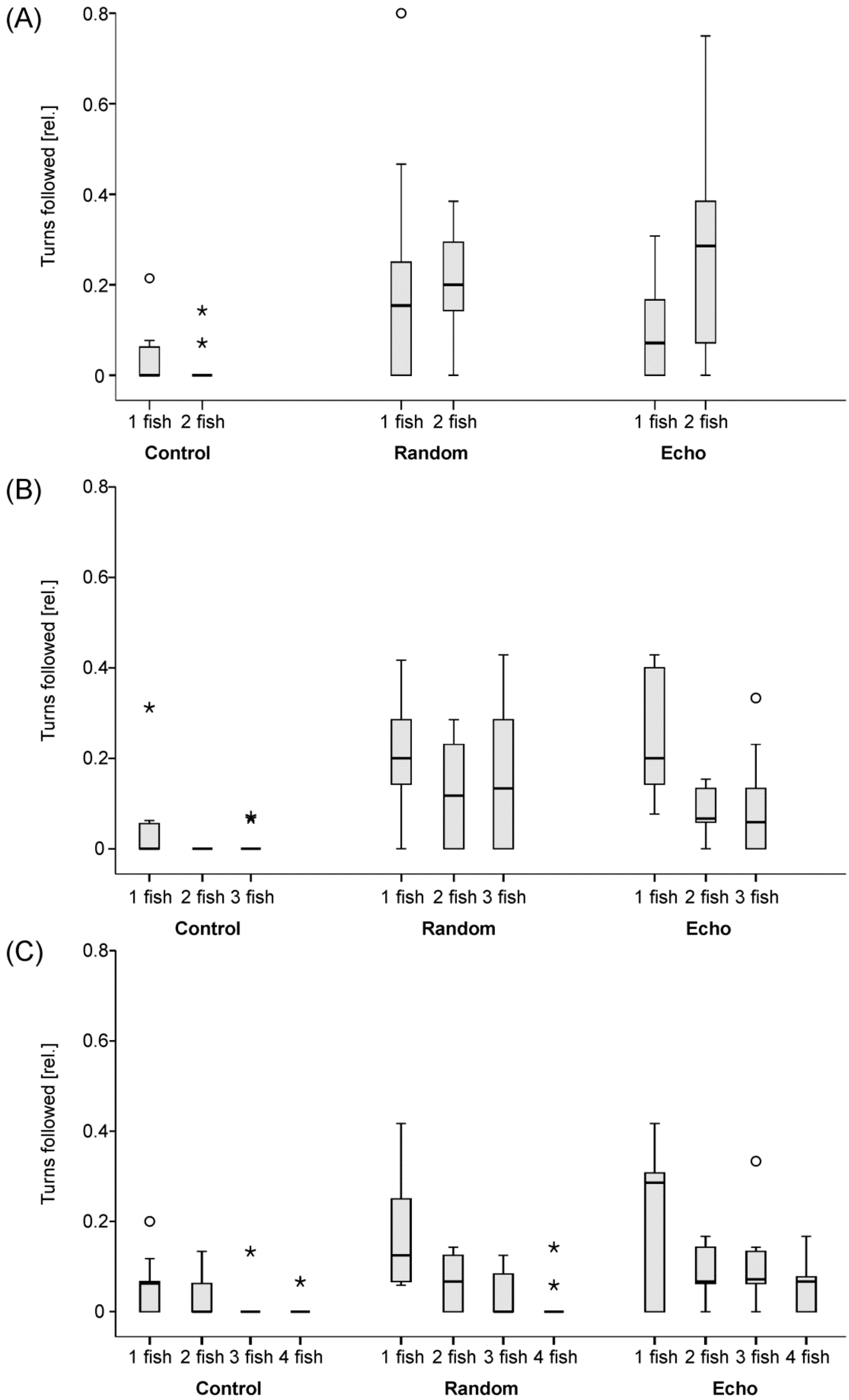

Figure 11.13: Amount of fish following the dummy's turns in groups of different sizes. Box plots indicate the relative number of the dummy's turns that were followed by the fish, broken down to the number of following fish in response to the electrically silent control condition, the static random condition, and the dynamic playback condition for groups of two (A), three (B), and four (C) individuals.

Electric discharge synchronizations were investigated in more detail in mixed groups of two live fish and the dummy. Results are summarized for the static random playback condition (Table 11.1) and the dynamic echo playback condition (Table 11.2). These episodes were specifically chosen to represent events of interactive behavior of both fish with the dummy. The duration of these episodes is listed in the columns on the right-hand side of Table 11.1 and Table 11.2. In total, 112.4 seconds were analyzed for the static random playback (no suitable episode was observed during experiments with group 7) and 127 seconds for the dynamic echo playback. Discharge synchronizations were quantified based on cross-correlation analysis between all possible pairs of IDI-sequences, which were recorded during these events. A threshold criterion of a correlation coefficient greater than 0.3 for a duration longer than 500 ms was set to isolate and quantify sequences of relatively high synchronization. During the experiments with the static random playback, this criterion was never met by randomly occurring correlations of the dummy's signals with the discharge sequences of either of the two fish. However, both fish synchronized their discharges, both to the playback and to the respective other individual (Table 11.1). During experiments with the dynamic random playback, synchronizations of the dummy's signals with those of live fish were also observed (Table 11.2).

Table 11.1: Number of sequences with relatively strong synchronization in response to the static random playback. The table summarizes the number of sequences during which synchronization in *n* = 9 mixed groups of two fish and the dummy exceeded a correlation coefficient of 0.3 for at least 500 ms. Sequences are specified for the six possible pairings between the dummy (D), fish #2, and fish #3. The column on the right-hand side gives the total amount of time in seconds that was analyzed for the respective group. It represents episodes of following-behavior during the experiment with that group.

Random	Sequences with correlation ≥ 0.3 and duration ≥ 500 ms						
Group	**D vs. #2**	**#2 vs. D**	**D vs. #3**	**#3 vs. D**	**#2 vs. #3**	**#3 vs. #2**	**Analyzed [s]**
1	0	2	0	1	1	3	13.0
2	0	0	0	1	1	0	12.4
3	0	2	0	0	2	3	12.0
4	0	3	0	0	1	0	13.0
5	0	2	0	0	0	4	12.0
6	0	4	0	2	1	2	13.0
7	-	-	-	-	-	-	-
8	0	4	0	2	0	8	18.0
9	0	3	0	2	4	2	19.0
Sum	**0**	**20**	**0**	**8**	**10**	**22**	**112.4**

Table 11.2: Number of sequences with relatively strong synchronization in response to the dynamic echo playback. The table summarizes the number of sequences during which synchronization in $n = 9$ mixed groups of two fish and the dummy exceeded a correlation coefficient of 0.3 for at least 500 ms. Sequences are specified for the six possible pairings between the dummy (D), fish #2, and fish #3. The column on the right-hand side gives the total amount of time in seconds that was analyzed for the respective group. It represents episodes of following-behavior during the experiment with that group.

Echo	Sequences with correlation ≥ 0.3 and duration ≥ 500 ms						
Group	**D vs. #2**	**#2 vs. D**	**D vs. #3**	**#3 vs. D**	**#2 vs. #3**	**#3 vs. #2**	**Analyzed [s]**
1	0	0	0	1	5	2	13.0
2	1	1	0	1	0	0	14.0
3	3	4	0	0	2	2	14.0
4	2	2	1	2	1	1	13.0
5	0	1	1	1	0	1	6.0
6	2	4	1	4	0	3	14.0
7	5	3	0	2	1	3	18.0
8	2	2	0	0	2	3	15.0
9	1	2	2	5	4	2	20.0
Sum	**16**	**19**	**5**	**16**	**15**	**17**	**127**

The definition of threshold criteria for relatively high correlations between two IDI-sequences allowed to analyze the behavioral patterns that had simultaneously been displayed by the two synchronizing individuals during that part of an episode. An exemplary set of simultaneously occurring pairs of IDI-sequences and the corresponding cross-correlation diagrams are presented for both playback types in Figure 11.14 and Figure 11.16. Representations of the behavioral interactions the fish engaged in at the moment when synchronizations first exceeded the threshold criteria are drawn to scale in Figure 11.15 and Figure 11.17, respectively. These illustrations demonstrate that a mobile dummy can be introduced into a small group of weakly electric fish and generate behavioral patterns similar to those observed among live fish with regard to physical and electric signaling interactions. In particular, switching synchronization partners within the mixed group frequently included the mobile dummy.

The episode selected from the experiments with the static random playback (Figure 11.14 and Figure 11.15) started with fish #2 synchronizing its discharges to those of the mobile dummy (Figure 11.14A (a) and (b)) while approaching (Figure 11.15 (a)) and eventually following (b) the dummy from behind. Fish #3 approached from a position further away and eventually started synchronizing with fish #2, who reciprocated the signaling display, culminating in two successive sequences of strong mutual synchronization of accelerated and regular IDI-sequences (Figure 11.14C (c) and (d)). While fish #3 initiated the first sequence of mutual synchronization from a position laterally behind fish #2 (Figure 11.15 (c)), the second sequence was initiated by fish #2, who by that time had fallen back into a similar position laterally behind fish #3. Fish #3 in turn had started to swim towards the dummy (Figure 11.15 (d)). Fish #3 then caught up with the dummy, followed a turn and started synchronizing its discharges to the playback sequence (Figure 11.14B (e)), while fish #2 acted as a bystander (Figure 11.15 (e)). After completing the turn, the dummy moved away from the fish, and fish #2 started synchronizing discharges to fish #3 (Figure 11.14C (f)) while approaching it from behind (Figure 11.15 (f)). Fish #3, however, moved away from fish #2 and followed the dummy, synchronizing its discharge activity to the playback sequence while still being synchronized to by fish #2 (Figure 11.14B and C (g)). This episode was reminiscent of the single file swimming behavior described by Moller (1976) in *Marcusenius cyprinoides* (Figure 11.15 (g)).

The episode selected from the dynamic echo playback experiments (Figure 11.16 and Figure 11.17) additionally features synchronization sequences of the dummy's playback EODs to the signals of the fish, by which they were triggered as an artificial echo response when the fish discharged near the dummy. At the beginning of the episode, fish #2 synchronized its discharges from a distance to the playback signals emitted by the dummy (Figure 11.16A (a); Figure 11.17 (a)). Fish #2 then approached the dummy more closely from behind (Figure 11.17 (b)), thereby triggering a sequence of mutual discharge synchronization with the playback (Figure 11.16A (b)). As the dummy then stopped and turned, still being followed by and synchronized to by fish #2, fish #3 approached it from the front and also started synchronizing its discharges to the playback signals (Figure 11.16B (c) and Figure 11.17 (c)). Upon completion of the turn, both fish still synchronized with the playback, and synchronization by the dummy was again triggered mainly by fish #2 (Figure 11.17 (d)). The dummy then moved away, leaving fish #3 in a position orthogonally behind fish #2 (Figure 11.17 (e)) from where it started synchronizing to the signals of fish #2 (Figure 11.16C (e)). Fish #2 then turned and began pursuing fish #3 while initiating a sequence of mutual discharge synchronization that was based on aggressive bursts (Figure 11.16C (f) and Figure 11.17 (f)).

The two exemplary episodes described above demonstrate electric discharge synchronizations in a mixed group of two weakly electric fish and a mobile dummy. They provide insight into the behavioral situations this signaling strategy was used in, which is during relatively close social interactions, usually when following or approaching another individual.

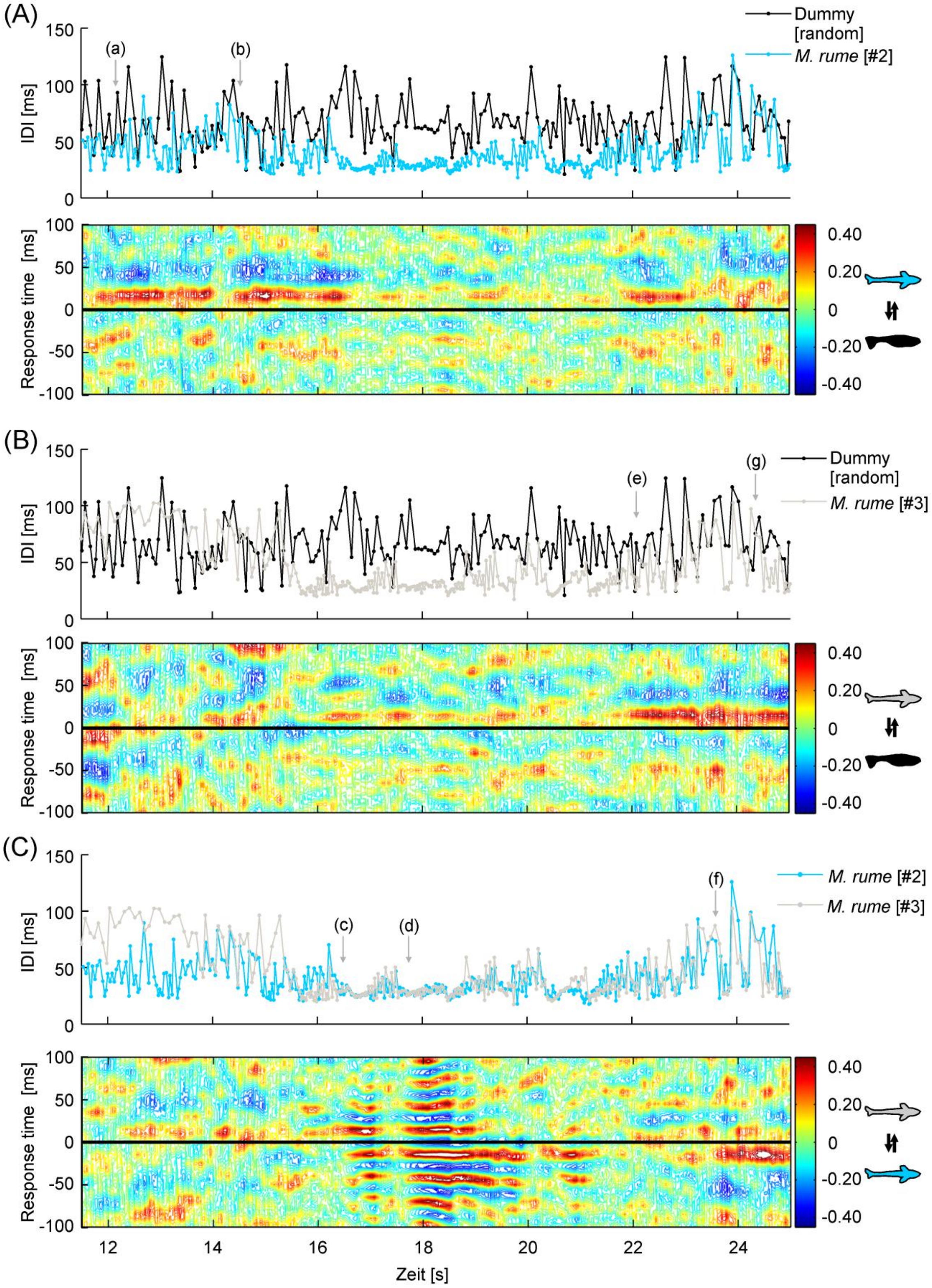

Figure 11.14: Exemplary episode of a mixed group responding to the static random playback: Electric discharge synchronizations. The upper panels in A, B, and C depict IDI-sequences emitted during the same time frame during which a group of two *M. rume* interacted with the mobile dummy emitting a static random playback. (A) IDI-sequences of dummy (black) and fish #2 (blue), (B) IDI-sequences of dummy (black) and fish #3 (grey), (C) IDI-sequences of fish #2 (blue) and fish #3 (grey). The lowercase

letters in brackets mark time points at the beginning when correlation coefficients first exceed 0.3 for a period of at least 500 ms and are referenced in Figure 11.15. The lower panels are cross-correlation diagrams of the respective pairs of IDI-sequences with color-coded correlation coefficients for a response time of ±100 ms. High correlations at positive response times result from discharge synchronizations of fish #2 with the dummy (A), fish #3 with the dummy (B), and fish #3 with fish #2 (C). The opposite case is represented by high correlations at negative response times. High correlations of the dummy's playback with the discharge sequences of fish #2 (A) and fish #3 (B) can only occur randomly due to the non-interactive nature of the static random playback. In (C), high correlations at negative response times represent discharge synchronization of fish #2 with fish #3.

In total, 127 behavioral episodes were observed during which discharge synchronizations by *M. rume* to either the playback signals or the EODs of the respective other fish exceeded the threshold criteria. Of the 60 episodes observed during experiments with the static random playback, four were discarded because the response time, at which synchronization occurred, did not correspond to the latency at which echo responses occur in *M. rume*, and because the animals did not show any interactions at the time. Of the remaining episodes, 73% were associated with situations where the synchronizing individual approached either the dummy or the other fish from behind. Swimming in parallel to the trajectory of the dummy or the fish that was being synchronized to was observed in 41% of all episodes. Similar behavior was observed during episodes with the dynamic echo playback. Here, 61% of all episodes were associated with approaches from behind and 44% with swimming in parallel to the trajectory of the dummy or the other fish.

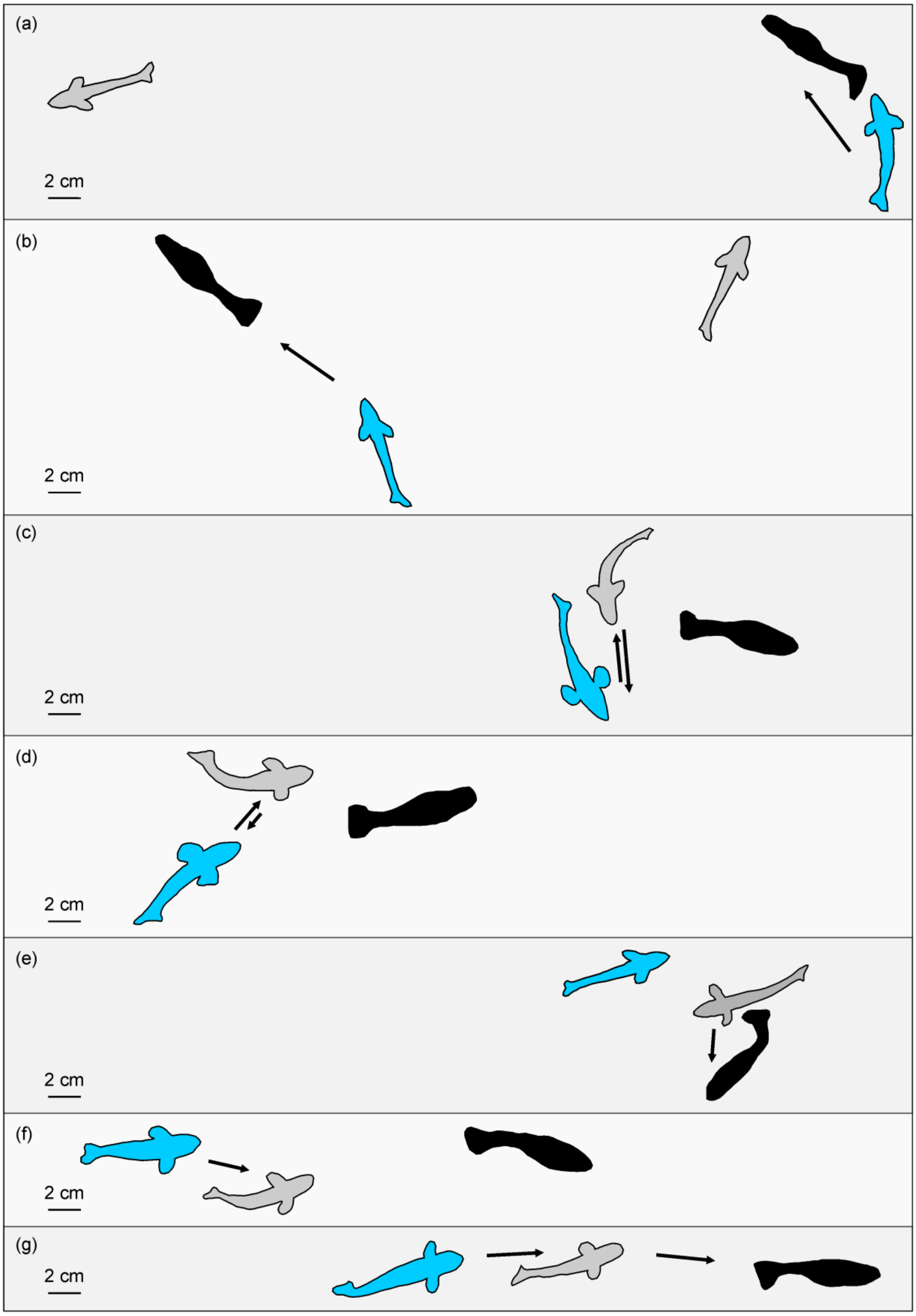

Figure 11.15: Exemplary episode of a mixed group responding to the static random playback: Spatial interactions. Panels are marked by lowercase letters corresponding to the time points in Figure 11.14 and depict interactions between the dummy (black), fish #2 (blue), and fish #3 (grey). Pictograms are drawn to scale and represent the spatial relationship of fish and dummy at the onset of relatively strong synchronization episodes (correlation coefficient ≥ 0.3 for at least 500 ms). Black arrows point from synchronizing individuals to the respective synchronization partners.

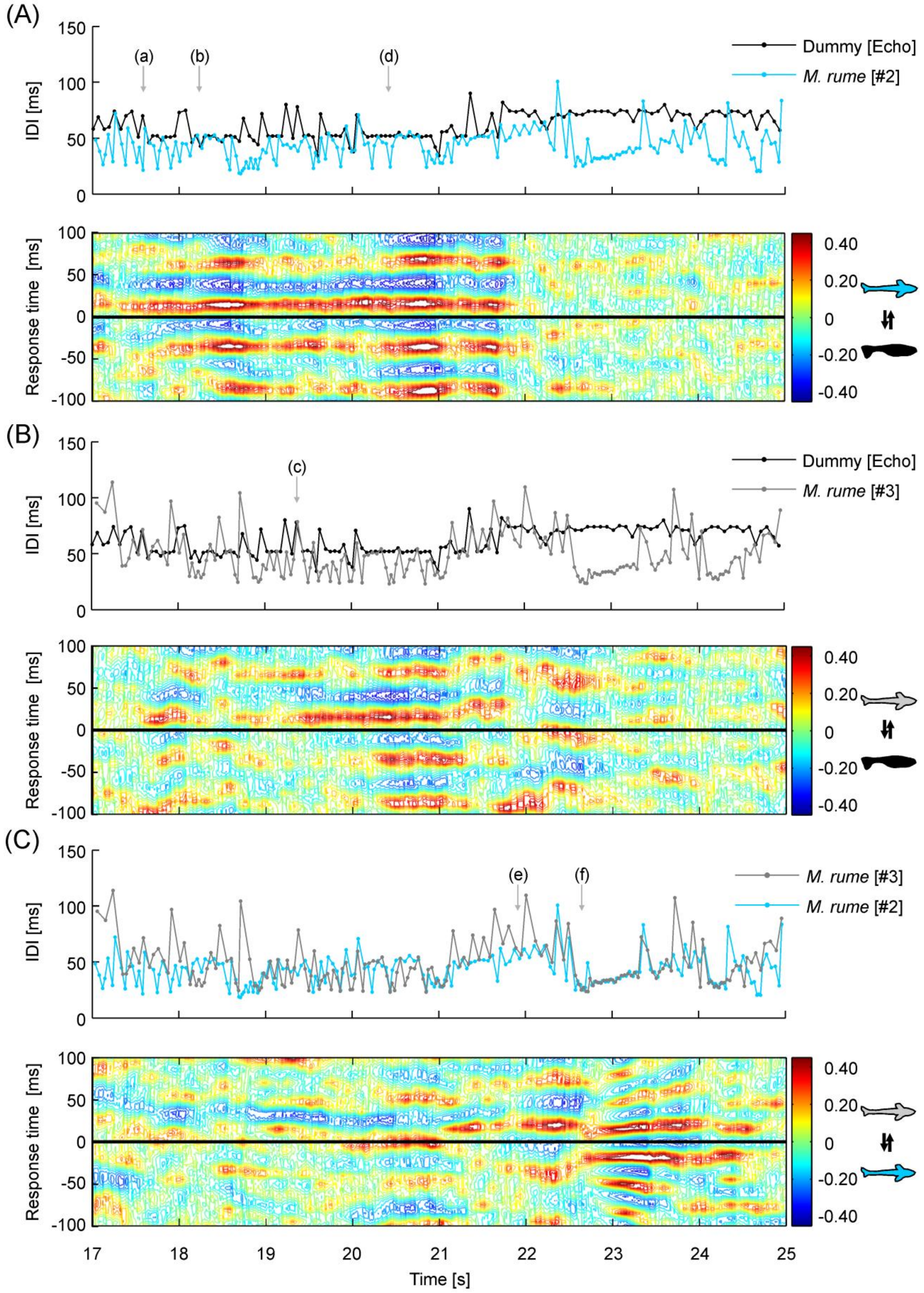

Figure 11.16: Exemplary episode of a mixed group responding to the dynamic echo playback: Electric discharge synchronizations. The upper panels in A, B, and C depict IDI-sequences emitted during the same time frame during which a group of two *M. rume* interacted with the mobile dummy emitting a dynamic echo playback. (A) IDI-sequences of dummy (black) and fish #2 (blue), (B) IDI-sequences of dummy (black) and fish #3 (grey), (C) IDI-sequences of fish #2 (blue) and fish #3 (grey). The lowercase

letters in brackets mark time points at the beginning when correlations first exceed 0.3 for a period of at least 500 ms and are referenced in Figure 11.17. The lower panels are cross-correlation diagrams of the respective pairs of IDI-sequences with color-coded correlation coefficients for a response time of ±100 ms. High correlations at positive response times result from discharge synchronizations of fish #2 with the dummy (A), fish #3 with the dummy (B) and fish #3 with fish #2 (C). The opposite case is represented by high correlations at negative response times. High correlations of the dummy's playback with the discharge sequences of fish #2 (A) and fish #3 (B) result from the interactive nature of the dynamic echo playback. In (C), high correlations at negative response times represent discharge synchronization of fish #2 with fish #3.

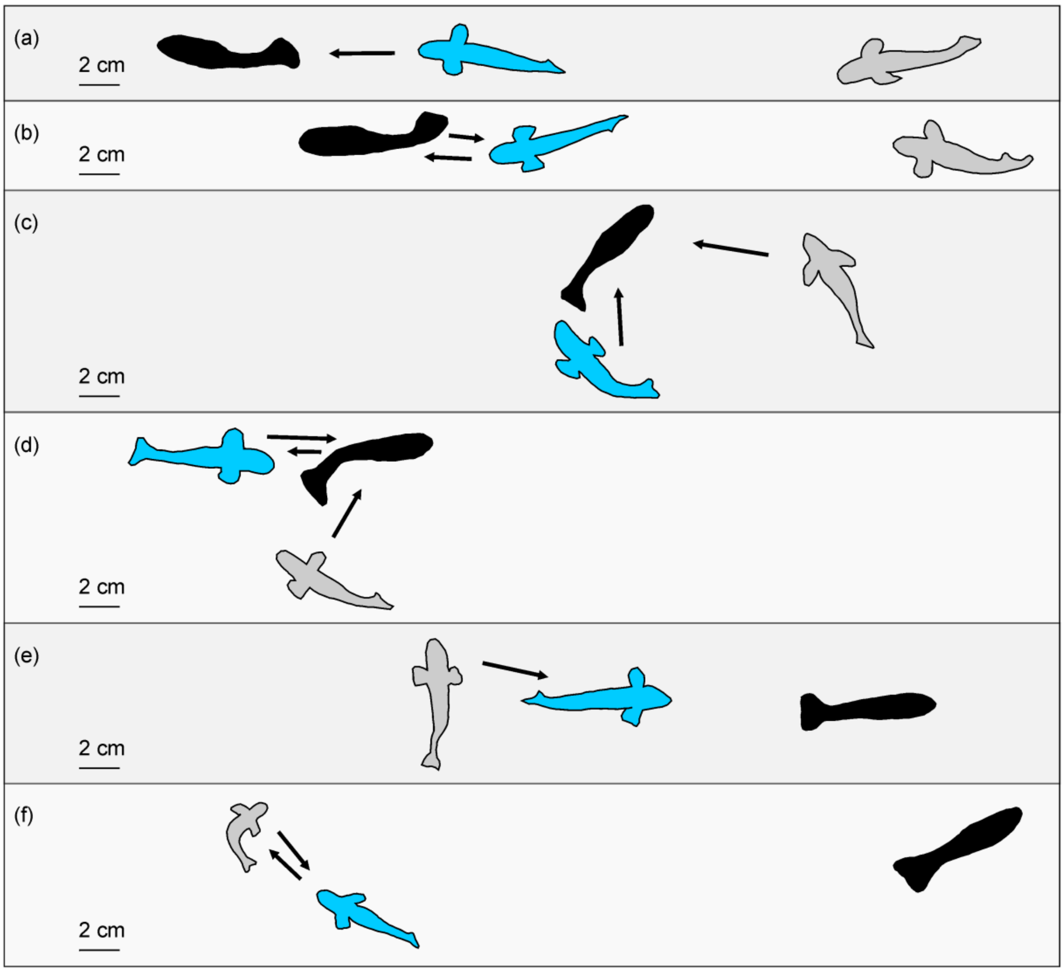

Figure 11.17: Exemplary episode of a mixed group responding to the dynamic echo playback: Spatial interactions. Panels are marked by lowercase letters corresponding to the time points in Figure 11.16 and depict interactions between the mobile dummy emitting a dynamic echo playback sequence (black), fish #2 (blue), and fish #3 (grey). Pictograms are drawn to scale and represent the spatial relationship of fish and dummy at the onset of relatively strong synchronization episodes (correlation coefficient ≥ 0.3 for at least 500 ms). Black arrows point from synchronizing individuals to the respective synchronization partners.

Two typical behavioral sequences were described in greater detail to illustrate these observations (Figure 11.18 and Figure 11.19). Relatively strong synchronization episodes often occurred in situations during which a synchronizing individual approached its synchronization partner from behind, moved closer, and then into a more lateral position next to the other individual, often in parallel to its swimming trajectory. This behavioral sequence was frequently also directed towards the dummy and is illustrated in Figure 11.18. It shows IDI-sequences of a static random playback and the responding fish (Figure 11.18A) as well as the corresponding cross-correlation diagram, which reveals discharge synchronization of the fish to the playback. Synchronization began approximately at 16.3 seconds (Figure 11.18B) when maximum correlation values exceeded the 0.3 threshold criterion (Figure 11.18C). Detailed interactions between the fish and the dummy are drawn to scale in (Figure 11.18D) and represent selected time points referenced in (Figure 11.18A). At the beginning of the episode, the fish was located at a distance from the dummy (a), approached quickly from behind, and started synchronizing its discharges to the playback when it was less than one body length away from the dummy (b). This one-sided synchronization persisted for the remainder of the whole episode, during which the fish first followed directly behind the dummy (c) and eventually caught up into a more lateral position next to the dummy, which then slowed down and turned (d).

Of particular interest were episodes with mutual IDI-synchronization sequences between two fish. In total, nine episodes (one of which was discarded because synchronization did not occur at the response time characteristic for echoing, and animals did not obviously interact) were observed, during which both live fish simultaneously synchronized their discharges to one another according to the threshold criteria. The remaining eight episodes were associated with behavioral situations during which one fish followed right after the other (one episode) or eventually caught up to a more lateral position relative to the ahead swimming individual (seven episodes). All these episodes were associated with situations during which the following fish initiated the mutual sequence of discharge synchronization.

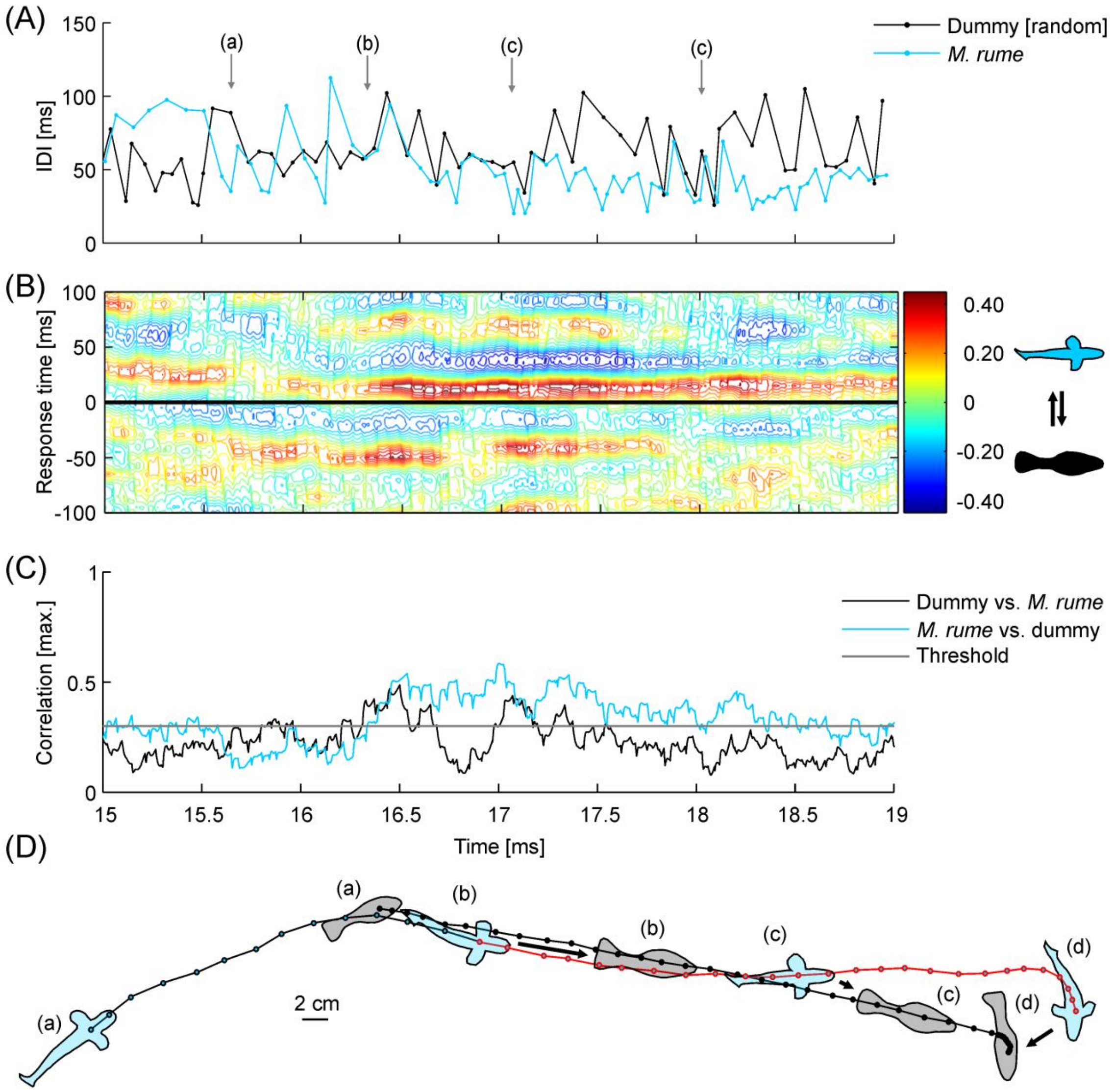

Figure 11.18: Exemplary illustration of an episode with discharge synchronization of *M. rume* to a static random playback. (A) IDI-sequences of the fish (blue) and a static random playback (black) emitted by the dummy during a short interaction period. (B) Cross-correlation diagram of the sequences depicted in (A) with color-coded correlation coefficients for a response time of ±100 ms. High correlations at positive response times represent discharge synchronizations of the fish (blue) with the dummy (black) at that response time. High correlations at negative response times can only occur randomly due to the non-interactive nature of the playback. (C) Maximum correlation coefficients within the 100 ms response-time window in (B) plotted over the same time frame for correlations of the signal sequences of the fish with the dummy (blue) and vice versa (black). The horizontal grey line delineates the 0.3 threshold indicative of relatively high correlation. (D) Illustrations of the interaction of fish (blue) and dummy (grey) drawn to scale along with their trajectories during the same time. Lowercase letters in brackets assign drawings from the same video frames and correspond to the time points marked in (A). Trajectories marked in red indicate that the fish was synchronizing its discharges to those of the dummy with a correlation coefficient of 0.3 or higher as a part of a coherent synchronization sequence of at least 500 ms. Black arrows indicate discharge synchronization in a given situation.

An example of a mutual synchronization episode between two fish is illustrated in Figure 11.19. Here, IDI-sequences of two interacting fish are plotted together (Figure 11.19A), revealing simultaneous regularization patterns of both fish shortly after the beginning of the episode. The corresponding cross-correlation diagram (Figure 11.19B) reveals mutual synchronization, initiated by fish #3, simultaneous with the regularized sequences. Maximum correlation values are plotted in (Figure 11.19C), marking the point in time when the 0.3 correlation threshold criterion was exceeded. Detailed trajectories and interactions of the two fish are drawn to scale at several points in time (Figure 11.19D), and are referenced by lowercase letters in (Figure 11.19A). Before the synchronization episode, fish were located more than a body length apart (a). Fish #3 then approached from behind to a lateral, almost parallel position and started synchronizing (b). This was reciprocated shortly after that by fish #2 and culminated in a short sequence of mutual discharge synchronization between the two fish (c). Synchronization was terminated by fish #3 (d) shortly before the trajectories of the two fish crossed and they eventually stopped interacting (e).

Angular relationships between synchronization partners were determined for the moment an individual reached the threshold criterion for relatively strong synchronization. This way, quantitative data describing the behavioral interactions during discharge synchronization in groups could be obtained. Absolute counts of the angular relationship between the swimming direction of the synchronizing individual and the direction towards its synchronization partner are presented in Figure 11.20A. Both for interactions between two fish and for interactions of fish with the dummy, the higher occurrence of low angles is consistent with spatial relationships where the synchronizing fish was headed towards the individual (fish or dummy) it synchronized its discharges to at the time. Results of the same evaluation are presented in Figure 11.20B for the angle between the swimming direction of the individual (or the dummy) receiving synchronization and the direction towards the synchronizing fish. The higher occurrence of angles close to 180° indicates that the synchronizing individual approached the receiver of synchronization from behind in the majority of cases. Center distances between individuals at the time the threshold criterion for synchronization was met were 111 ± 43 mm (*mean* ± *s.e.*) during experiments with the static random playback and 114 ± 59 mm (*mean* ± *s.e.*) during experiments with the dynamic echo playback. Electric discharge synchronizations thus seemed to occur during situations when an individual approached another one from a distance, and were often followed by social interactions. Synchronization of EODs trough echoing may thus be used to specifically single out and address another individual within a social group and communicate an intent to interact with that individual.

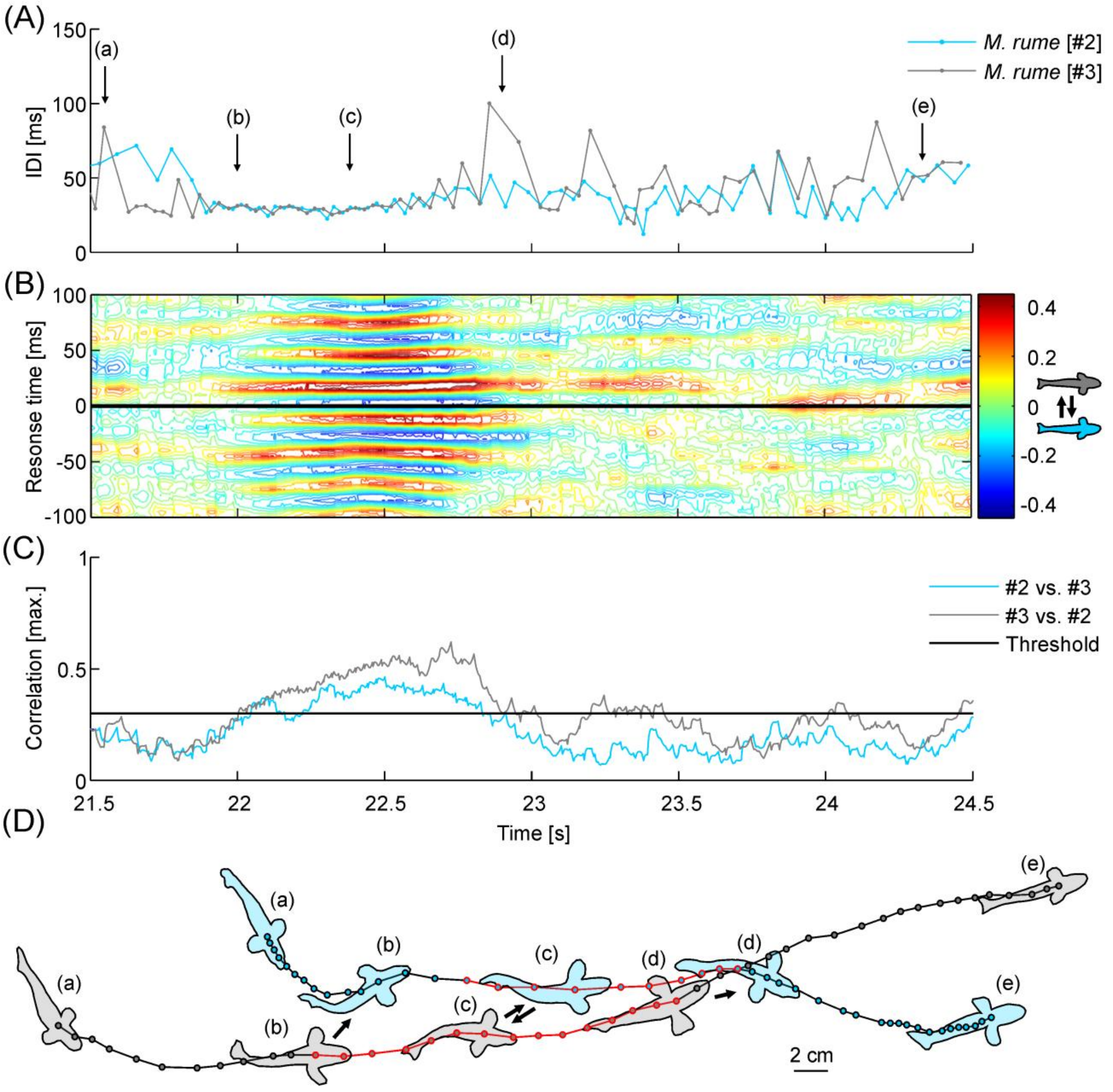

Figure 11.19: Exemplary illustration of a sequence with mutual discharge synchronization between two fish. (A) IDI-sequences of fish #2 (blue) and fish #3 (grey) during a short interaction period. (B) Cross-correlation diagram of the sequences depicted in (A) with color-coded correlation coefficients for a response time of ±100 ms. High correlations at positive response times represent discharge synchronizations of fish #3 (grey) with fish #2 (blue). High correlations at negative response times represent synchronization of fish #2's discharges with those of fish #3 at that response time. (C) Maximum correlation coefficients within the 100 ms response-time window in (B) plotted over the same time frame for correlations of the signal sequences of fish #2 with fish #3 (blue) and vice versa (grey). The horizontal black line delineates the 0.3 threshold indicative of relatively high correlation. (D) Illustration of the interactions of fish #2 (blue) and #3 (grey) drawn to scale along with their trajectories during the same time frame. Lowercase letters in brackets assign drawings from the same video frames and correspond to the time points marked in (A). Trajectories marked in red indicate that the fish was synchronizing its discharges to those of the other individual with a correlation coefficient of 0.3 or higher as a part of a coherent synchronization sequence of at least 500 ms. Black arrows indicate which fish engaged in discharge synchronization in a given situation.

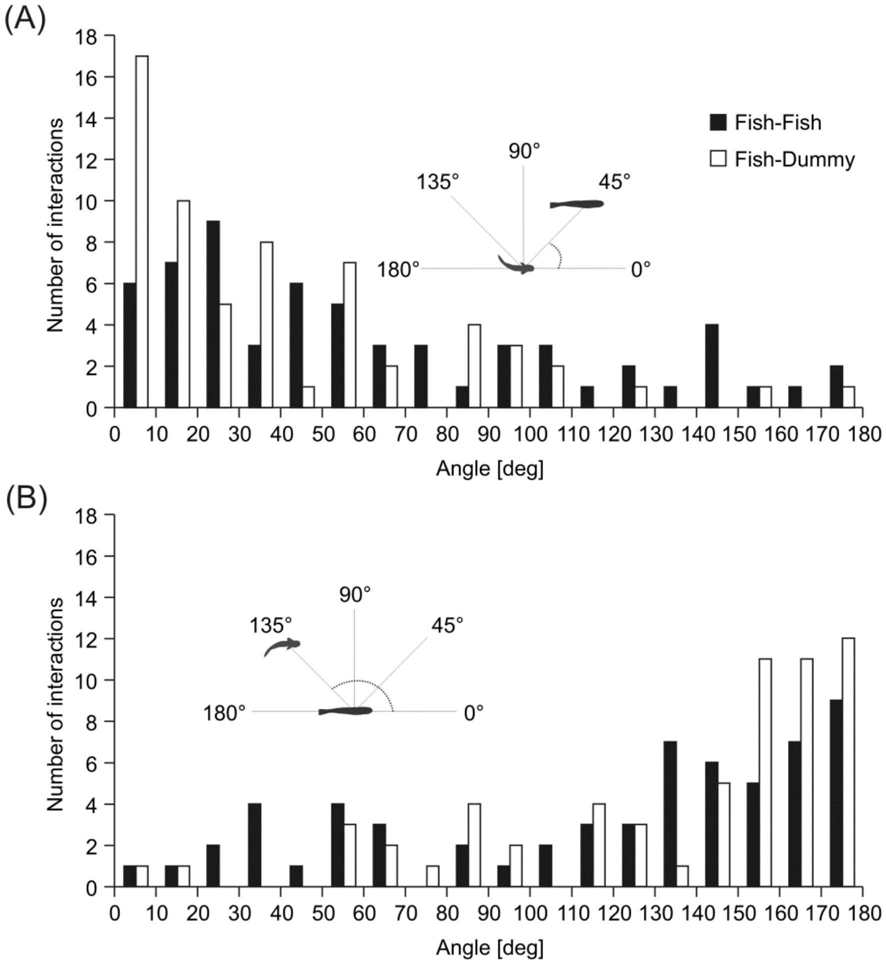

Figure 11.20: Spatial interactions during electric discharge synchronizations in mixed groups. Angular relationship of two fish (black) or a fish and the dummy (white) at the onset of sequences with strong discharge synchronizations defined by a cross-correlation coefficient ≥ 0.3 and a duration of at least 500 ms. (A) Histogram quantifying the frequency of angular values for the angular difference between the swimming direction of the synchronizing fish and the connecting line to its synchronization partner. The higher incidence of low values indicates that fish synchronized more frequently while they were faced towards their synchronization partner. (B) Histogram quantifying the frequency of angular values for the angular difference between the connecting line between the synchronizing fish and its synchronization partner, and the swimming direction of the individual whose discharges were being synchronized to by the focal fish. The higher incidence of high values indicates that individuals were being synchronized to more frequently by individuals that approached them from behind. Angular relationships of synchronization partners are illustrated for a fish–dummy interaction in the insets of (A) and (B). No differentiation was made between the two playback conditions during the quantification of fish–dummy interactions. Bin size: 10 degrees.

11.4 Discussion

The results of this section extend the observations made in chapter 5 from single fish to small groups of up to four *M. rume* and demonstrate that the mobile dummy exerted influence on motor and electromotor behavior of live animals based on electric playback generation. Similar to the interactions with single fish, the influence of the electro-communicating dummy on the swimming behavior of individuals in groups was reliable, but no significant difference occurred based on the playback pattern that was presented. Whether the dummy produced a static random playback sequence or a dynamic echo playback did not influence motor behavior in groups. Electrical discharge synchronizations by individual fish occurred in response to both playback types and were investigated in mixed groups of two fish and the dummy. Detailed analyzes of behavioral interactions during episodes of strong discharge synchronizations support the idea that mormyrids use the echo response to generate a shared social attention framework for the exchange of information during electrocommunication.

The general influence of the dummy on following-behavior and group dynamics was reliable but declined with increasing group size. Although this effect of group size could not be explicitly tested statistically due to the limited amount of experimental animals, it was observed with regard to the number of followed turns (Figure 11.12), nearest-neighbor distances (NND; Figure 11.6) and orientation (Figure 11.11). Independently of what measure was used for quantifying the fish's responses to the dummy, the strongest reactions were always observed in single fish. Larger groups of fish hardly followed for extended time periods (Figure 11.13). This difference may reflect that live fish were more attractive than the dummy in experiments with more than a single test fish, but could also hint towards a quorum rule for decision-making in groups (Sumpter *et al.*, 2008). Fish were more attracted to conspecifics compared with the electrically silent dummy, but this preference was less evident during playback presentations (Figure 11.8). Because the robot never moved as close to the tank wall as the fish, individuals had to weigh the social attraction towards the electrically signaling dummy against taking the risk to abandon the relative safety of the walls when swimming into the open area. In sticklebacks (*Gasterosteus aculeatus*), risky decisions were only made if they were initiated by a certain proportion of fish. Single individuals and pairs of fish readily followed after a stickleback replica, whereas collective swimming in groups of four and eight fish could only be induced by introducing a second dummy (Ward *et al.*, 2008). Such quorum rules can prevent groups of animals from taking wrong decisions because the probability that misinformed behaviors of a sufficiently large number of individuals get amplified into a group response decreases with group size (Ward *et al.*, 2008). However, one characteristic of quorum decision making is that if animals follow, they do so collectively as a group (Sumpter *et al.*, 2008). This was not often observed during the experiments with *M. rume*. A definite demonstration of quorum rules in mormyrids would require the introduction of additional dummies to initiate collective following-behavior.

Entering an open, featureless, and unfamiliar environment is risky behavior that is avoided by many animals, including fish (Maximino *et al.*, 2010). From the trials without the dummy (Figure 11.4; Figure 11.5; compare also Figure 5.3 and Figure 5.4A), it became evident that animals preferred the proximity to the tank walls and frequently grouped in the corners. This behavior may have reflected a desire to seek shelter among conspecifics (Hamilton, 1971). In a threatening situation, such behavior should be more pronounced than the otherwise strong tendency of *M. rume* to engage in competitive behaviors.

Although the dummy reliably attracted some fish from groups of all sizes, instances, when all members of a group followed in a polarized, school-like formation, were quite rare. A high degree of polarization characterizes shoals in which animals adopt a similar orientation, which is a prerequisite for behavioral synchrony at the global level in large schools of fish (Pitcher and Parrish, 1993). Independently of orientation, group coherence can be characterized by looking at nearest neighbor distances (NND). Strongly schooling pelagic mackerel (*Scomber japonicus*) kept nearest neighbor distances of 0.4 body lengths and the individual deviation from the mean swimming direction of the group was on average 8°, whereas in the somewhat less organized schools of silversides (*Atherinops affinis*), directional deviations of 17° and an average NND of 1.0 body length were measured (van Olst and Hunter, 1970). Compared with these data, the small groups of *M. rume* did not show particularly prominent shoaling tendencies. Group polarization was not particularly high in general, and the dummy had no significant influence on the polarization among fish (Figure 11.10). The ability of the dummy to recruit fish that then followed with the same orientation decreased rapidly with group size (Figure 11.11). When taking the median body length of 85 mm as a basis, the median of the mean NND was always greater than one body length in groups of *M. rume* (Figure 11.7).

These results raise the question to what extent mormyrids in general, and *M. rume* in particular, actually shoal in their natural habitat. Can the concept of optimal group size (Krause and Ruxton, 2002) be applied to these animals, and, if so, what is their elective group size (Pitcher and Parrish, 1993) in different naturally occurring contexts? Unfortunately, there is no knowledge about the social behavior of *M. rume proboscirostris* in the wild, and field reports of shoaling behavior in mormyrids are mostly anecdotal. Kirschbaum (1995) and Hopkins (1986) described anadromous behavior in mormyrids, who migrate in large schools into flooded areas to spawn during the rainy season. According to Kramer (1996), mostly the small species below 20 cm gather in schools. Whether mormyrids form schools may thus be size or age-dependent, and subject to environmental conditions and the life cycle of an animal. But shoaling tendencies are also species-dependent. Hopkins (1980) reported ecology and EOD-characteristics of 23 mormyrid species in Gabon, West Africa, and found that particularly members of the genera *Marcusenius* and *Petrocephalus* formed mixed species shoals in sheltered areas. Large schools of *Petrocephalus bovei* were also observed by Kramer (1990) during daylight. Hopkins (1980) further noted that shoaling species produced short-duration EODs, whereas EODs of longer duration were associated with a solitary lifestyle.

Carlson (2016) systematically compared population density, social behavior and electric signaling characteristics of sympatric *Petrocephalus degeni* and *Gnathonemus victoriae* and found the former to be social, whereas the latter displayed competitive behavior. He attributed these differences in social behavior to neuroanatomical differences in the nucleus exterolateralis (EL), which roughly divide mormyrids into two clades. Members of clade A, such as *Gnathonemus* and *Mormyrus*, have a neuroanatomical differentiation in the EL that makes them capable of EOD-waveform discrimination, with important implications for social communication (Carlson *et al.*, 2011) (see section 1.3). This specialization is absent in non-clade A members like *Petrocephalus*, for which vision appears to be of higher importance during social behavior (Stevens *et al.*, 2013). This implicates that clade A species may not be particularly well suited to observe schooling behavior. However, mormyrid social behavior is also likely to vary depending on diel activity patterns. Many mormyrids live territorial and cryptic during the day and forage at night (Moller *et al.*, 1979), but it is mostly unknown to what extent foraging behavior occurs in group formation. In the case of *Mormyrops anguilloides*, the notion that these larger species, in particular, are solitary and territorial (Kramer, 1996), did not provide a complete picture of their behavioral repertoire because they in fact form hunting groups at night (Arnegard and Carlson, 2005).

When considering schooling as a strategy against visual predators in featureless open-water habitats (Parrish, 1992), such behavior seems unlikely to be observed during the nocturnal activity period of mormyrids that hide during daylight hours. Whether any of the anti-predator benefits of shoaling described in section 11.1.1 would be effective against nocturnal predators, like for example electroreceptive catfish (Hanika and Kramer, 2000; Merron, 1993), remains to be investigated. Interestingly, mormyrids do not produce 'Schreckstoff' (Pfeiffer, 1977), which is released from the injured skin in many fishes and mediates an alarm response that, among other effects, also increases shoaling tendencies (von Frisch, 1941). It would, therefore, be interesting to test whether flight-associated electric discharge patterns (Kramer, 1976c) are socially transmitted as signals, and acted upon by individuals in the vicinity of a mormyrid that is attacked by a predator.

In the laboratory, several factors can influence whether group behavior occurs. Khait *et al.* (2009) observed group cohesion in juvenile *M. rume proboscirostris* and found the parameters of spacing to be dependent on group size, light levels, and the ontogenetic development of the EOD. However, older individuals were much less social and displayed aggressive behavior to form hierarchies and compete for resources (Kersten, 2017a) (see section 4.3.1). This transition is a commonly observed phenomenon in fishes. Shoaling during the juvenile phase occurs in about half of all fish species, whereas only 25% maintain this behavior throughout their entire lives (Shaw, 1978). Which behavior is observed can depend on a multitude of conditions such as the availability of shelters, fish density, dominance relationships, size differences between individuals, as well as the novelty of the environment, which may increase stress levels and induce anxiety (Becco *et al.*, 2006; Carlson, 2016; Kersten, 2017a; Maximino *et al.*, 2010; Moller, 1995). Electrocommunication not only adds a layer of complexity to mormyrid social behavior but also to the design of behavioral experiments aiming to uncover the significance of communication in groups. In laboratory experiments on social behavior of different mormyrid species, group sizes were chosen by experimenters to enable the assignment of EODs to individual group members. This task increases in difficulty with increasing group size and has consequently not been performed for groups of more than five fish (Gebhardt *et al.*, 2012a). While this approach provides insights into electric signaling interactions of individuals in small groups (Gebhardt *et al.*, 2012b; Kupschus, 2017; Neusel, 2014; Smeets, 2013), it does not allow to determine the elective group size of these animals and may therefore not represent their natural behavior patterns.

This methodological dependency on relatively small groups is an important constraint, because collective behavior may not be entirely understood by studying individual based interactions without feedback from group-level effects (Couzin and Krause, 2003). Both the absolute number and the density of fish in a shoal are likely to be relevant parameters. By studying minnows (*Phoxinus phoxinus*), Partridge (1980) found that pairs of fish behaved qualitatively different than groups of three or more individuals. This means that it is not necessarily possible to conclude from the behavior in dyadic interactions on the behavior of larger groups of fish by extrapolation alone. In the field, the formation of oceanic herring schools was not triggered before the population density reached a critical value (Makris *et al.*, 2009), and similar density-dependent transitions into polarized groups were observed in tilapia (*Oreochromis niloticus*) in the laboratory (Becco *et al.*, 2006). It is, therefore, possible that groups of four *M. rume* were too large to be collectively influenced by a single dummy but too small for typical shoaling behaviors to emerge.

Group sizes in the current experiment were constrained by the availability of experimental animals necessary to obtain independent data for valid statistical comparisons, and by the aim to analyze motor and electromotor behaviors with high precision and with the highest possible degree of automation. Despite the low light levels, animals were seemingly agitated when transferred to the novel environment of the experimental tank, where they preferentially grouped in the corners. However, an increase in acclimatization time usually led to increased levels of competitive behavior and aggression, which were absent when animals were tested directly after their release from the start box. A simple strategy to prevent animals from aggregating in the corners of the tank is to use a circular testing area (Jun *et al.*, 2013). The observation that animals showed a tendency to seek shelter further emphasizes the suggestion argued for in section 10.3, which is that experiments should be performed in the absence of visible light by relying entirely on infrared illumination for video recordings. In chapters 4 and 9 it was shown that *M. rume* made use of their electroreceptive modalities and used actively generated electrical information to mediate social coherence under non-visual conditions. Complete elimination of visually perceived information could, however, result in qualitatively different behaviors that are governed by distinct sets of local interaction rules in groups of *M. rume*. A hypothesis would be that mormyrids that move in groups adjust their inter-individual spacing to keep directional information and magnitude of the electrical field vector of a conspecific's EOD constant at defined electroreceptive regions of their body. Due to the pulse-type nature of the mormyrid EOD and the curvilinear structure of the resulting dipole field, the question how animals extract spatial information from electric signaling sequences of a moving conspecific is not trivial (Hopkins, 2005).

Other influences that should be considered are disturbances generated by the robot's movement, and how well the dummy mimicked the behavior of a fish. Electrical playback generation by the dummy was validated by demonstrating that fish responded with social signaling such as double pulses, regularizations and discharge synchronizations (see chapters 4, 5, and 9). Visual information did not seem to be of particular importance in chapter 8 and was controlled for experimentally (chapter 9). Although measures were taken to dampen the noise generated by the moving robot, and animals were also given time to habituate to the acoustic disturbance it caused, the robot (Landgraf *et al.*, 2012a) likely affected fish behavior during the experiments.

Aspects that were not taken into consideration are the motility patterns of the dummy with respect to swimming speed and dynamics. In zebrafish (*Danio rerio*), swimming speed of a mobile robot influenced group cohesion (Butail *et al.*, 2013), and guppies (*Poecilia reticulata*) were more attracted to a mobile fish replica that mimicked the natural motion phases of live fish when moving (Landgraf *et al.*, 2016). In groups of both mosquitofish (*Gambusia holbrooki*) and golden shiners (*Notemigonus crysoleucas*), it was found that adjustments of swimming speed were essential determinants of group cohesion (Herbert-Read *et al.*, 2011; Katz *et al.*, 2011). The parameters of swimming style should therefore be incorporated into models of shoaling behavior (Lopez *et al.*, 2012), as well as into the behavioral repertoire of mobile dummy fish for experimental investigation and validation of such models in groups of live fish (Bonnet *et al.*, 2016; Landgraf *et al.*, 2016). During the experiments with *M. rume*, linear speed and swimming dynamics could not be precisely matched to those of live fish (Appendix B; Figure B.2). The wheels of the robot underneath the tank could be actuated neither dynamically nor differentially during forward motion, and the robot, therefore, had to come to a complete stop each time before it could initiate a turn. Although the attractiveness of the dummy was mainly determined by the presence of electrical playback signals, refining the dynamics of the dummy's swimming behavior may, nevertheless, increase the likelihood to initiate and sustain coordinated movements in mixed groups of mormyrids and mobile fish robots in interactive experiments on electrocommunication.

Independently of the exact conditions under which mormyrids may or may not engage in shoaling, or even form polarized schools, their social behavior is highly complex. This is evident from their elaborate electrocommunication strategies, which are based on both EOD-waveform and temporal variations within discharge sequences (see section 3.5). IDI-based electrocommunication strategies can be based on discharge frequencies, temporal patterns, and latency interactions between individuals, and were investigated in great detail in pairs of live fish and mobile dummies in chapters 4 and 5. The difficulty to systematically study electrocommunication based on the temporal signaling sequences of different individuals increases with group size, but looking at dyadic interactions is, as argued above, likely insufficient to conclude on the significance of electrocommunication in groups. Here, electrocommunication was investigated in mixed groups of two live fish and a mobile dummy, which generated either a static random playback or a dynamic echo playback. By imitating the echo response of *M. rume*, dynamic playback generation enabled discharge synchronizations between live individuals and the dummy, and thus the comparison of artificially synchronized episodes with similar interactions from pairs of live fish. The characteristics of echoing and discharge synchronizations have been investigated in dyadic interactions in chapters 4 and 5, and the implications of these findings for electrocommunication were discussed in section 6.3.

Based on the results of chapter 5, a function of synchronized discharge episodes in selectively allocating social attention between individuals has been proposed. The group experiments in the current chapter support this interpretation. By defining and identifying episodes of relatively strong correlation between simultaneous signaling sequences, a reversed process was used to observe and analyze how fish behaved during EOD-synchronization. In line with the results of previous experiments (Figure 5.10), synchronization episodes were considered to be relatively strong if correlation coefficients exceeded a value of 0.3 for 500 ms or more. Especially the moments when this threshold was exceeded were analyzed in more detail (Figure 11.20). Two observations supported the social attention hypothesis. First, as already indicated by the correlation between spatial relationships and the magnitude of EOD-synchronization of fish and dummy in chapter 5 (Figure 5.13), the onset of relatively strong correlations in the current experiments was frequently associated with approach configurations. In these situations, *M. rume* often approached either the dummy or a conspecific in a stereotyped manner by coming from behind and then catching up into a lateral position (Figure 11.18). Second, during episodes of mutual echoing in pairs of live fish, it was mostly the approaching individual that initiated this synchronization (Figure 11.19). It is thus argued that the ability of mormyrids to selectively synchronize their signaling by mutually generating echo responses to each other's EODs (Arnegard and Carlson, 2005; Gebhardt *et al.*, 2012a), and the observation that the resulting episodes of synchronized discharge activity can rapidly switch between group members (Gebhardt *et al.*, 2012b), represent a communication strategy that allows individual fish to address each other in a wide variety of social contexts (see sections 5.4 and 6.3). This mechanism is arguably most useful in group constellations, where it may enable the exchange of information during electrocommunication by establishing a link that depends on very fast and precise time-locking of the EODs of two individuals.

Behavioral synchronization during signal generation with implications for social communication occurs within many domains of the animal kingdom and can also be observed in visual and acoustic displays (Buck, 1988; Hartbauer, 2008; Reaney *et al.*, 2008). In the primate central nervous system, neural synchrony of activity patterns between different regions of the brain has been associated with visual attention (Gregoriou *et al.*, 2009). Mormyrid weakly electric fish offer the opportunity to study such mechanisms non-invasively in freely interacting animals, which may allow concluding on more general neurobiological principles.

V. General Conclusion

The central theme of this thesis was to explore the feasibility of using mobile fish dummies for the investigation of IDI-based electrocommunication in a mormyrid weakly electric fish. One of the objectives for using this methodological approach was the hypothesis that the embodiment of a source of electrical playback signals as a moving dummy might give a more comprehensive account of the communicative repertoire of live mormyrids compared with a set of stationary playback electrodes. The reason for this assumption was the rationale that signal generation and behavior of a mobile dummy fish can match, and, ideally, respond to the behavior of live fish. Thus, dummy experiments should provide a framework for the study of electrocommunication while simultaneously avoiding the difficulties associated with the assignment of EODs to their respective sender in experiments with more than one freely behaving fish. The underlying hypothesis was that playback sequences of species-specific EODs are key stimuli that make it possible to integrate a mobile dummy fish socially into a group and make it 'accepted' as an artificial conspecific by live individuals. This integration would eventually enable the creation of mixed societies of real and artificial mormyrids and open exciting possibilities for research projects in the field of animal–machine interaction (Halloy *et al.*, 2013).

Two setups for behavioral experiments were designed and implemented to test these possibilities in the weakly electric fish *Mormyrus rume*. These setups allowed the generation of motor and electromotor behavioral output using mobile dummies with different degrees of freedom and different levels of interactivity with live fish. It was shown that electric communication signals are sufficient as stimuli to induce following-behavior in single individuals and small groups of *M. rume*, and that electrical signaling was more relevant for behavior than visual stimuli and motility cues. Even after reducing the dummy to only the EOD from the fish's perspective, following-behavior and motor interactions persisted. The assumption that EODs are key stimuli that trigger social behavior was thus confirmed and a dummy could be socially integrated with live fish based on electrical signaling.

Behavioral interaction occurred at the motor level, but also at the level of electrical signaling. Three types of electrocommunication strategies were studied in more detail and interpreted with respect to their adaptive value. Based on hierarchy relationships, habituation, and a set of dyadic contest experiments, double-pulse patterns were classified as conventional threat signals of aggressive motivation that the fish use at the beginning of a sequential assessment strategy during competitive encounters (Enquist and Leimar, 1983). Discharge regularizations, which facilitate active electrolocation, can also be beneficial in social contexts, especially during the early stages of mutual assessment. During such interactive encounters, the ability of mormyrids to also interact electrically through echoing each other's EODs may facilitate the evaluation of physical condition and waveform information of another individual, simultaneously (Arnegard and Carlson, 2005; Terleph, 2004). Thus, synchronized discharge regularization, mediated by mutual echo responses, likely facilitates the assessment of conspecifics and helps to avoid unnecessarily costly conflicts.

Interactive playback that simulated the echo latency of *M. rume* provoked more echo responses from live fish compared with static sequences of random IDIs. Because EOD-synchronizations were increased during approach configurations, it was hypothesized that echoing represents a more general mechanism that can be utilized by mormyrids to allocate social attention between individuals. Such a strategic function of discharge synchronization is not necessarily restricted to the communication of identity information or behavioral states and context-dependent motivations. Instead, the proposed strategy provides a framework in which mormyrids can exchange information by establishing a relatively private communication channel between individuals, which would be particularly useful in groups of several signaling fish. This strategy could, in principle, be used in a variety of behavioral contexts from aggressive to cooperative interactions.

It was also shown that passive perception of a conspecific's EOD, likely enabled by the knollenorgan receptors that mediate electrocommunication, provide spatial information during social interactions with a moving playback signal. From an evolutionary perspective, the echo response could represent a mechanism to avoid jamming of this passive sensory capability. This mechanism could then have ritualized into a communication strategy because it ensures that the respective other fish detects that it is subject to social intentions by an individual that locks on to its EODs by echoing. This hypothesis unifies the opposing interpretations of the function of echoing as a jamming avoidance mechanism during active electrolocation and as a strategy for social communication (Heiligenberg, 1976; Kramer, 1974; Russell *et al.*, 1974).

At the level of individual interactions, mobile dummy fish that emitted electrical playback signals reliably induced social behavior in live animals. The influence on groups, however, declined rapidly with increasing group size. Possible reasons for this are a quorum decision mechanism, which means that animals only respond if a certain fraction of individuals initiates a particular behavior, and the relatively small group sizes that were investigated. Because the problem of assigning EODs to individual fish reemerges in groups and increases rapidly with increasing groups size, this means that there is a tradeoff between the possibility to observe emergent shoaling behaviors and the ability to efficiently analyze electric signaling behavior in a group context. Collective behaviors in natural and mixed societies emerge as a result of non-linear positive feedback between individuals that follow simple interaction rules and can lead to the solution of complex problems that are beyond the capacities of any individual member of the group (Mondada *et al.*, 2013).

Many instances where solutions of complex problems are collectively solved were observed and investigated in social insects (Deneubourg and Goss, 1989). Ioannou (2017) argues that in fish, and vertebrates in general, mechanisms other than self-organized collective intelligence are likely to account for many of the observed group phenomena. He claims that this is in part because behavior in vertebrates is more complicated at the individual level and because individuals are genetically less similar than social insects, which should result in higher competition between members of a group.

In mormyrids, electrocommunication provides a unique opportunity to integrate an artificial conspecific with live fish, particularly at the level of individual interactions. But electrocommunication also adds complexity to the social behavior in groups of weakly electric fish. More needs to be known about the natural shoaling tendencies of different mormyrid species, the situations in which schooling occurs, and the adaptive advantages of group behavior for individual fish to carry the full potential of a mormyrid mixed society into effect.

VI. Summary

Mormyrid weakly electric fish possess a specialized electrosensory system. During the process of active electrolocation, these animals perceive self-generated electric organ discharges (EOD) and are thereby able to detect objects in their nearby environment. The EOD is a short, biphasic pulse, which is simultaneously used to communicate with conspecifics. There are two principles according to which information exchange occurs during electrocommunication. The waveform of the EOD constitutes a relatively stable identity marker that signals species, gender, and status of an individual. In contrast, the temporal sequence of inter-discharge intervals (IDI) is highly variable and encodes context-specific information. Modifications of IDI-duration not only alter the instantaneous discharge frequency but also enable the generation of specific signaling patterns and interactive discharge sequences. One such interactive discharge behavior is the so-called echo response, during which a fish responds with a constant latency of only a few milliseconds to the EOD of a conspecific. Animals can synchronize their signaling sequences by mutually generating echoes to each other's signals over a coherent period. Although active electrolocation and electrocommunication are mediated by different types of electroreceptor organs and neural pathways, an unambiguous assignment of electromotor behavior to only one of the two functions is often problematic.

In this thesis, the significance of IDI-based signaling sequences during motor and electromotor interactions of the mormyrid fish *Mormyrus rume proboscirostris* were investigated. To this end, different electrical playback sequences of species-specific EODs were generated via mobile fish dummies, and the motor and electromotor responses of live fish were analyzed.

In Part One of this thesis, electrocommunication strategies of the fish were analyzed, and particularly the functions of double pulses, discharge regularizations, and echo responses were examined in an adaptive context. Double pulses were classified as an aggressive motivation signal, whereas regularizations may have a communicative function during the early stages of the sequential assessment of a potential opponent. In this context, discharge synchronization by means of echo responses may enable a mutual assessment for the net benefit of both contestants. Because echo responses occur in various behavioral contexts, and artificial echoes of the dummy evoked increased echoing by the fish, it was hypothesized that the echo response serves a more general purpose by enabling mutual allocation of social attention between two fish.

In Part Two of this thesis, a biomimetic robotic fish was designed to investigate the sensory basis on which fish followed the dummy. It was shown that electrical playback signals induced following-behavior in live fish, whereas biomimetic motility patterns had no effect. By subsequently reducing the mobile dummy to only the electric signaling sequence from the perspective of the fish, it could be shown that passive perception of electrical communication signals is also involved in mediating the spatial coordination of social interactions. This passive perception is likely mediated by the same electroreceptor organs that are used during electrocommunication. The EOD can therefore be considered to be an essential social stimulus that makes it possible to integrate a dummy into a group of weakly electric fish as an artificial conspecific.

The influence of an interactively signaling mobile dummy fish on small groups of up to four individuals was investigated in Part Three of this thesis. Typical schooling behavior was a rare occurrence in this context. However, EOD-synchronizations through mutual echo responses between two fish, or between a fish and the interactive dummy, were frequently observed during social interactions in small groups. Motor interactions during synchronization episodes supported the hypothesis that mormyrids may use discharge synchronizations between individuals to allocate social attention, and the echo response may thus adopt a particularly useful function during communication in groups.

VII. Zusammenfassung

Schwach elektrische Fisch aus der Familie der Mormyriden verfügen über ein spezialisiertes elektrosensorisches Sinnessystem. In einem Prozess, der als aktive Elektroortung bezeichnet wird, sind diese Tiere in der Lage, selbstgenerierte elektrische Organentladungen (EOD) wahrzunehmen, und dadurch Objekte in ihrer unmittelbaren Nähe zu detektieren. Das EOD ist ein kurzer bipolarer Puls, der gleichzeitig auch zur Kommunikation mit Artgenossen dient. Informationsaustausch während der Elektrokommunikation basiert auf zwei verschiedenen Prinzipien: Die Wellenform des EOD stellt einen relativ konstanten Identitätsmarker dar, der beispielsweise Art, Geschlecht und Status eines Individuums signalisiert. Die zeitliche Abfolge der Intervalle zwischen den EODs ist hingegen höchst variabel und kodiert kontextspezifische Information. Durch Modifikation der Intervalldauer ändert sich nicht nur die Entladungsfrequenz, sondern es können auch spezifische Signalmuster und interaktive Entladungssequenzen generiert werden. Ein interaktives Entladungsverhalten stellt beispielsweise die Echoantwort dar, bei der ein Fisch mit einer konstanten Latenz von wenigen Millisekunden auf das EOD eines Artgenossen reagiert. Zwei Tiere können ihre Entladungssequenzen synchronisieren, indem sie ihre Signale über einen kohärenten Zeitraum gegenseitig mit Echos beantworten. Obwohl aktive Elektroortung und Elektrokommunikation über unterschiedliche Rezeptororgansysteme und neuronale Pfade vermittelt werden, ist eine eindeutige Zuordnung der elektromotorischen Verhaltensäußerungen der Fische zu nur einer der beiden Funktionen oft problematisch.

In der vorliegenden Arbeit wurde die Bedeutung intervallbasierter EOD-Sequenzen für motorische und elektromotorische Interaktionen des Mormyriden *Mormyrus rume proboscirostris* erforscht. Hierzu wurden verschiedene elektrische Playbacksequenzen artspezifischer EODs generiert und durch mobile Fischattrappen wiedergegeben. Die motorischen und elektromotorischen Verhaltensreaktionen der Fische wurden analysiert.

Im ersten Teil der Arbeit wurden Elektrokommunikationsstrategien der Fische analysiert und die adaptive Funktion insbesondere von Doppelpulsen, Entladungsregularisierungen und Echoantworten untersucht. Doppelpulse wurden als aggressives Motivationssignal kategorisiert, wohingegen die Kommunikationsfunktion von Regularisierungen im gegenseitigen Einschätzen zu Beginn einer kompetitiven Begegnung zu liegen scheint. Entladungssynchronisation durch gegenseitige Echoantworten kann dabei eine Einschätzung des Gegenübers zum Vorteil beider Parteien erleichtern. Da Echoantworten in verschiedenen Verhaltenssituationen auftreten und artifizielle Echoantworten der Attrappe vermehrt zu Echos vonseiten der Fische führten, wurde postuliert, dass die Echoantwort eine generellere Funktion bei der Fokussierung gegenseitiger sozialer Aufmerksamkeit übernehmen kann.

Im zweiten Teil der Arbeit wurde ein biomimetischer Fischroboter konstruiert, um zu untersuchen, auf welcher sensorischen Grundlage die Fische der Attrappe folgen. Es konnte gezeigt werden, dass elektrische Playbacksignale, nicht aber biomimetische Bewegungsmuster, Folgeverhalten der Fische induzieren. In einem weiteren Schritt konnte durch die Reduktion der Attrappe auf die elektrischen Signalsequenzen aus der Perspektive der Versuchsfische gezeigt werden, dass passive Wahrnehmung elektrischer Kommunikationssignale auch bei der räumlichen Koordination sozialer Interaktionen von Bedeutung ist. Dies wird mutmaßlich über die gleichen Rezeptororgane vermittelt, die auch für die Elektrokommunikation verantwortlich sind. Das EOD kann daher als ein soziales Signal betrachtet werden, das es ermöglicht, eine Attrappe als künstlichen Artgenossen in eine Gruppe schwach elektrischer Fische zu integrieren.

Der Einfluss einer elektrisch interaktiven mobilen Fischattrappe auf kleine Gruppen von bis zu vier Individuen wurde im dritten Teil der Arbeit getestet. Typisches Schwarmverhalten konnte in diesem Zusammenhang nur selten beobachtet werden. In kleinen Gruppen kam es während sozialer Interaktionen jedoch häufig zu EOD-Synchronisationen durch Echoantworten zwischen zwei Fischen, oder zwischen einem Fisch und der interaktiven Attrappe. Motorische Verhaltensinteraktionen im Zeitraum dieser Synchronisationen stützen die Hypothese, dass Mormyriden durch elektrische Entladungssynchronisation soziale Aufmerksamkeit zwischen Individuen herstellen können, und die Echoantwort somit besonders in Gruppen eine nützliche Kommunikationsfunktion übernehmen kann.

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Abbreviations

CN	medullary command nucleus
DC	Direct current
DP	Thalamic dorsal posterior nucleus
ELa	Nucleus exterolateralis pars anterior
ELL	Electrosensory lateral line lobe
ELp	Nucleus exterolateralis pars posterior
EMN	Electromotor neuron
EOD	Electric organ discharge
ESS	Evolutionary stable strategy
fps	Frames per second
IDI	Inter-discharge interval
JAR	Jamming avoidance response
MHC	Major Histocompatibility Complex
nELL	Nucleus of the electrosensory lateral line lobe
NND	Nearest neighbor distance
PCN	Mesencephalic precommand nucleus
RCS	Relative cumulative sum
RHP	Resource holding power
SIDs	Sharp increases decreases in EOD rate
TTL	Transistor-transistor logic
VPd	Dorsal region of the ventroposterior nucleus
V_{p-p}	Peak-to-peak voltage

Appendix A

Table A.1: Properties of the seven IDI-sequences that were used during the playback experiments in chapter 4.

Playback	**Context**	**IDI min [ms]**	**IDI max [ms]**	**IDI mode [ms]**	**IDI mean [ms]**	**IDI var [ms]**	**Freq. mean [Hz]**	**Freq. var [Hz]**	**Special**
F_1	Foraging in a group	17	80	31	34	91	31.0	53.6	---
F_2	Following after a moving fishing lure	33	93	64	65	127	15.8	8.6	---
F_3	Slowly swimming	44	150	94	94	329	11.1	5.4	---
F_4	Resting	67	308	260	239	1924	4.5	2.9	---
P_S	Subordinate	63	1356	149	---	---	---	---	Cessations
P_A	Aggressive interaction	17	101	26/ 50	---	---	---	---	Accelerations
P_D	Reaction to playback signals	20	109	22/ 44/ 91	---	---	---	---	Double pulses

Video examples of experimental trials from chapter 4 featuring typical instances of the quantified motor interactions 'cut off,' ‘circling,’ ‘lateral probing,’ ‘lateral va-et-vient,’ ‘radial va-et-vient,’ ‘head butt,’ and ‘touch’ (compare Figure 4.20) can be found online:

https://doi.org/10.1371/journal.pone.0184622.s007

Appendix B

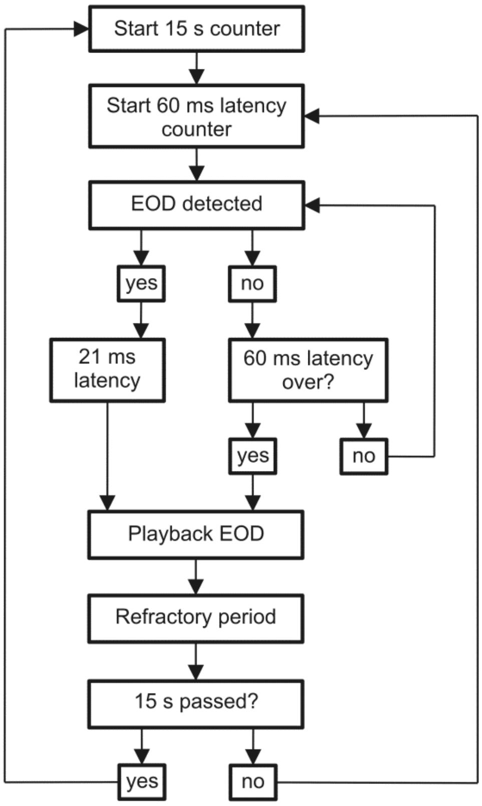

Figure B.1: Dynamic echo playback generation. Flowchart illustrating the generation of the dynamic echo playback sequence used in chapters 5 and 11. Artificial echoes were triggered by EODs that were emitted by *M. rume* close to the receiving electrodes of the mobile dummy fish.

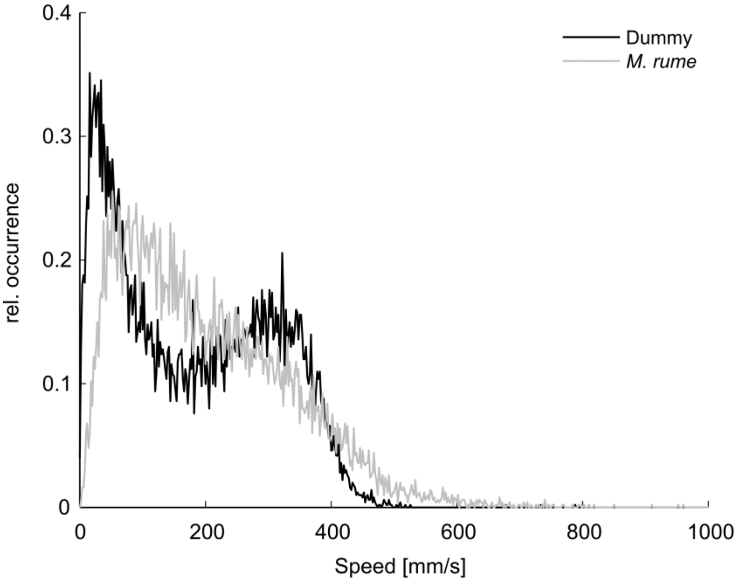

Figure B.2: Distributions of swimming speed. Relative occurrence of speeds of the dummy (black) and all fish (grey) during the presentation of the static random playbacks. Bin size: 2 mm s^{-1}.

Appendix C

Characterization of the acoustic noise that was generated by the tail-fin oscillation of the biomimetic robot fish used in chapter 8. Figure C.1 represents a short period of oscillation with a frequency of 1.5 Hz. Sound was recorded underwater with a hydrophone (Brüel & Kjær Type 8103; charge sensitivity 97 x 10^{-3} pC/Pa) and a charge amplifier (Type 2635, Brüel & Kjær, Nærum, Denmark; lower frequency limit: 1 m/s^2; upper frequency limit: 30 kHz) via an MME sound card at 88200 Hz using Audacity version 2.1.2. The power spectrum of the same sequence is shown in Figure C.2.

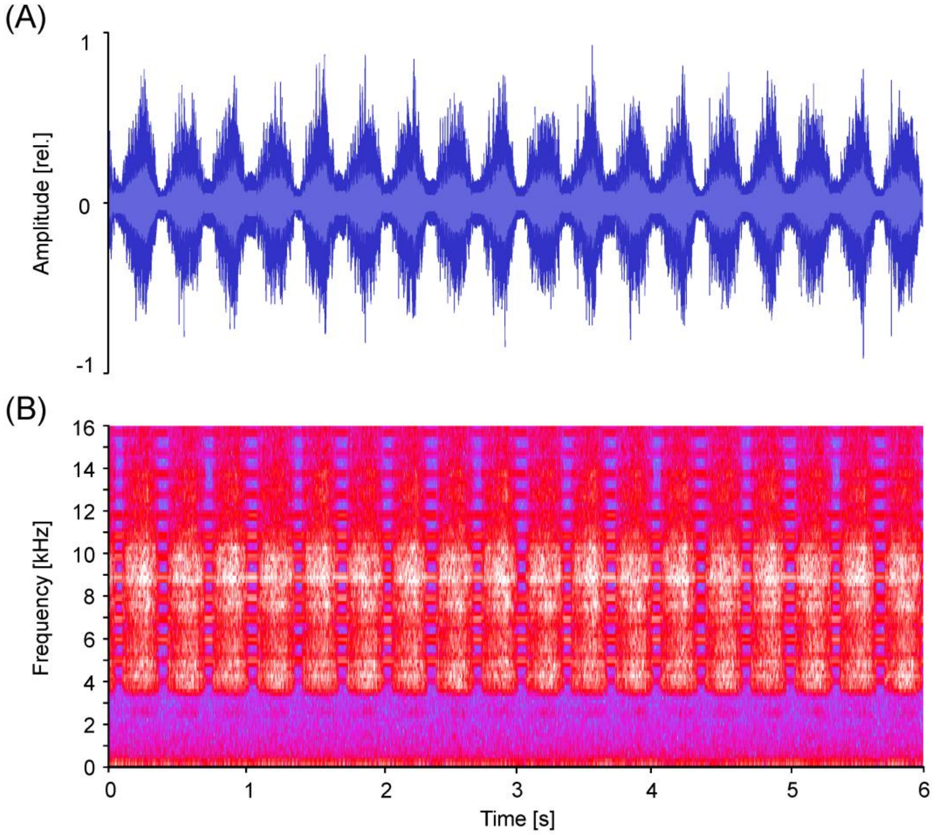

Figure C.1: Acoustic noise made by the biomimetic robotic dummy fish. (A) Waveform data showing relative amplitude and periodicity of the sound of the dummy's the tail-fin oscillation. (B) Spectrogram of the sequence shown in (A). Sound levels are color-coded with lighter colors representing higher intensities. Most noise was generated at frequencies between 4 and 10 kHz.

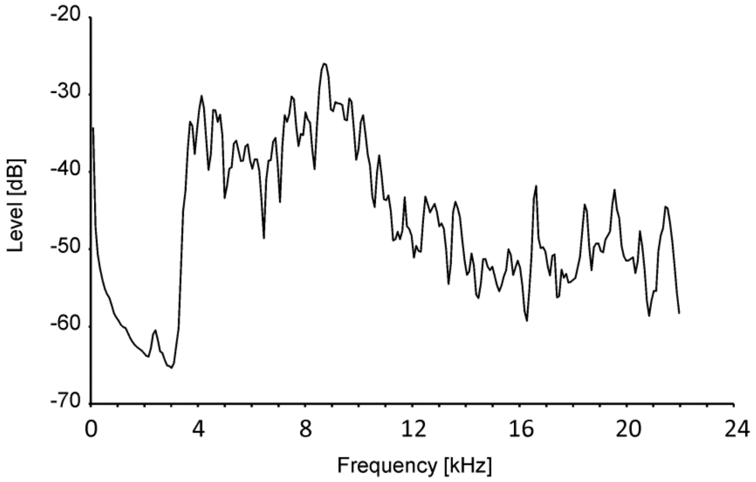

Figure C.2: Characterization of acoustic noise. Power spectrum of the data shown in Figure C.1.

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Publikationen

Research Papers

Worm M, Landgraf T, Prume J, Nguyen H, Kirschbaum F, von der Emde G (2018): Evidence for mutual allocation of social attention through interactive signaling in a mormyrid weakly electric fish. *Proceedings of the National Academy of Sciences* (Accepted).

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